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Minimally Processed Refrigerated Fruits and Vegetables

Second Edition

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Editors

Minimally Processed Refrigerated Fruits and Vegetables

Second Edition

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I dedicate this book to my mother, father, wife, children, and grandchildren.

Preface to the Second Edition

Never in the history of mankind were human food intake, nutrition, health, environment, and lifestyles so much interrelated and researched, as it is done today, in the twenty-first century. Never in human experience has food been available in this abundance and variety, as seen today, in the world. Humans (an average person) can eat convenient, refined, highly processed food with great speed, enabling them to consume about 3000 calories in 10–15 min. But, approximately, it takes a marathon (5 h) to burn 2600 calories (100 calories/mile/10 min).

Advances in food technologies in the last 200 years resulted in efficient food production and centralized, standardized processing systems, which enormously improved the general health, quality of life, and abundance of foods, eradicated many infectious and deficiency diseases, and increased life expectancy of majority of the people. Processing is accomplished by using one or more of a range of operations, including washing, grinding, mixing, cooling, storing, heating, freezing, filtering, fermenting, extracting, extruding, centrifuging, frying, drying, concentrating, pressurizing, irradiating, microwaving, and packaging. Recent advances in emerging food-processing technologies, such as high hydrostatic pressure or high-intensity electric field pulses, and minimal processing allow targeted and sophisticated modification and preservation of foods.

The researches in food science and nutrition over the last 200 years focused on five major areas:

(a) Basic nutrient (carbohydrates, proteins, lipids) preservation and microbial safety of foods.
(b) Micronutrient deficiency disease prevention with vitamins and minerals.
(c) Public health efforts at eradicating nutrient deficiency by the voluntary addition of iodine to salt (1922). The fortification of other foods were also used to address public health problems such as rickets (vitamin D), beriberi (thiamin), pellagra (niacin), and dental caries (fluoride) and folate fortification for pregnant woman diet (1998). Since the initiation of fortification policies in the United States, as in many parts of the world, clinically evident nutritional deficiencies have been virtually eliminated.

(d) Food and chronic diseases: the role of food in the cause of and treatment for chronic disease. Disease patterns shifted from infectious and nutrient deficiency diseases to increasing rates of cardiovascular disease, diabetes, cancer, obesity, neurological diseases, and osteoporosis.
(e) Nutrition for optimal lifelong health: superfood, phytochemicals, nutraceuticals, and bioactive food components.

The chronic conditions of cardiovascular diseases, cancers, neurodegenerative diseases, obesity, osteoporosis, arthritis, diabetes, and chronic respiratory diseases emerged as the major causes of morbidity and mortality. Nutritional and epidemiological research implicated food as a major factor in the etiology of diseases.

In response to consumer demand, the food processors produced a range of new processes and products; attention is being increasingly focused on food which can confer specific health benefits, so-called functional foods, superfoods, phytochemicals, and nutraceuticals, whose further development may help the population to attain even greater health in the twenty-first century.

Foods rich in whole and unrefined vegetables, fruits, legumes, nuts, and seeds contain high concentrations of antioxidant phenolics, fibers, and numerous other phytochemicals that may be protective against chronic diseases. Whole foods are foods that are unprocessed or minimally processed, before being consumed.

Minimally processed refrigerated (MPR) fruits and vegetables are fresh, raw, whole or cut, safe foods which are usually processed and sold to consumers in a ready-to-eat, ready-to-use, ready-to-cook, ready-to-serve forms (Wiley 1994). The fresh-cut produce industry has been the fastest-growing portion of the food retail market during the past 10 years, providing consumers with convenient and nutritious food. However, fresh-cut fruits and vegetables raise food safety concerns, because exposed tissue may be colonized more easily by pathogenic bacteria than intact produce. This is due to the higher availability of nutrients on cut surfaces and the greater potential for contamination because of the increased amount of handling.

This book is an outgrowth of our research and studies at the University of Maryland, USA; INRA Avignon, France; and the METU, Turkey, over the last 35 years. The first edition of this book was the first book written on the subject of minimally processed fruits and vegetables, about 25 years ago. It was and still is a new major development in food science.

This volume contains 4 parts and 22 chapters. The book starts with an introduction and definition of the concept. The first part deals with the fundamentals of minimal processing technologies in seven chapters. The second part of the book gives some common commodities currently minimally processed in the market in eight chapters. The third part has three chapters and gives information about the emerging and new technologies in the sector. The fourth part of the book gives some information related to safety, health, and nutritional aspects of the minimal processing in five chapters.

This volume is written for food processors, engineers, and technologist, as well as nutrition experts, medical doctors, consumers, regulatory officials, graduate and undergraduate students, researchers, and other stakeholders on the subject.

I hope the book will serve to its purpose by and large.

Gölbaşı, Ankara, Turkey Fatih Yildiz
January 10, 2017

Preface to the First Edition

The objective of this book is to introduce, organize, and document the scientific, technical, and practical aspects involved with the manufacture, storage, distribution, and marketing of minimally processed refrigerated (MPR) fruits and vegetables. The overall function of these foods is to provide a convenient, fresh product for food service and retail consumers. High levels of quality accompanied by superior safety are essential requisites of MPR fruits and vegetables. Since refrigeration or chilling is essential to the quality and safety of these food products, "refrigeration" is included in the title of this book, i.e., *MP Refrigerated Fruits and Vegetables*.

This *swiftly* emerging area of processing requires organization and unification of thinking concerning fruit and vegetable food products which are not considered commercially sterile from a classical standpoint. Fruits and vegetables require very special attention because of the multitude of enzymic and respiratory factors as well as microbiological concerns which impact on the safety of low-acid and acidified vegetables and on the economic viability of high-acid fruit products of all kinds.

The name of this field, minimally processed (MP) fruits and vegetables, deserves attention in that there is little agreement among processors, produce dealers and merchants, and research workers regarding the proper term for these products. Many names are used as synonyms for MP fruits and vegetables, and these include ready-to-use, precut, lightly processed, fresh-cut, etc.; I think it behooves the food industry to settle on a single name and agree on a standard definition of this product. Doing so would benefit research and development efforts, data base searches, nutritional information needs, and the like.

The term "refrigerated" as opposed to chilled foods seems to be slightly confusing. These terms are synonymous, but probably one or the other should be selected to avoid confusion. Although the "chilled food" term may be easier to say than "refrigerated food," in the United States (US) at least, "refrigerated" may be more recognizable by consumers.

This volume is designed to serve primarily as a reference book for those interested and involved in the minimally processed refrigerated or chilled fruit and vegetable industry. There has been an attempt to bring together historical information available from many fields developed long before the concept of "minimally

processed" foods was considered a viable field of endeavor. I have tried to gather as much knowledge as possible regarding this field but realize that there is much more research and development to be completed and that great opportunities exist in this area of food technology. The lack of information in certain areas has hampered the authors of some of the chapters. If I have been able to summarize the present knowledge of MPR fruits and vegetables and stimulate others to develop this important field in a uniform and concise manner, I think we will all feel successful.

I thank all of the contributors to this volume and thank the following individuals for reviewing chapters: Timothy P. Lydane, Imperial Produce; Dr. John Y. Humber, Kraft General Foods; Dr. Dennis C. Westhoff, University of Maryland; Dr. Harold R. Bolin, USDA-ARS; Dr. Bernard A. Twigg, University of Maryland; Dr. Charles A. McClurg, University of Maryland; and Dr. Charles R. Barmore, W.R. Grace and Co. Thanks also go to Kathleen Hunt, Robert Savoy, Lovant Hicks, David Jones, Ester Lee, and all others who read manuscripts, worked with tables and artwork for figures, entered information and data into the computer, and generally made this volume possible.

Finally, I thank Joy Wiley for her help and encouragement during the time that this work was being produced.

Robert C. Wiley, September 22, 1993

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Prof. Dr. Fatih Yildiz Dr. Yildiz received his B.S. degree from Atatürk University in Erzurum, Turkey. He started his postgraduate studies at the University of Wisconsin, Madison. He got his M.S. and Ph.D. degrees in food biochemistry from the University of Maryland in the USA. He worked as a faculty member at the University of Maryland for 5 years. He was the founding faculty member of the Department of Food Engineering, Department of Biotechnology, and Department of Biochemistry at the Middle East Technical University, Ankara, Turkey. Dr. Yildiz worked as a professor at the University of Minnesota, Department of Food Science and Nutrition, in the USA. He also has done research at INRA, France, as a visiting professor. He supervised more than 120 graduate students in these universities.

He has done research projects with FAO, UNIDO, UNICEF, OECD, and NATO as project director in the past.

Currently, he is the director of the Mogan International Research Center, Ankara, Turkey, giving lectures, organizing conferences, and supervising projects throughout the world.

Dr. Yildiz has published more than 150 research and review papers, in international and national journals, and chapters in several books as the major author, in addition to editing four international books. He was the coauthor of the book entitled *Minimally Processed and Refrigerated Fruits and Vegetables* published in 1994, which was a new concept in the food industry. He is the editor of the first book entitled *Phytoestrogens in Functional Foods* published by CRC and *Advances in Food Biochemistry and Development and Manufacture of Yogurt and Other Functional Dairy Products*. All these books brought new dimensions to food science.

His current research interests include health (phytochemicals), nutrition, and safety attributes of foods. He is the editor in numerous journals and a member of 10 scientific and academic organizations in the USA, EU, and Turkey. Dr. Yildiz is married, has two children, and three grandchildren.

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Part I
Fundamentals

Chapter 1
Introduction to Minimally Processed Refrigerated (MPR) Fruits and Vegetables

Robert C. Wiley and Fatih Yildiz

1.1 Introduction

Minimally processed refrigerated (MPR) fruits and vegetables are an important and rapidly developing class of foods. These convenience foods are being produced by unique applications of the basic and food sciences and their supporting technologies and engineering. MPR fruits and vegetables have attracted the interest of many facets of the food industry including such diverse areas as food manufacturers, retail food stores (deli departments), restaurants, carry-out establishments, and commissary units. Much of the developmental work in this field is now being carried out in western Europe, Japan, and the United States in response to strong consumer demand, both individual and institutional, for new types of like-fresh high-quality convenience and safe foods. The purpose of MPR foods is to deliver to the consumer a fresh-like fruit or vegetable, product with an extended shelf-life, and at the same time ensure food safety and "maintain sound nutritional and sensory quality. MPR fruits and vegetables received their original impetus from institutional users, but retail applications are gaining favor and are expected to expand rapidly.

Many countries are making significant strides toward solving the myriad of problems associated with the manufacturing, distribution, and marketing of MPR fruits and vegetables.

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This progress appears to be due to strong governmental and food industry support for MPR food applications, a conducive regulatory climate in preparation for the expanding food markets that will be available within the European Community (EC) since the trade and monetary barriers are removed.

It is clear that investigators in the fields of food science, nutrition, technology, related disciplines, and engineering have studied and conducted research on the various facets of MPR food for many years, especially in the cold preserved and raw fresh foods areas (Fig. 1.1). The early work by Smock and Neubert (1950) on controlled atmosphere (CA) storage of apples is a good example of the type of research that is now utilized and is being greatly refined with regard to MPR fruits and vegetables. It is now time to look at this field as a concise discipline investigating the continuum of product flow from harvest to consumption (Fig. 1.2). As seen in Fig. 1.2, the discipline effectively links product production (horticulture production) with manufacturing, packaging, distribution, and consumption. See Morris (1991) and Anon (1991) for good manufacturing practices (GMP) and Hazard Analysis Critical Control Point (HACCP) Systems for MPR fruits and vegetables and chilled foods in general. A unified approach and thinking of MPR foods as a specific food preservation industry/method as contrasted to canning, freezing, or drying would greatly assist in its development, which is now growing rapidly in many parts of the world.

As shown in Fig. 1.1, the major differences between MPR fruits and vegetables and raw fruits and vegetables are the rather specific processing and preservation steps taken with MPR foods. The MPR fruits and vegetables are usually living respiring tissues (Rolle and Chism 1987), but respiration is greatly "increased by cutting, slicing, low temperature heat treatments, and preservatives (Figs. 1.3 and 1.4, Anon 1989a). As seen in Fig. 1.3, the intact cell is expected to be much more resistant to oxidative browning and entrance of bacteria as compared to the cut cell. Figure 1.4a shows the intact vegetable product in a package, and Fig. 1.4b shows the condition of some cut surface cells with the majority of intact interior cells. The latter situation may greatly complicate the modeling of gas exchange in polymeric packages as suggested by Mannapperuma and Singh (1990). Refrigeration and packaging may be optional for raw fresh intact fruits and vegetables but are mandatory for MPR fruits and vegetables.

In this book, the attempt is made to differentiate between intact fresh fruits and vegetables and MPR fruits and vegetables even though the latter are somewhat difficult to define exactly. For example, whole intact apple fruit in CA storage, described by Smock and Neubert (1950), would not be considered an MPR product, whereas sliced, refrigerated apple slices treated with ascorbic acid and calcium salts (Ponting et al. 1972) would easily fall into the MPR category. As seen in Fig. 1.5, the vacuum-infused peeled oranges prepared using pectinase are a good example of minimally processed fruit Baker and Bruemmer (1989). The fruit can be segmented or handled in a tray pack as a whole fruit. Waldorf salad with diced apples, celery, walnuts, etc., in a dressing and sous vide with a portion of prepared fruits or vegetables would be considered an MPR fruit and vegetable product to carry the concept

Food Preservation and Processing Categories	Not Preserved, May be home Cooked	Minimally Processed, Refrigerated,May Be Home Cooked	Cold Preserved, Processed Frozen Storage, May be Home Cooked	Irradiation, electronic processing methods	Dehydrated	Heat Processing Canning,Sterilization, Pasteurization,Ultra High Temperature Processing,Frying	*Non-thermal and Electrotechnologies of food processing and preservation
Food Quality Color,Flavor, texture	Fresh	Like-Fresh	Slightly to fully Modified**	Slightly Modified**	Slightly to Fully Modified**	Fully Modified**,but Safe, no microbial contamination	Slightly Modified**
Storage, Shelf-life	May not be refrigerated	Requires Refrigerated, Transportation, Storage	Requires Frozen or Refrigerated storage,Transportation, and Market display	Requires Refrigerated storge	Shelf Stable at Ambient Temperatures	Long Shelf life at Ambient Temperatures	Shelf Stable, requires Refrigerated Transportation, Storage
Packaging	May not be Packaged	Requires Packaging	Requires Packaging	Requires Packaging	Requires Packaging	Requires Hermetic, Aseptic packaging	Requires Packaging
Cell Integrity	No Change	No change	Changes	Changes	Changes	Total destruction	Changes
Enzymes(Activity and structure)	No Change	No Change	Changes	Changes	Changes	Total destruction	Changes
Phytochemicals, Vitamins, Proteins	No Change	No Change	Changes	Changes	Changes	Highly changed	Changes
Dietary Advanced Glycation End Products(dAGEs), Formation before home cooking	None	None	Some dAGEs formation	dAGEs formation	dAGEs formation	High Quantity of dAGEs Formation	Some dAGEs formation
Free Radical Formation	None	None	Some Free radical	Free radicals formation	Some Free radicals fomation	Free radicals formation	Free radicals formation

*Non-thermal and electrotechnologies of food processing and preservation includes:
a-High Pressure, Ultra-high Pressure(HPP), b-Plasma, c-Pulsed Light and Pulsed electric fields, d-Ohmic Heating, e-Ionizing radiation, f-Pulsed X-ray, g-Ultrasound, h-High-voltage arc discharge, i-Magnetic fields, j-Dense phase carbon dioxide, k-Microvawes, l-Electron Beam Processing.
**Product Freshness

Fig. 1.1 Spectrum of food preservation systems related to minimally process refrigerated (MPR) fruits and vegetables (*Product freshness)

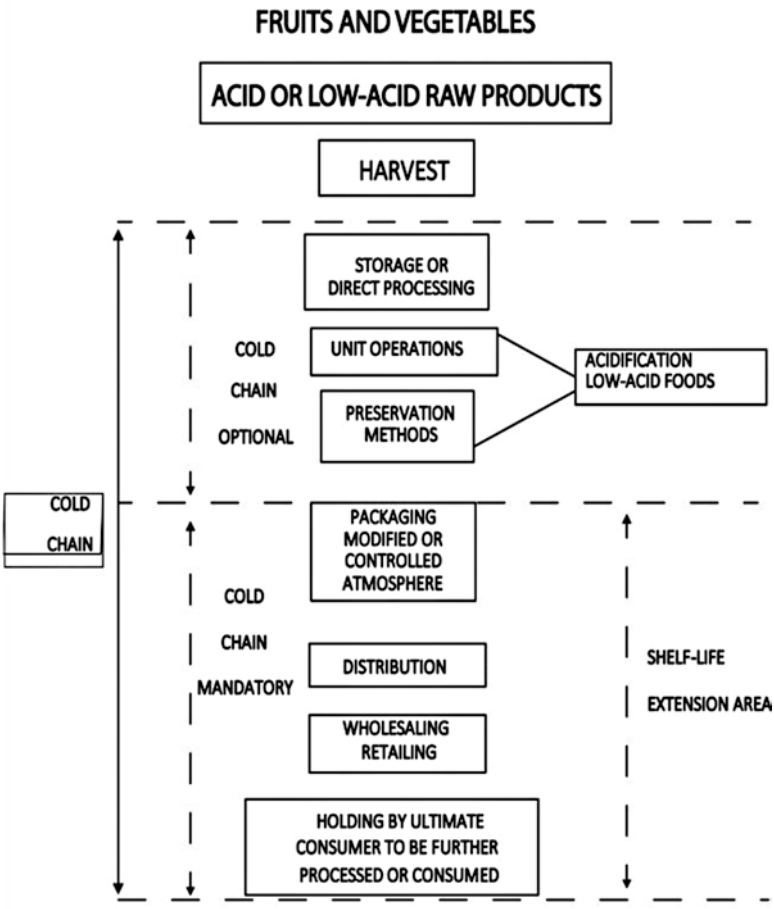

Fig. 1.2 General flow sheet for minimally processed refrigerated fruits and vegetables

into prepared foods. See Part 2 for an extensive listing of MPR fruits and vegetables.

A more difficult to define, gray area might involve the classification of washed, waxed cucumbers. These products would probably not be considered MPR foods because although they are packaged (waxed), they are also intact." Waxed intact cucumbers could be contrasted with precut or sliced cucumbers which have a relatively short shelf-life and are considered MPR foods. MPR fruits and vegetables should not include fresh intact fruits and vegetables which may undergo normal postharvest handling treatments such as sizing, grading, washing, waxing, CA, or modified atmosphere (MA) storage (see Fig. 1.1).

Frozen foods make up a portion of products normally classified as cold preserved foods. However, MPR foods are dissimilar to frozen foods in that frozen foods must be transported and stored at −10 °C or lower, whereas MPR foods require cold-chain

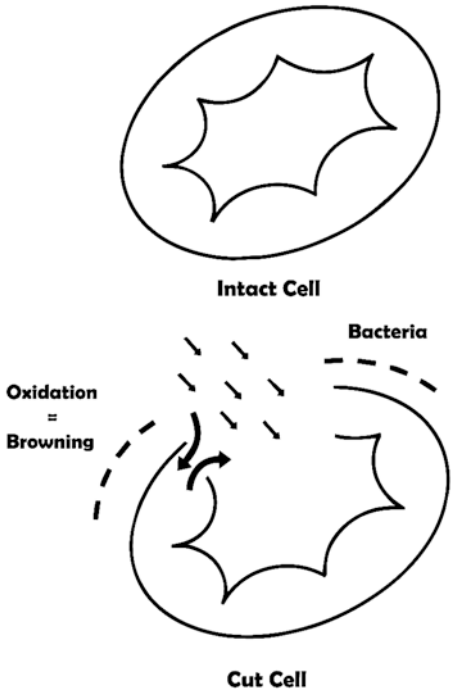

Fig. 1.3 Diagrams of intact and processed (cut) fruit or vegetable cell (From CTIFL, France)

refrigerated or slightly higher temperatures depending on the commodity or mixtures of commodities (Anon 1989b). The overall quality of frozen food is different than the like-fresh quality of MPR food. Many times the consumer can discern differences in texture between frozen and MPR foods. Some manufacturers of sous vide meals, for example, freeze their product, which makes the meal little less than a frozen food in most respects.

MPR fruits and vegetables are different from dehydrated fruits and vegetables in terms of texture and water activity (a_w). MPR fruits and vegetables which normally have a_w around 0.97–0.99 are very sensitive to a_w reduction. However, preservatives such as ascorbic acid and citric acid used on dehydrated fruits and vegetables are also used on MPR fruits and vegetables. Dehydrated foods are considered shelf-stable at ambient temperatures and thus do not require the cold-chain delivery system used for MPR foods (Fig. 1.2).

An important difference between MPR and thermally processed foods is that MPR foods do not exhibit "commercial sterility" as defined for thermally processed foods. This definition states thermally processed foods are free of microorganisms capable of reproducing under nonrefrigerated conditions and that no viable microorganisms (including spores) of public health significance can reproduce. This "sterility" may be acquired by control of water activity, reducing pH, or application of heat. There is some likelihood that the "commercially sterile" concept may be assigned to those MPR fruits and vegetables that are deemed to be safe. The microbial and

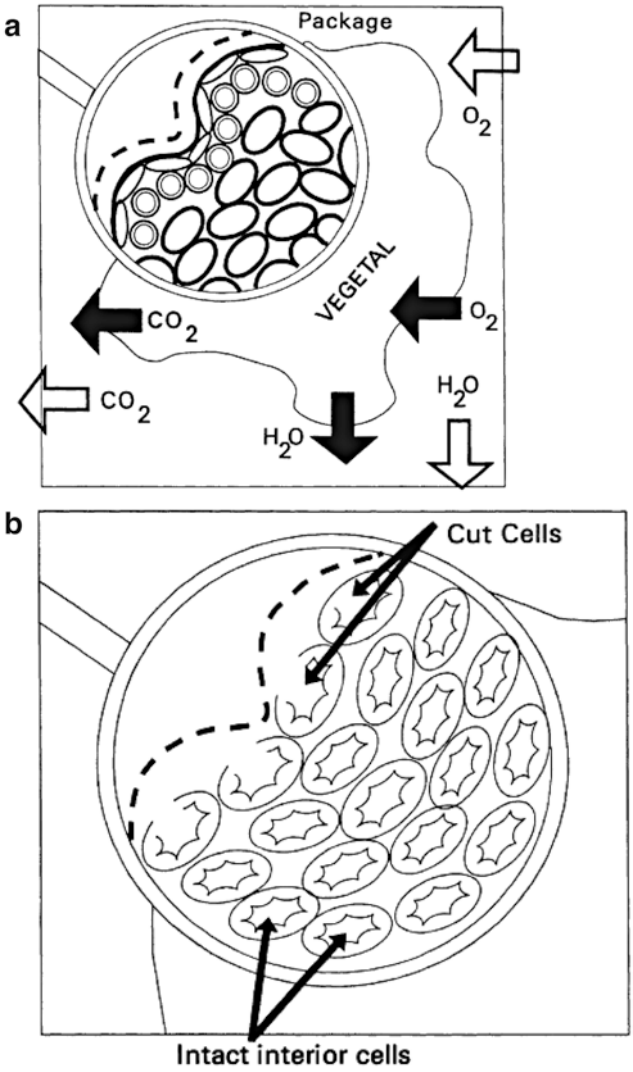

Fig. 1.4 Diagram of product in package and product with surface modification such as from peeling or slicing (From CTIFL, France)

enzyme limits for "commercial sterility" or "microbial and enzymatic safety" of MPR fruits and vegetables are yet to be developed, if in fact this term is accepted and is germane to these foods. Thermally processed food cannot be claimed to be like-fresh. Nevertheless, the tremendous amount of research conducted on handling, processing, and preservation of fresh, frozen, irradiated, dehydrated, and thermally processed fruits and vegetables can be applied where appropriate to the relatively new concept of MPR foods Kader (1986), Kantor (1989).

Fig. 1.5 Example of vacuum-infusion peeling of oranges by pectinase (From Agricultural Research Service, USDA)

A major advantage in the development and careful classification of MPR foods is that they provide a basis for separation of the traditional cold preservation field, as described by Potter (1968), into two distinct areas, that is, frozen foods and refrigerated foods. As shown in Fig. 1.1, separation of these two areas would greatly simplify the frozen food/refrigerated food classification by placing all frozen foods together and all refrigerated foods in a refrigerated category (Appendix 1). No doubt refrigerated as well as other preservation categories should be subclassed into commodity and prepared foods areas. One noticeable difference between MPR fruits and vegetables and many other refrigerated foods is the "living tissue" concept which must be considered in dealing with certain types of MPR fruits and vegetables. The shelf-life of MPR fruits and vegetables also should be limited to 3–6 months from harvest to consumption due to the living tissue and active enzymes concept.

There is a need to update and organize current information developed over many years of research in food science and technology that may apply to MPR foods. In addition, there are demands for research to make MPR foods comparable to the currently more actively used preservation methods for which so many food processing industries are named. The food industry seems interested in making the large family of MPR foods more intensively competitive. It would require several volumes to cover adequately the various food product types that could be interpreted as using the technologies and packaging systems required to produce MPR foods. To narrow the scope of this volume, emphasis will be placed on MPR fruits and vegetables, although in some chapters of the book, particularly in the regulatory and quality control areas, it is difficult to separate MPR fruits and vegetables from other MPR foods. This burgeoning area of food science requires the expertise of horticulturists, plant physiologists, molecular biologists and pathologists, biochemists, biotechnologists, microbiologists, food scientists, and food engineers and packaging experts, to name only a few specialties. A truly interdisciplinary effort will be needed to deliver to the markets MPR fruits and vegetables that will have extended high-quality shelf-life beyond 8–10 days, which is usually satisfactory for the retail European market. MPR fruits and vegetables will probably require a minimum shelf-life of 21 days to compete satisfactorily in US markets (Lioutas 1988). This author is likely referring to name-brand products requiring national distribution and not to regional operations.

1.2 Definitions of MPR Fruits and Vegetables

There have been a number of articles which have defined minimal processing. For example, Rolle and Chism (1987) suggest that minimal processing "includes all unit operations (washing, sorting, peeling, slicing, etc.) that might be used prior to blanching on a conventional processing line." They feel all these products are living tissues. A slightly different approach was taken by Huxsoll and Bolin (1989) in which they felt the "minimally processed product is raw and the cells of the tissues are alive but these characteristics are not required." There was no agreement on the definitions for minimally processed fruits and vegetables at a recent American Chemical Society symposium (Hicks and Sapers 1991). Cantwell (1991) for one, at the symposium, called these products "cut fruits and vegetables which are lightly processed."

It is generally conceded that MPR fruits and vegetables are products that contain live tissues or those that have been only slightly modified from the fresh condition and are fresh-like in character and quality. These tissues do not exhibit the same physiological responses as normal (raw) untreated intact live plant tissues (Fig.1.3 and 1.4). The cutting, abrasion, or minimal heating of these tissues can cause broadly different responses in various environmental and packaging situations. However, some fruits and vegetables that show little or no physiological activity should also be included in the MPR category. The cold preservation category (Fig. 1.1) now encompasses some of these types of foods. MPR fruits probably should include products such as chilled peaches in glass containers in which complete inactivation of cellular metabolism has recently occurred, and the product has been quickly transferred to the market in the cold chain. Sous vide dishes which may include preheated vegetables or fruits should be included in the MPR food area. In terms of physical state, the tendency has been to include only solids, semisolids, and semiliquids as MPR fruits and vegetables, but refrigerated liquids both cloud and clarified juices should be included in this class of foods. The freshly squeezed orange juices and others that are chilled and packaged in plastics, paperboard, or glass are good examples of this type of product. This type of food should probably be included with refrigerated fruit and vegetable products under the like-fresh minimal processing column (Fig. 1.1). The USFDA is still considering whether these products should be labeled fresh.

MPR fruits and vegetables (for the purposes of this book) are defined as those prepared by a single or any number of appropriate unit operations such as peeling, slicing, shredding, juicing, etc., given a partial but not end-point preservation treatment including the use of minimal heat, a preservative, or radiation. The preservation or hurdle treatment may include pH control, antioxidants, chlorinated water dips, or a combination of these or other treatments (see Chap. 3). It is important to take advantage of the synergies of all preservation treatments. The initial preparation and preservation treatments are usually followed by some kind of controlled/modified atmosphere and vacuum packaging and subjected to reduced temperatures above the freezing point during storage, distribution, marketing, and just prior to

preparation for consumption. For safety and greatest retention of sensory and nutritional quality, these products must be distributed and marketed in the cold chain.

Some precut fruits and vegetables (examples are cucumber, eggplant, tomatoes, and tropicals, and subtropicals) have to be handled at higher temperatures to avoid chilling injury (Appendix, Tables 2 and 3). It should be clear that MPR fruits and vegetables are not intended for ambient shelf-life stability as expected from canned, retorted, or aseptic processed and packaged fruits and vegetables. Nor are they protected from spoilage or quality changes by freezing and being held in frozen storage. There is a need for *agreement* by workers in the food field as to the proper description (title) for these foods and definitions thereof. To be successful, MPR foods will have to be of high quality, convenient, healthier, fresh-like aroma, active phytonutrients, and safe and reduce the risk of chronic diseases. Currently, there is no other food processing methods that have all these properties.

1.3 Quality of Minimally Processed Fresh Produce and Other Plant Foods

Several parameters affect quality and shelf-life of MPR produce from field to the fork as summarized in Table 1.1 and Fig. 1.6. Quality aspects of MPR fruits and vegetables are studied and published by many researchers (Mercedes Diaz-Mendoza et al. 2016; Anna Kårlund et al. 2014). Leaf senescence is a physiological process critical for plant survival. It is characterized by the dismantling of cellular structures, massive degradation of macromolecules, and efficient relocation of nutrients from senescing leaves to growing tissues or sink organs. Shelf-life extension will be very closely related to the control of senescence and cell membrane peroxidations in MPR fruits and vegetables (Mercedes Diaz-Mendoza et al. 2016; Yildiz et al. 2007).

Health-promoting quality of minimally processed fruits and vegetables has been emphasized by many researchers in many countries in the last 20 years (Crowe et al. 2011; Carter et al. 2010). There is an increasing epidemiological and experimental

Table 1.1 Summary of the factors affecting total quality of the MPR fruits and vegetables

Parameters	Affects
Preharvest agricultural practices	Composition, phytonutrients
Growing crops practices	Size, shape, scars, pesticide residues
Harvest practices	Maturity, brix/acid, color
Postharvest handling	Mechanical damage, biochemical changes
Processing	Microbial growth, browning, weight loss
Packaging, handling, and storage	Spoilage, weight, loss, softening, surface drying
Physiological changes from harvest to fork (respiration rates of F&V O_2/CO_2)	Senescence, changes in respiration rates, ethylene production, yellowing, loss of water, size changes, loss of phytonutrients, shelf-life

Fig. 1.6 Senescence of broccoli (changes in lipids, chlorophylls, chloroplast, ethylene, and proteins metabolism during postharvest storage, yellowing)

data indicating that the consumption of vegetables and fruits confers many health benefits (Hughes et al. 2010; Cho et al. 2010). There are more than 10,000 phytochemicals in plant foods which include antioxidants, anti-inflammatory substances, and many others (Trim 2006; World Cancer Research Fund/American MPR) fruits and vegetables:Institute for Cancer Research 2007). For example, polyphenols are antioxidants and cardioprotective in humans (Anna et al. 2014).

1.4 Approach to Studying MPR Fruits and Vegetables

It is becoming increasingly recognized that low temperatures are not the defenses against foodborne illnesses that were previously accepted by the food industry and governmental agencies. The emergence to prominence of non-spore-forming psychrophilic pathogens such as *Listeria monocytogenes* and *Yersinia enterocolitica* can cause enormous problems in refrigerated foods (Chap. 19). Many vegetables, mushrooms, and legume sprouts are considered to be low-acid foods by the USFDA (pH 4.6 and over); thus, special measures will have to be taken to make these types

of foods free from type B and type E C. *botulinum* toxin formation in anaerobic situations brought on by packaging, product density, deaeration, or inert gas injection.

MPR fruits and vegetables have necessarily been studied first on an individual commodity basis and not as mixtures such as precut salads, pizza vegetables, or stir-fry mixtures, sous vide, and the like. A good example of the individual approach is the work on grated carrots in modified atmosphere packaging in France (Carlin et al. 1990). In this research, only gaseous packs of thin strips 1.5 mm × 1.5 mm sections of carrots were studied. There is a need for studies of convenience foods, that is, complex food mixtures in salads and soup mixtures and the various new products being developed for the market. Also every commodity and its individual cultivars need to be investigated for their applications to MPR foods. It is not clear at this time, for example, whether strawberries for the fresh market or for freezing would be the best suited as a MPR product. Perhaps plant breeders, biotechnologists should be looking for cultivars that are especially suited for minimal processing and refrigerated products. This idea seems to be supported by Rolle and Chism (1987), who have stated that MPR fruits and vegetables are all living respiring tissues in an "energized state." The individualistic concept is also suggested by Labuza and Breene (1989), who feel that is all important to define the end point of high-quality life for each type of fruit and vegetable. Both cultivar and maturity play a very important role in determining these end-point values. It is necessary to collect data on individual commodities, their cultivars, and maturity levels before attempting to work with either simple or complex MPR food mixtures, although this will eventually have to be done by computer modeling. There is no question that complex minimally processed food mixtures are being found in the markets at this time, but little information seems to be available about their shelf-life characteristics.

Rolle and Chism (1987) suggested live tissues that have the largest energy reserves such as white potato, topped beetroot, and apple have the longest shelf-life (postharvest) and that the greatest problems are being found with those commodities such as sweet corn/eggplant, raspberries, and strawberries that have the smallest "reserves." Probably more important than the lack or presence of "reserves" is the physical injury received during size reduction operations and the series of preservation steps that will set up a complex series of physiological and microbiological events (Figs. 1.3 and 1.4).

It appears that MPR fruits and vegetables of the high-reserve type and high respiration rates will have a shorter shelf-life than the intact raw or fresh product, for example, controlled atmosphere stored apples vs. the presliced refrigerated MPR) fruits and vegetables:fruit. The former may exhibit 6–8 months or longer satisfactory storage period, whereas refrigerated presliced apples may not be usable for more than 2–3 weeks if untreated or up to the average of 10 weeks if treated with 0.5% ascorbic acid and 0.1% Ca (Ponting et al. 1972). Low-reserve fruits and vegetables are very sensitive" to further processing as a means to extend storage and shelf-life and present complex research challenges. There are currently research studies in many parts of the world to address these problems, but in most cases, the findings are not published or available to the public.

The study of MPR fruits and vegetables will be divided into introductory information, preparatory operations, preservation methods, packaging, biological aspects, and regulatory implications for MPR food products.

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Chapter 2
Aspects of the Biology and Physics Underlying Modified Atmosphere Packaging

Theophanes Solomos

2.1 Introduction

The practice of modified atmosphere packaging (MAP) for fresh and minimally processed refrigerated (MPR) fruits and vegetables is widespread, particularly for commodities with a relatively short storage life (Cameron 1989; Chinnan 1989; Hayakawa et al. 1975; Hobson and Burton 1989; Kader 1986). The subject has been reviewed in the past from both a practical and a theoretical standpoint (Chinnan 1989; Mannapperuma et al. 1991). The beneficial effects of MAP are due in part to the decrease in O_2 and the increase in CO_2 levels, and in part to the decrease in water loss (Ben-Yehoshua et al. 1983; Biale 1946, 1960; Fidler et al. 1973; Isenberg 1979; Kader 1980, 1986; Kidd and West 1945; Lipton and Harris 1974; Smock 1979). In fact, in non-climacteric fruits such as citrus, the prevention of water loss is the main factor contributing to the extension of their storage life (Ben-Yehoshua et al. 1983) (see Chaps. 5, 6 and 7 for packaging materials).

In order to develop an appropriate modified atmosphere (MA) environment, the rates of O_2 uptake and CO_2 evolution, along with the permeability to O_2 and CO_2 of the film, must be known. In addition, the tolerance of the plant materials to the levels of CO_2 and O_2 engendered by MA must also be considered. The optimum levels of O_2 and CO_2 are known for a number of commodities (Fidler et al. 1973; Isenberg 1979; Kader 1985; Saltveit 1989; Smock 1979). In the case of bulky plant organs such as fruits, it is advantageous to determine the diffusivity of O_2 and CO_2 through their skin and flesh in order to avoid the creation of partial anoxia at the center of the tissue as this would be expected to contribute to spoilage and development of off-flavors during extended shelf life periods (Kader 1986).

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In this chapter, we discuss aspects of both the biology and physics involved in MAP. We also attempt to address some problems encountered in the generation of non-steady-state predictive models.

2.2 Biological Responses of Plant Tissue to Low O_2 or High CO_2

2.2.1 Effects of Low O_2 on Senescence of Detached Plant Tissues

The effects of O_2 on fruit ripening include (1) a diminution in the rate of respiration, (2) a delay in the climacteric onset of the rise in ethylene, and (3) a decrease in the rate of ripening (Blackman 1954; Burg and Burg 1967; Fidler et al. 1973; Kader 1986; Kanellis et al. 1991; Mapson and Robinson 1966; Smock 1979; Solomos 1982; Yang and Chinnan 1988a, b). It was observed by Blackman (1954) that the respiratory isotherms of O_2 uptake as a function of the external O_2 concentration are biphasic in nature in that they include an initial gradual decrease at relatively high O_2 levels, followed by a rapid decline as the levels of O_2 approach zero. The isotherm of CO_2 output follows that of O_2 up to the point where the rate of decline diminishes; in fact, it may even increase as the O_2 level approaches zero (Biale 1960). The rise in CO_2 evolution at low levels of O_2 is obviously caused by the expected Pasteur effect which results in an increase in fermentation. As far as prolonging storage life is concerned, the range of O_2 levels that would be expected to be beneficial must be in the region between the point that induces the initial decline in respiration and that at which it generates partial anoxic environments. It should be underlined that in this region of O_2 levels, the tissue does not experience anoxia because (1) there is no accumulation of ethanol (Table 2.1) and (2) no symptoms of low-O_2 injury develop even after lengthy storage (Fidler et al. 1973; Kader 1986; Lougheed 1987).

Table 2.1 Ethanol content (mM)

Apple no.	Air	Apple no.	1.5% O_2
1	3.294	1	1.32
2	3.422	2	0.56
3	9.264	3	0.09
4	5.361	4	2.18
5	5.584	5	0.54
Avg	5.385	Avg	0.938

"Gala" apples were kept for 180 days in air and 1.5% O_2

Table 2.2 Internal O_2 concentration, rate of respiration, and percentage of V_{max} of oxidases with different $K_m^{O_2}$

Intercellular partial pressure of O_2 kPa	CO_2 output ($\mu l \cdot g^{-1} \cdot h^{-1}$)	$K_m^{O_2}$ (μM)				
		0.05	2.2	2.5	3	4
		Percentage of V_{max}				
19.25	5.92	99.98	99.45	99.30	99.10	98.54
6.50	5.90	99.94	99.13	97.52	96.92	95.14
4.91	4.92	99.91	97.47	96.65	95.85	93.52
4.13	4.40	99.90	96.96	95.99	95.03	92.28
2.23	3.34	99.78	93.81	99.91	90.10	85.04
0.92	3.30	98.82	73.60	67.65	62.59	51.11
0.49	2.20	85.63	16.57	12.91	19.65	6.93

The data were calculated from the rate of respiration and diffusion coefficient of O_2 through the skin and flesh of "Gold" apples (Solomos 1987)

The biphasic nature of the O_2 isotherm as a function of external O_2 concentration has been attributed in turn to:

1. The existence of regulatory enzyme(s) that perceive(s) the level of O_2 and exert(s) a feedback inhibition on the initial steps of glucose oxidation, thus lowering respiration (Blackman 1954; Solomos 1982; Tucker and Laties 1985)
2. The effect of resistance to the diffusion of O_2 through the tissue (Chevillotte 1973; James 1953)
3. The presence of a terminal "oxidase" with an affinity for O_2 much smaller than that of cytochrome oxidase (Mapson and Burton 1962)

Work with apples and avocados (Solomos 1982; Tucker and Laties 1985) has shown that suggestion (2) is not a viable explanation of the biphasic nature of the O_2 isotherm. Suggestion (3), that the decrease in respiration at relatively high O_2 levels is due to the presence of an oxidase other than cytochrome oxidase, is difficult to assess. It is fair to say that neither cytochrome oxidase nor the alternative oxidase is expected to be curtailed by the levels of O_2 that initiate a diminution in the rate of respiration. Table 2.2 presents data that indicate that the apparent K_m for O_2 of the putative oxidase must be larger than 4 μM in order to produce an experimentally detectable decrease in CO_2 evolution. It is known that the K_m for O_2 of the cytochrome oxidase is about 0.05 μM, whereas that of the alternative oxidase is 10- to 15-fold higher than that of cytochrome oxidase (Douce 1985; Siedow 1982; Solomos 1977; Tucker and Laties 1985). The data thus preclude the curtailment of either of the known mitochondrial terminal oxidases by relatively high O_2 concentrations. Plant tissues, however, contain terminal "oxidases" that are resistant to the combined inhibition of both cytochrome and alternative oxidase (Laties 1982; Theologis and Laties 1978). Neither the nature of the residual oxidases nor the degree of their participation in plant respiration is known with any degree of precision. Suffice it to say that they are predominantly cytosolic in origin, with rather a low affinity for O_2 (Solomos 1988). Because of this low O_2 affinity, it could be argued that these

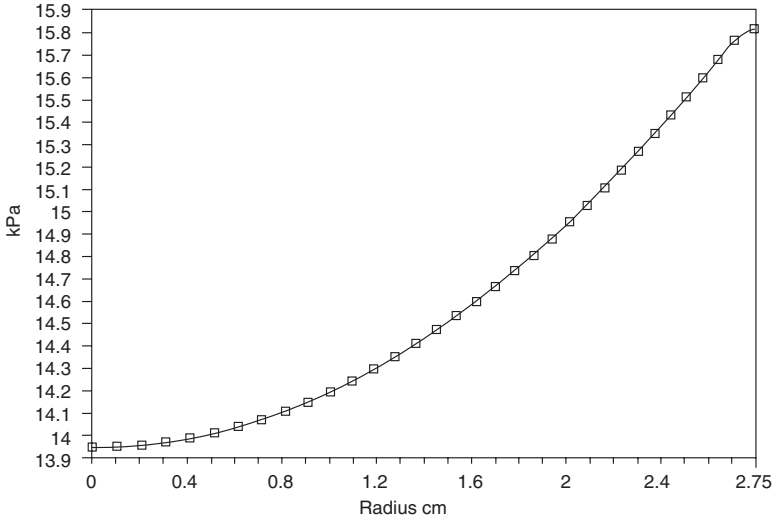

Fig. 2.1 Oxygen distribution along the tuber. The oxygen distribution was calculated from the data concerning the diffusivity of CO_2 in the skin and flesh of potato tubers (Tables 2.11 and 2.12) and the assumption that the sum of the internal partial pressures of CO_2 and O_2 equals the partial pressure of the latter in the ambient atmosphere

"oxidases" may not be contributing to the decrease in respiration with decreasing external O_2 levels. It should be emphasized that in actively respiring plant tissues, such as avocados, the O_2 level is low enough to preclude any appreciable participation of the residual oxidases in the respiration of the fruit. Even in potato tubers, which have much lower rates of respiration than avocado fruits (Solomos and Laties 1976), the oxygen level at the center of the tuber drops to about 13% (Fig. 2.1), a concentration that would be expected to severely curtail the engagement of these "residual" oxidases (Solomos 1988). In addition, their curtailment must exert a feedback restraint on the initial steps of glucose oxidation, yet this is not compatible with the most likely known regulatory mechanisms of plant respiration (Davies 1980; Solomos 1988; Turner and Turner 1980; Wiskich 1980). In the case of climacteric fruits, it may be suggested that the effect of low O_2 on respiration is due to the diminution of ethylene action (Burg and Burg 1967). Therefore, on the basis of the above discussion, it appears that suggestion (1) is the most likely explanation of the effects of low O_2 on fruit respiration.

It was assumed in the past that the accompanying decrease in respiration in response to a lowering of the external O_2 levels was an important facet of the mode of action of low O_2 in prolonging the storage life of fruits (Burton 1974). However, one may argue that the decrease in respiration reflects a metabolic depression engendered by hypoxia. In the first place, hypoxia affects metabolic events in tissues where ripening is not an issue and where ethylene is not involved. For instance, hypoxic conditions inhibited the accumulation of RNA, protein, and DNA synthesis associated with the wounding of potato tubers (Butler et al. 1990). We have also

Table 2.3 Effect of 1.5% O_2 on sugar accumulation and activity of the alternative oxidase in potato tubers stored at 1°C

	Sugars (μmoles·g^{-1})			Alternative oxidase nanomoles O_2·min^{-1}·mg protein^{-1}		
Days	10 °C Air	1 °C Air	1 °C 1.5% O_2	10 °C Air	1 °C Air	1 °C 1.5% O_2
0	16.9	–	–	0.0	–	–
20	19.6	91.5	32.9	0.0	46.63	4.24
30	18.4	113.4	34.4	0.0	60.79	4.16

observed that hypoxia (1.5% O_2) prevented the accumulation of simple sugars and the induction of the alternative oxidase associated with storage of potato tubers at 1 °C (Table 2.3). It should be pointed out that in potatoes, chilling temperatures do not induce the biosynthesis of ethylene. Undoubtedly the delaying effects of low O_2 on the senescence of detached plant organs in general, and fruit ripening in particular, must involve a decrease in ethylene accumulation. In a perceptive paper, Burg and Burg (1967) suggested that the ethylene receptor contains a metal and when it is in its oxidized state, the binding of ethylene is enhanced. However, the effect of hypoxia on ethylene biosynthesis and action may be indirect through the suppressive effects of hypoxia on the induction of 1-amino-cyclopropane-1-carboxylic acid (ACC) synthase and/or synthesis of transducer(s) of ethylene action. It has been reported previously that, in avocados, O_2 concentrations in the range of 2.5–5.5% suppress the activity, appearance of exoenzymes, and accumulation of proteins of cellulose and polygalacturonase, associated with normal ripening (Kanellis et al. 1991). The suppressive effects on the cellulase protein were also reflected in the accumulation of its mRNA. Further, the intensity of inhibition of the synthesis of these hydrolases was inversely related to the levels of O_2 under which the fruits were kept. In addition, the same range of O_2 concentrations that suppressed the synthesis of the hydrolases induced the appearance of anaerobic isoenzymes of alcohol dehydrogenase (ADH). The rates of increase in the levels of cellulose, its mRNA and polygalacturonase, and the disappearance of the anoxic isoenzymes of ADH on reexposure of the fruits to air were directly related to the previous levels of O_2 (Kanellis et al. 1991). The fact that similar ranges of O_2 concentrations on one hand suppressed the rise in the enzymes associated with normal ripening, while at the same time inducing the synthesis of anoxic isoenzymes of ADH, indicates that the O_2 sensing mechanism is common for both processes. The induction of anoxic isoenzymes in response to hypoxia is easily understood because it is advantageous for the tissue to synthesize enzymes that increase the production of ATP in anoxia before oxygen is completely depleted. However, the extension of the storage life of fresh fruits and vegetables must be the consequence of metabolic depression which, unlike anoxia, is not deleterious to the long-term survival of the tissue. Metabolic depression is the most important adaptation for survival of intertidal organisms, which experience frequent transitions from normoxia to hypoxia (Storey and Storey 1990). Because the intensity of respiration could be considered to reflect the intensity

Table 2.4 Days to climacteric under different O_2 concentrations

		Treatment					
Year	Harvest date	Air	8% O_2	6% O_2	4% O_2	3% O_2	2% O_2
1987	8–24	19	24	40	66	109	>194
1988	8–26	22	–	–	89	>280	–
1989	8–22	21	–	45	76	103	–
1990	8–24	16	–	–	32	–	105
1991	8–27	9	–	–	–	–	45

of cellular metabolism, and because low oxygen invariably decreases the rate of respiration of such detached plant organs as fruits, flowers, and leaves, this indicates that hypoxia, by an as yet unknown mechanism, produces a decrease in metabolism, which in turn results in diminishing the rate of plant development and hence senescence. In short, the decrease in respiration may not be the cause of the decline in the rate of senescence, but rather a response to a metabolic depression, which diminishes the demand for biological energy.

It has been pointed out that low O_2 in preclimacteric tissues delays the onset of the climacteric rise in ethylene evolution (Mapson and Robinson 1966). Experimental results concerning the range of O_2 concentrations that delay the onset of ripening are limited. It appears that in the case of "Gala" apples, the external O_2 concentration must fall below 8% in order to prolong the preclimacteric stage of the apples (Table 2.4). In short, the system that is involved in the induction of ACC synthase, a key regulatory enzyme in ethylene biosynthesis (Yang and Hoffman 1984), is saturated at O_2 levels above 7–8%. The data of Table 2.4 show, as expected, that the effect of low O_2 on the timing of the onset of ripening differs with the season.

Quantitative data concerning the effect of low O_2 on the rate of ripening are rather difficult to establish. At present it is not possible to describe unequivocally the relationships between O_2 concentration and rate of ripening. Suffice it to say that low O_2 does indeed delay ripening, as has been amply demonstrated in a variety of fruits (Kader 1986; Knee 1980; Kanellis et al. 1991; Liu and Long-Jum 1986; Quazi and Freebairn 1970; Yang and Chinnan 1988a). Yang and Chinnan (1988b) developed a mathematical expression for predicting the changes in the color of tomato fruits as a function of O_2 concentration.

A critical parameter that must be taken into consideration in designing suitable MAP is the limit of O_2 below which the produce cannot be safely stored. This limit, as expected, varies with the produce, but it is important to realize that levels of O_2 that induce partial anaerobiosis will be detrimental to both longevity and quality of the produce. This limit can be assessed experimentally by measuring either the values of the respiratory quotient (RQ) or, preferably, the increase in ethanol content of the tissue. The latter may be a more reliable indicator than the RQ values, especially at levels of O_2 that initiate partial anaerobiosis, and that will be somewhat difficult to detect from the changes in the RQ. Because of its volatility, ethanol can be detected in the ambient atmosphere of MAP by removing a gas sample and determining the ethanol content using gas chromatography (Nakhasi et al. 1991).

2.2.2 *Effects of CO_2 on Senescence of Detached Plant Tissues*

The mode of action of CO_2 on senescence is unclear. Burg and Burg (1967) suggested that CO_2 is a competitive inhibitor of ethylene. Recent experimental evidence indicates that CO_2 may indeed diminish the action of ethylene provided the concentration of the latter is less than 1 $\mu l \times L^{-1}$. In the case of apples, CO_2 enhances the inhibitory effects of low O_2 on respiration (Fidler et al. 1973), whereas CO_2 concentrations in the range of 1–27% do not affect the rate of respiration of peaches (Deily and Rizvi 1982). It should be pointed out that CO_2 is a metabolically active molecule participating in a number of carboxylating reactions. In addition, it is expected that high concentrations of CO_2 could alter the pH of the cytosol, which in turn may affect plant metabolism (Siripanich and Kader 1986). Anoxic conditions generated by CO_2 induce changes in a number of intermediate metabolites that differ from those observed when the tissue is kept under nitrogen instead (Kader, personal communication). CO_2 is also required for the action of ACC oxidase (Kuai and Dilley 1992).

It is well known that tolerance to CO_2 varies greatly, not only between species but also between cultivars of the same species. For instance, "Golden Delicious" apples can tolerate high CO_2 concentrations, whereas "McIntosh" apples are damaged by even 3% CO_2 (Fidler et al. 1973). Strawberries can tolerate CO_2 levels as high as 20%, and storage of peaches in 10–15% CO_2 is beneficial (Deily and Rizvi 1982). In apples, high CO_2 concentrations appear to inhibit succinic acid dehydrogenase (Hulme 1956). Storage of lemons under high concentrations of CO_2 leads to an accumulation of organic acids (Biale 1960). In lettuce, high CO_2 concentrations affect the metabolism of phenolic compounds (Siripanich and Kader 1985a, b). Another beneficial effect of high CO_2 levels is their antimicrobial activity. At present it is impossible to predict the tolerance of a particular tissue to high levels of CO_2 (Kader 1986).

2.2.3 *Effects of Slicing on Tissue Metabolism*

The effects of wounding on plant metabolism have been studied extensively in tissues prepared from bulky plant organs such as tubers and roots. The vast literature on this subject has established that slicing induces profound quantitative and qualitative changes in tissue metabolism (Kahl 1974; Laties 1978). The observed changes include a rise in respiration, DNA and RNA synthesis, induction of new enzymes, membrane degradation, and the appearance of novel mRNA (apRees and Beevers 1960; Butler et al. 1990; Clicke and Hackett 1963; Kahl 1974; Laties 1978). The effect of slicing on respiration is probably the most extensively studied aspect of wounding in bulky plant organs (Laties 1978). These investigations have shown that slicing induces a three- to fivefold rise in respiration over that of the parent-plant organ. With aging there is a further two- to threefold increase in respiration (Laties 1978). This rise in respiration with aging of slices is critically dependent on

protein and RNA synthesis since the addition, within 8–10 h of slicing, of either protein or RNA synthesis inhibitors prevents the development of the respiratory rise with aging (Clicke and Hackett 1963; Kahl 1974). Neither the cause of this rise in respiration nor its metabolic significance is clear. However, the data indicate that inhibitors of respiratory development also inhibit a number of biochemical events, such as suberin formation and synthesis of phenolics, associated with aging of potato slices (Kahl 1974; Laties 1978).

Several experiments show that the nature of both respiratory substrates and pathways changes with aging of slices. In particular, in fresh potato slices, most of the respiratory CO_2 is derived from the α-oxidation of fatty acids arising out of the attendant breakdown of phospholipids in response to slicing, whereas carbohydrates are respiratory substrates of aged slices (Jacobson et al. 1970; Laties 1978). It has also been reported that in slices other than potatoes, a large portion of CO_2 is produced by the pentose phosphate pathway (PPP) (apRees and Beevers 1960). In addition it should be mentioned that temperature and gas composition affect both respiratory substrates and pathways. Thus, when potato slices are aged either in air, in the presence of 10% CO_2, or in a bicarbonate solution, suberin formation is prevented and the tissue develops callus (cf. Laties 1978). Moreover, the respiration of aged slices is manolate resistant and is presumed to comprise the PPP (Kahl 1974). This observation is important from the point of view of MAP because aging in high CO_2 levels may prevent the formation of color in potato slices.

The effects of hypoxia on minimally processed produce, in combination with high CO_2 concentrations, have not been studied in detail. However, based on the observations that hypoxia inhibits the synthesis of DNA, protein, and novel mRNA in potato slices (Butler et al. 1990), it may be anticipated that these conditions repress the synthesis of those enzymes that are considered to exert adverse effects on the quality of tissue slices, for example, phenylalanine ammonia lyase (PAL), this being considered to increase the content of phenolics in the tissue, which in turn tend to increase in wounded plant tissues (Kahl 1974; Uritani and Asahi 1980). In addition MAP environments may suppress the rise in amylases, thus diminishing the breakdown of starch prevalent in potato slices (Kahl 1974).

A number of experimental observations indicate that regardless of the origin of the respiratory reducing equivalents, the terminal electron acceptor is predominantly cytochrome oxidase, even in tissues that possess substantial cyanide-resistant respiration (Laties 1978; Solomos 1988). If this is the case in tissue slices, the oxygen concentration can be reduced to very low levels because of the high affinity for O_2 of the cytochrome oxidase, and because of the short diffusion path available to gases. For instance, in the case of sweet potato slices suspended in air at 25 °C, the rate of O_2 uptake is of zero order with respect to its external concentration until the latter drops to about 0.4% (Fig. 2.2). The ability to decrease the O_2 concentration to such low levels may be beneficial because it is expected to reduce the browning due to polyphenol oxidases (PPO), as the latter have a rather high K_m for O_2 (Beevers 1961).

At present there are no detailed studies concerning the effect of ranges of O_2 and/or CO_2 concentrations on either metabolism, longevity, or quality of cut tissue segments. It is to be expected, as in the case of intact tissues, that O_2 concentrations

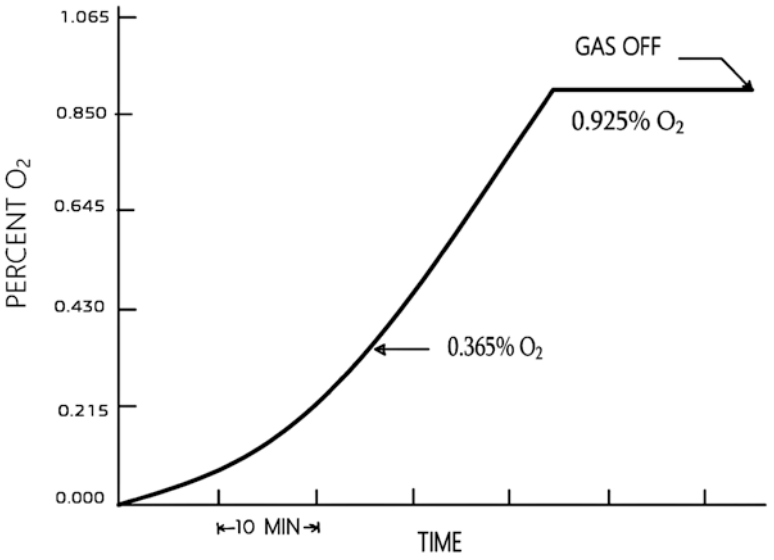

Fig. 2.2 The rate of O_2 uptake of slices suspended in air was followed polarographically. The slices were initially maintained under 0.925% O_2. At the indicated point, the gas was turned off. The rate of O_2 uptake between 20.946% and 0.925% was of zero order

that engender partial anoxia will be detrimental to longevity and quality of the produce. This low limit of O_2 can be assessed in a manner identical to that described earlier for intact tissues.

2.3 Determination of Gas Diffusivities in Plant Tissues

2.3.1 General Considerations

In attempting to generate predictive MAP models, it is useful to know the tissue's permeability to gases in order to calculate their concentration at the center of the organ, particularly when bulky fruits or vegetables are used. The diffusion barriers of a plant organ include the skin, the intercellular spaces, the cell walls, and plasmalemma. The diffusion of gases through bulky plant organs such as fruits, roots, and tubers follows Fick's first law, and the diffusion channels are predominantly gaseous in nature (Burg and Burg 1965; Burton 1974; Cameron and Yang 1980; Solomos 1987). Simple calculations with apples have shown that, assuming an aqueous diffusion barrier, the maximum radius that could maintain 1% O_2 at the center of the fruit would be about 0.7 cm (Solomos 1987). For fruits with rates of O_2 uptake much larger than that of apples, the radius would be even smaller. Similar observations have also been reported for potato tubers and apples (Burton 1974).

Table 2.5 Relationship between external pressure and internal concentration of C_2H_4

External pressure (kPa)	Internal ethylene concentration (μl)
101.3	472
76	319
37.3	190
25.3	88

From Burg and Burg (1965)

The most convincing evidence for the gaseous nature of the diffusion paths is that provided by Burg and Burg (1965). These authors showed that the diffusivity of gases was inversely related to the external pressure (Table 2.5), as would be expected from the ideal gas law. If the barrier was liquid in nature, the changes in external pressure would not be expected to affect the length of the mean free path because of the incompressibility of water.

Fick's first law states that the flux normal to the surface of a metabolically inert gas is given by (Crank 1970):

$$J = AD\frac{\partial c}{\partial x} \tag{2.1}$$

where J, in μmoles sec^{-1} per fruit, is the flux; A, in cm^2, is the surface available to diffusion; D, in $cm^2\ sec^{-1}$, is the diffusion coefficient; and $\partial c/\partial x$ is the concentration gradient with respect to distance. It is customary to replace $\partial c/\partial x$ with the difference in concentration $\Delta c/\Delta x$. This is permissible only when the change in concentration with distance is linear (Jacobs 1967; Nobel 1983). In order to determine D, the concentration gradient must first be determined. In the case of non-steady-state conditions, the change in concentration with respect to time should also be known. In order to calculate these gradients, it is necessary to solve the equation for Fick's second law (Crank 1970; Jacobs 1967). The general equation for Fick's second law for a metabolically active gas in three dimensions is (Crank 1970):

$$\frac{\vartheta c}{\partial t} = D\left[\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} + \frac{\partial^2 c}{\partial z^2}\right] \pm v \tag{2.2}$$

where v is the specific rate of evolution (+) or uptake (−) of the gas under consideration. The analytical solutions of Eq. (2.2) are numerous, depending on the boundary conditions and the initial distribution of the gas throughout the barrier. Equations (2.3) and (2.4) below represent the expression of Eq. (2.2) for a solid sphere and cylinder, respectively (Crank 1970; Jacobs 1967):

$$\frac{\vartheta c}{\partial t} = D\left[\frac{\partial^2 c}{\partial r^2} + \frac{2}{r}\frac{\partial c}{\partial r}\right] \pm v \tag{2.3}$$

$$\frac{\vartheta c}{\partial r} = D\left[\frac{\partial^2 c}{\partial r^2} + \frac{1}{r}\frac{\partial c}{\partial r}\right] \pm v \tag{2.4}$$

where r, in cm, is the radius of the sphere and cylinder. In cases where the peel of the tissue is the main parameter, these equations must be solved for hollow spherical and cylindrical shells. For nonmetabolic gases, there are analytical solutions for a hollow sphere and cylinder (Crank 1970).

Apart from the mathematical complexities, determining the diffusivity of gases under dynamical conditions introduces a number of uncertainties because of the nonhomogeneous nature of the diffusion barriers of a plant organ. For instance, the diffusion coefficients of CO_2 in the skin and flesh of potato tubers are about 6.90×10^{-7} and 2.50×10^{-4} $cm^2 \cdot sec^{-1}$, respectively. The existence of such a barrier in the flesh will generate appreciable concentration gradients within the flesh when the efflux of a gas is measured. To demonstrate this point, it is assumed that CO_2 is diffusing in an infinite cylinder of unit cross-sectional area, with a D_{CO_2} similar to that of potato tuber flesh. Table 2.6 describes the percentage distribution of a quantity M of CO_2 deposited at $x = 0$ and $t = 0$. The change in concentration with time and distance is given by (Crank 1970):

$$C(x,t) = \frac{M}{2\pi (Dt)^{1/2}} \times \exp\left[-\frac{x^2}{4Dt}\right] \tag{2.5}$$

It may be seen from Table 2.6 that the concentration gradient is substantial. Therefore, the assumption that the concentration of a metabolically inert gas is uniform throughout the flesh is not valid (Cameron and Yang 1980).

Another uncertainty of the efflux method is the assumption that the equilibrium between the cellular solution and intercellular spaces is instantaneous. However, a number of observations indicate that this may not be the case. It was shown by Burton (1950) that the evacuation of O_2 from a small plug of potato tissue was a lengthy process. In addition, indirect experimental evidence indicates that the resistance to gas diffusion from the cell to the intercellular spaces may not be negligible (Chevillotte 1973). It is also expected that the solubility of the gas in aqueous solutions would affect the equilibrium distribution between the cell and the intercellular spaces, especially where short time intervals are concerned. A case in point is the changes in RQ in the course of the rapid climacteric rise in respiration. In precli-

Table 2.6 Concentration of CO_2 with distance and time as percent of initial amount deposited at the center of an infinite cylinder with a diffusion coefficient of $2.5 \times 10^{-4} \cdot cm^{-2} \cdot sec^{-1}$

Time (min)	Distance (cm)				
	1	1.5	2	2.5	3
10	12.688	1.370	0.061	0.001	0.000
11	14.223	1.880	0.111	0.003	0.000
15	18.755	4.253	0.533	0.037	0.001
20	21.854	7.182	1.512	0.204	0.018
30	24.009	11.433	4.046	1.064	0.208
40	24.119	13.826	6.344	2.330	0.685
50	23.581	15.109	8.102	3.636	1.365
60	22.843	15.763	9.378	4.809	2.126

macteric avocados, the RQ is close to unity, changes to less than one at the climacteric peak, and then returns toward unity at the postclimacteric stage (Solomos and Laties 1976). It was found, however, that in bananas this pattern of changes was not metabolic in nature, but rather the result of the difference in the respective solubilities of CO_2 and O_2 in water (McMurchie et al. 1972). Because of this difference in solubilities, O_2, which has a smaller solubility in water than does CO_2, equilibrates with the intercellular spaces faster than does CO_2. Further, the efflux method requires a precise knowledge of the volume of the intercellular spaces and the solubility of the gas in the cellular liquid.

However, if appropriate experimental precautions are taken, it may be feasible to obtain reasonable approximations of gas diffusivities through the skin of a plant organ by following the efflux of metabolically inert gases. For instance, if the skin is thin, if the tissue is loaded with relatively high concentrations of the inert gas, if the volume of the vessel is small, and if the diffusion in the flesh is much larger than in the skin, then this method could give reasonable approximations of gas diffusivity through the skin. In order to avoid the generation of the concentration gradient along the flesh of potato tubers, the resistance to diffusion was calculated by considering only the initial linear part of the efflux isotherm of ethane (Banks 1985). This approach, however, introduces some uncertainties concerning the origin of the gas. It was assumed that the gas originated under the skin, which may not be correct because ethane, being nonpolar, is expected to dissolve in the waxy layers of the cuticle. In tissues with thick skin, the volume of the waxy layer can be appreciable. For instance, in a cylindrical tuber of radius 2.8 cm, length 12 cm, and skin thickness 0.012 cm, the volume of the phellem is about 2.5 ml. It is also possible that some of the initial gas efflux may originate from the gas adsorbed on the tuber surface or present in gaseous cavities. This approach could be compared to the ion fluxes, where the initial flux contains a large component of the apparent free space and does not measure fluxes across the cellular membranes, these being the main barrier to ion fluxes between cells and ambient environment (Briggs et al. 1961). It should be emphasized that any determination of gas diffusion is *meaningless* unless it is verified experimentally.

In view of the uncertainties and mathematical complexities involved in the determination of the D of gases under non-steady-state conditions, we shall here consider only steady-state situations.

The steady-state solution of Eq. (2.2), $((\partial c)/\partial t = 0)$, in a one dimensional, plane sheet, for a metabolically active gas is (Hill 1928):

$$C(x) = \frac{v}{2D}x^2 - \frac{\ell v}{D}x + C_0 \tag{2.6}$$

where v, in μmoles $cm^{-3}\cdot sec^{-1}$, is the constant rate of output (+) or uptake (−) of the gas per unit tissue volume; ℓ, in cm, is half of the tissue thickness; and C_0, in μmoles cm^{-3}, is the concentration of the gas at $x = 0$, that is, the ambient atmosphere. Thus, the concentrations of CO_2 and O_2 at the center of the tissue C_i are:

$$C_i = C_0 \pm \frac{v}{2D}\ell^2 \tag{2.7}$$

The concentrations of CO_2 and O_2 at the center of a sphere and cylinder are given by Eqs. (2.8) and (2.9), respectively (Hill 1928):

$$C_i = C_0 \pm \frac{v}{6D} R^2 \tag{2.8}$$

$$C_i = C_0 \pm \frac{v}{4D} R^2 \tag{2.9}$$

where R, in cm, is the radius of either the sphere or cylinder. The other notations have been defined earlier.

In the case of metabolically inert hollow spherical and cylindrical shells, the flux of CO_2 per unit time at their surfaces is given by Eqs. (2.10) and (2.11), respectively (Crank 1970):

$$J_{r=R} = 4\pi D \frac{C_i - C_0}{R - R_i} \tag{2.10}$$

$$J_{r=R} = 2\pi Dh \frac{C_i - C_0}{\ell n\left(R / R_i\right)} \tag{2.11}$$

where R and R_i, in cm, are the outside and inside radii, respectively. In the case of a thin spherical wall (Eq. 2.10), it is assumed that $R{\cdot}R_i \approx R_2$. The other notations have been defined earlier. It should be pointed out that Eq. (2.11) may not be very accurate unless the surfaces of the cylindrical bases are small in comparison with the cylindrical surface, and the length is much larger than the radius. In the case of oxygen, the order of the concentration differences in Eqs. (2.10) and (2.11) is reversed, for example, $C_0 - C_i$. It is obvious from Eq. (2.11) that for an accurate determination of D, the values of R and R_i must be known with some degree of precision. It is customary to use Eq. (2.12), instead of Eq. (2.11):

$$J_{r=R} = 2\pi rh \frac{C_i - C_0}{\Delta r} D \tag{2.12}$$

and to determine the apparent diffusion coefficient, $D' = (D/\Delta r)$. This could, depending on the dimensions, introduce appreciable error because $D' = R \times (D/\Delta r)$ (Abdul-Baki and Solomos 1994).

Finally the flux of oxygen through a metabolically inert plane sheet is given by Eq. (2.13) (Jacobs 1967):

$$J = \frac{AD\left(C_0 \quad C_i\right)}{\Delta x} \tag{2.13}$$

As already mentioned, gases diffuse in and out of plant organs in gaseous channels. Thus, the usually observed low diffusivities are due to the fact that only a small

fraction of the tissue surface is available to gas diffusion. In "Russet Burbank" potato tubers, the fraction of the surface permeable to gases varies with the tuber from 4.22×10^{-6} to 7.8×10^{-6}, the average being 6.22×10^{-6} (Abdul-Baki and Solomos 1994). It is thus apparent that Eq. (2.1) should be written as:

$$J = ADN\frac{dc}{dx}$$

where N is a number between 0 and unity representing the fraction of the surface that is permeable to gases (Burg and Burg 1965). On the basis of microscopic and gas diffusion measurements, it was calculated that only 1/1000 of the cross section of the flesh of potato tubers is permeable to gases (Woolley 1962).

The diffusivity of gases through plant tissues could also be decreased by the degree of tortuosity of the path. However, in most plant tissues of interest for consumption, the skin is quite thin; hence, the effect of a tortuous path will probably be insignificant.

It was mentioned earlier that for the determination of the diffusion coefficient of the gases for the cases considered above, one must measure the flux and the concentrations of the gases inside and outside the tissue.

2.3.2 Measurements of Intercellular Gases

Several methods have been used in the past to ascertain the internal concentration of gases in various fruits and vegetables. These methods include evacuation, manometric techniques, use of oxygen microelectrodes, and the removal of plugs of tissue which are then sealed in airtight vials (Solomos 1987).

The evacuation technique introduces the following uncertainties. In the first place, the values reflect the overall concentration of the gases in the tissue and not that at a particular point, for example, at the center, under the skin, etc. Further, the evacuated gases will contain not only those present in the intercellular spaces, which is required, but an unknown portion of the dissolved gases in the cellular sap. This in turn requires corrections that must include solubility of gases in the cell liquid and also, depending on time and intensity of respiration, the production or utilization of the gas within the time interval. The use of O_2 microelectrodes has produced measurements of some large O_2 gradients within the tissue (Brädle 1968). Readings of an oxygen microelectrode will vary greatly depending on whether the electrode is submerged in liquid or is in a gaseous phase. The use of manometric techniques, though reliable, requires the construction of special apparatus which makes it difficult to use for a large number of samples (Hulme 1951). Removal of plugs is a destructive method. In addition, it introduces uncertainties in the subsequent analysis of the gases similar to those given above for the evacuation technique.

Banks and Kays (1988) affixed small vials on the surface of potato lenticels and followed the changes in the concentration of CO_2 and O_2. It is expected that these concentrations in the vials will reflect that under the skin. This method is an

improvement on any of the previous techniques, but the uncertainty exists that the gases may diffuse laterally to adjacent lenticels if the pressure in the vial increases, or the lenticel is partially blocked. Waldraw and Leonard (1939) removed small plugs of tissue to create cavities into which the inserted tubes were in turn sealed airtight. It is anticipated that, with time, the composition of the gas atmospheres of the tubes will equilibrate with the internal gas atmosphere of the tissue. Thus, the composition of the fruit gases can be determined by analyzing the gas in the tubes. Trout et al. (1942) showed that the removal of up to 10 ml of gas from the internal cavity of apple fruit generated no appreciable drop in pressure, and the system returned to equilibrium quite rapidly. However, this may not be the case for plant organs with small intercellular spaces. With improved analytical techniques for measuring gases, a small volume of samples – between 25 and 50 μl – can be used to accurately determine the concentrations of metabolically active gases, that is, O_2, CO_2, and C_2H_4. Several investigators have inserted hypodermic needles into the locule cavities of apple fruits to determine the internal concentration of C_2H_4 (Burg and Burg 1965). The hubs of the needles are sealed with a vaccine cap, and samples are withdrawn from the needle using an airtight syringe. The concentration of C_2H_4 is then measured by gas chromatography (Burg and Burg 1965).

This method can be improved upon further by gluing a chromatographic septum to the calyx of the fruit and inserting the needle through the septum and into the locules (Solomos 1989). This technique facilitates sequential sampling, and the needle can be replaced easily if it becomes blocked. The method has the additional advantage that it inflicts minimal injury, and the needle can be kept in the fruit for longer periods so that the effect of injury is dissipated. In the case of fruits, such as apples, with large locular cavities, the injury effect may not be an issue. Further, because of the small volume of the needle, it is expected that its gas space will come into rapid equilibrium with the intercellular gases, and, in addition, the removal of small volumes of sample gas is expected to represent that in the intercellular spaces adjacent to the needle tip. In this way, gradients across the tissue can be ascertained.

Previous work established, as expected, that the total internal gas pressure equals that of the ambient environment (Hulme 1951; Trout et al. 1942). That requires that the sum of the internal partial pressures of the gases equals the surrounding atmosphere, approximately 1 atm. This is indeed the case for apple fruit (Table 2.7). This in turn suggests that the sampling method does not introduce appreciable experimental error. The rate of gas exchange can be measured either by a static or a flow-through system (Henig and Gilbert 1975; Solomos 1987).

Table 2.7 Internal partial pressure of gases in the atmospheres inside "Gala" apples

	CO_2	O_2	N_2	Total $CO_2 + O_2 + N_2$ + argon + water vapors
Experiment 1	0.022	0.181	0.772	1.002*
Experiment 2	0.026	0.183	0.777	1.012
Experiment 3	0.022	0.188	0.777	1.014

*Each reading represents the average of five apples

2.3.3 *Experimental Determination of CO_2 Diffusivity in Apples and Potatoes*

As mentioned above, the diffusion barriers from the cell to the ambient atmosphere include the skin, intercellular spaces, cell walls, and plasmalemma. Most of the previous data were mainly concerned, apart from a couple of exceptions (Burton 1950; Solomos 1987; Woolley 1962), with measuring skin resistance (Banks 1985; Burg and Burg 1965; Cameron and Yang 1980). The main reason for this is that the experimental procedures that were used are not amenable to determining gas diffusion through the intercellular spaces of the flesh. Further, with a few exceptions, the calculated resistances have not been subjected to experimental verification. We shall here briefly describe methods for evaluating gas diffusion coefficients through both skin and flesh, as well as experimental procedures for ascertaining their validity. Here we shall confine the discussion to apples and potato tubers.

2.3.3.1 Apples

We have used varieties of apples whose geometry approaches that of a sphere (Solomos 1987). Within the cultivar, we selected fruits whose equatorial and polar circumferences differed by <5%. In order to subject the data to experimental verification, we altered the rate of CO_2 output by decreasing the external O_2 concentration and comparing the values of CO_2 diffusivities. The experimental arrangements were those described by Burg and Burg (1965), with minor modifications (Solomos 1987, 1989). The geometrical configuration of the skin of the apple was assumed to be a hollow spherical shell. It is apparent from Eq. (2.10) that the concentration of CO_2 under the skin, along with the respiration rates and fruit dimensions, must be known, so that D' can be calculated. The concentration of CO_2 under the skin can be measured by inserting a hypodermic needle just under the surface of the fruit, while the concentration at the center is obtained by inserting a hypodermic needle through the calyx into the locules. In most of the apple cultivars we used, the gradient between the center and the subcutin was quite small (0.2–0.6%) (Solomos 1987). In the case of "Gala," whose data are presented here, the gradient of CO_2 was between 0.1% and 0.2%, which falls within the experimental error for measuring CO_2. It has been demonstrated that in the case of ethylene, the use of the concentration at the center to represent that under the skin introduced an insignificant error in the values of D' (Solomos 1989). Thus, the concentration of CO_2 under the skin is taken to be identical to that at the center. Table 2.8 shows the diffusion coefficient of CO_2 under different external O_2 concentrations. When the external O_2 concentration was decreased in steps from air to N_2, this of course affected the rate of CO_2 output. It may be seen from Table 2.8 that the diffusivities of CO_2 under different O_2 levels are in reasonable agreement.

Apple fruit pose problems for evaluating the diffusivity of their intercellular spaces. Because of the small difference in CO_2 concentrations between the center

Table 2.8 Diffusion coefficient ($cm^2 \cdot sec^{-1} \times \cdot 10^{-4}$) of CO_2 in the skin of "Gala" apples under different O_2 levels at 15 C

	Apple no.					
	1	2	3	4	5	CO_2 output (μl/G/H)
Air	1.67	1.28	1.28	1.50	1.51	6.08
O_2 (13.00%)	1.26	1.17	1.35	1.50	1.30	5.99
O_2 (6.78%)	1.18	0.98	0.99	1.28	1.19	4.29
O_2 (4.75%)	1.18	0.98	0.98	1.28	1.19	4.19
O_2 (1.59%)	1.16	1.48	1.00	1.30	1.47	3.54
O_2 (0.62%)	1.34	1.47	0.94	1.29	1.23	2.91
N_2	1.32	1.20	1.10	1.34	1.32	3.15
AVG	1.301	1.223	1.09	1.356	1.316	
STD	0.164	0.19	0.150	0.093	0.120	

Table 2.9 Diffusion coefficient of CO_2 in the flesh of "Gala" apples

Experiment	$cm^2 \cdot sec^{-1} \times 10^{-3}$
1	1.46 (0.27)
2	1.47 (1.27)
3	1.26 (0.27)
4	2.13 (0.95)

Each value represents the average of five apples. The number in parentheses is the STD

and subcutin, Eq. (2.8) cannot be used to calculate *D*. We thus proceeded to peel the fruit, blot it dry with filter paper, and then measure the rate of respiration and internal CO_2 concentration. Within about 6–8 h, the rate of CO_2 evolution was close to that of the intact fruit, probably because the dissolved CO_2 was dissipated. From the rate of CO_2 output and external and internal CO_2 concentrations, its diffusion coefficient in the intercellular spaces was calculated based on Eq. (2.8). The values obtained are presented in Table 2.9. Unfortunately the validity of these values cannot be tested experimentally because with time the outer layers of the fruit will form periderm, thus altering the internal concentration of CO_2.

2.3.3.2 Potato Tubers

We have used only "Russet Burbank" tubers because their geometry simulates a cylinder. (It should be stressed, however, that no tuber is exactly cylindrical.) The tubers were selected with the proviso that their length be greater than 11 cm and that the circumference, measured at several points along the tuber, not vary by more than 10% (Abdul-Baki and Solomos 1994). The CO_2 concentrations under the skin and at the center were measured by gluing two chromatographic septa, 11 mm in diameter, onto the surface in the middle of the tuber, each septum being 180° apart. Through the septa, two hypodermic needles were inserted, one at the center and the other under the skin. In addition the thickness of the lenticels was measured

microscopically. From the rate of CO_2 output and the concentration of CO_2 under the skin, the diffusion coefficient of CO_2 in the skin was calculated using Eq. (2.11). Table 2.10 shows the values of the D_{CO_2}. It may be seen that there is appreciable variability between the tubers. These values are close to those reported previously for O_2 (Burton 1950). The validity of the data was tested by transferring the tubers from 10 to 27 °C. From the observed values of CO_2 concentration under the skin, and the values of D_{CO_2} calculated from those obtained at 27 °C (Jost 1960), we calculated the rate of CO_2 output at 10 °C and compared it to that observed. The observed and calculated values of respiration are in good agreement (Table 2.11).

Table 2.10 Diffusion coefficient ($cm^2 \cdot sec^{-1} \times 10^{-7}$) of CO_2 in the skin of potato tubers

Tuber no.	10 °C	27 °C
1	7.16	8.21
2	6.73	9.33
3	6.03	6.04
4	6.04	7.97
5	6.37	7.77
6	4.19	5.12
7	5.56	5.98
8	7.79	7.62
Avg	6.24	7.26

The values of D_{CO_2} were calculated by inserting in Eq. (2.11) the observed fluxes and concentrations of CO_2 under the skin and ambient atmosphere, along with dimensions of the tuber and skin thickness (0.012 cm)

Table 2.11 Comparison of observed and calculated rates of CO_2 evolution at 10°C

27 °C	($\mu moles \cdot sec^{-1} \cdot 10^{-2}$)	
Tuber no.	Observed	Calculated
1	2.97	2.92
2	2.37	1.83
3	2.63	2.96
4	2.34	1.69
5	2.34	1.65
6	2.39	2.20
7	2.56	2.68
8	2.70	2.86
Avg	2.54	2.35

The theoretical fluxes were obtained by inserting in Eq. (2.11) the calculated values of D_{CO_2} at 10 °C from those observed at 27 °C along with the theoretical CO_2 concentration under the skin, calculated as in Table 2.13, the dimensions of the tuber, and the concentration of CO_2 in the ambient atmosphere

Table 2.12 Diffusion coefficient ($cm^2 \cdot sec^{-1} \times 10^{-4}$) of CO_2 in the flesh of potato tubers

Tuber no.	10 °C	27 °C
1	1.67	1.90
2	2.17	2.23
3	2.63	3.10
4	2.68	2.90
5	2.90	2.46
6	2.30	2.67
7	3.65	3.81
8	1.96	2.10
Avg	2.50	2.65

Table 2.13 Observed and calculated concentrations ($\mu moles \cdot cm^{-3}$) of CO_2 under the skin at 10 °C

Tuber no.	Observed	Calculated
1	1.90	1.89
2	1.42	1.51
3	2.66	2.64
4	1.85	1.87
5	1.87	1.86
6	2.93	2.96
7	2.53	2.52
8	2.10	2.10
Avg	2.15	2.17

The theoretical values were obtained by inserting in Eq. (2.9) the calculated values of D_{CO_2} from their value at 27 °C (Jost 1967), along with the observed CO_2 concentration at the center, and the specific respiration

The diffusion coefficient of CO_2 in the flesh was calculated from the values of CO_2 output, based on Eq. (2.9) (Table 2.12). The accuracy of these values was tested by calculating the concentration of CO_2 under the skin from Eq. (2.9) along with the observed concentrations of CO_2 at the center, and the calculated values of D_{CO_2} at 10 °C from the data at 27 °C corrected for temperature (Jost 1960). Here too, the observed and calculated values are in reasonable agreement (Table 2.13).

2.4 Modeling for Appropriate Gas Environment in MAP

2.4.1 General Considerations

MAP is an inexpensive way to generate controlled atmosphere (CA) conditions within the package. The CA environment is generated through the interactions between produce respiration, film permeability to gases, and the ratio between total film area and produce weight. MAP is a dynamic system in that the internal concentration of gases changes continuously until it reaches a steady state, i.e., where the

rates of O_2 and CO_2 fluxes equal their respective rates of utilization and production. Modeling thus includes a determination of the time it takes for the gases to reach their steady-state levels, which must equal their desired concentrations for the produce under consideration. However, the non-steady-state part of the modeling is of limited practical value and may be ignored. Modeling can be carried out under steady-state conditions.

At any time, the rate of changes in the concentrations of O_2 and CO_2 per unit volume of free gas space in the package can be expressed as:

$$\frac{d[O_2]}{dt} = \frac{P_{O_2} A\left\{[O_2]_{\text{out}} - [O_2]_{\text{in}}\right\}}{V} - \frac{R_{O_2} W}{V} \tag{2.14}$$

$$\frac{d[CO_2]}{dt} = \frac{-P_{CO_2} A\left\{[CO_2]_{\text{in}} - [CO_2]_{\text{out}}\right\}}{V} + \frac{R_{O_2} W}{V} \tag{2.15}$$

where $[O_2]$ and $[CO_2]$, in ml/cm^{-3}, are the concentrations of O_2 and CO_2, respectively; P_{O_2} and P_{CO_2}, in ml/h cm^2 ml cm^{-3}, are the permeabilities of the film to O_2 and CO_2; A, in cm^2, is the area of the film; R_{O_2} and R_{CO_2}, in ml kg^{-1}/h^{-1}, are the rates of O_2 uptake and CO_2 output, respectively; W, in kg, is the weight of the produce; and V, in cm^3, is the free gaseous volume of the package (void volume).

Obviously when the system reaches steady state, the changes in CO_2 and O_2 concentrations in the package with time are zero; hence,

$$R_{O_2} W = P_{O_2} A\left\{[O_2]_{\text{out}} - [O_2]_{\text{in}}\right\} \tag{2.16}$$

$$R_{CO_2} W = P_{CO_2} A\left\{[CO_2]_{\text{in}} - [CO_2]_{\text{out}}\right\} \tag{2.17}$$

2.4.2 Rate of Respiration

The solutions of Eqs. (2.14) and (2.15) require a precise knowledge of the rates of O_2 uptake and CO_2 evolution, which in turn vary with the concentrations of O_2 and CO_2, that is, $R_{O_2} = f(O_2 CO_2)$ and $R_{CO_2} = g(O_2 CO_2)$. Further, the effect of O_2 or CO_2 on the rate of respiration is also dependent on the stage of maturity. For instance, in preclimacteric "Gala" apples, the rate of CO_2 output decreases when the external O_2 concentration drops below 8.10 kPa (8%), whereas in the climacteric kind, the rate of CO_2 output is of zero order with respect to the external O_2 concentration up to 2.53 kPa (2.5%) (unpublished observations). The rates of O_2 uptake and CO_2 output can be determined using a flow-through system. Here, a stream of gas is passed through the tissue which is enclosed in a jar. The levels of O_2 and CO_2 in the outlet stream are monitored. This method is probably the most accurate. However, it is not practicable to measure the rate of respiration under a number of combinations

of O_2 and CO_2. Alternatively, the tissue may be enclosed in a vessel, and the changes in O_2 and CO_2 in the head space can be measured. At any instant, the change in the concentrations of O_2 and CO_2 will be dependent on the rate of respiration, the volume of the gas space in the vessel, and the weight of the tissue. Therefore,

$$R_{O_2} = \frac{V_0}{W} \frac{d[O_2]}{dt} \tag{2.18}$$

This method has the advantage that the rate of respiration can be determined under a variety of O_2 and CO_2 concentrations. However, if rapid changes in the rate of respiration are involved, they could introduce some uncertainty, especially with bulky plant organs, because of the large differences between the solubilities of O_2 and CO_2 in water. It is thus expected that the external O_2 levels will reach equilibrium between the concentrations in the intercellular gas spaces and the ambient atmosphere faster than will those of CO_2. It has been noted earlier that this can introduce appreciable experimental error in the values of RQ. Nevertheless, if appropriate ratios of the volume of the respiratory vessel to weight of tissue are chosen, it is possible that the concentration of gases in the ambient and fruit atmospheres will be close to equilibrium because the changes occur gradually. A number of authors have determined the rate of O_2 uptake by scrubbing the CO_2 in the vessel (Cameron et al. 1989; Henig and Gilbert 1975). Because of the absorption of CO_2, the pressure of the jar will decrease with time. This may introduce some experimental errors because of possible contamination from air during the withdrawal and subsequent injection of the gas into the gas chromatogram. Cameron (1989) measured the rate of O_2 depletion by enclosing an O_2 electrode in the jar, thus eliminating this source of experimental error.

Once the relationship between rates of O_2 uptake and CO_2 output as a function of both O_2 and CO_2 levels is determined, an expression is generated by various interpolation techniques. A number of interpolation methods have been used in the past to express the rates of O_2 uptake and CO_2 output as a function of O_2 and CO_2 concentrations. Henig and Gilbert (1975) divided the isotherms showing the percentage of gas versus time into linear and curvilinear segments. The latter part was plotted on semilog arithmetic paper, and both segments were subjected to regression analysis for the determination of the coefficients and intercepts. Hayakawa et al. (1975) expressed the rate of respiration in stepwise linear segments which were subsequently used to develop a predictive MAP model. Cameron (1989) fitted the O_2 depletion data to an exponential function, whereas Yang and Chinnan (1987, 1988a) used polynomial interpolations.

Unfortunately previously published modeling work was mainly concerned with intact tissue. There is a scarcity of experimental data regarding both the optimal MAP conditions and the effects of O_2 and CO_2 on the rate of respiration of tissue segments.

As mentioned above, slicing of tissues cut from such plant organs as tubers and roots invokes an immediate two- to fourfold increase in respiration over the parent organs (Laties 1978). In addition, there is a further two- to threefold increase with aging.

The latter increment depends on temperature and on whether the aging takes place with slices that are submerged in aerated liquid or in moist air (cf. Laties 1978). The facts that (1) slice respiration is mediated mainly by cytochrome oxidase with a K_m for O_2 of 0.05 μM (Solomos 1988; Theologis and Laties 1978) and (2) the resistance to diffusion through the flesh is lower than that through the skin indicate that tissue slices at relatively low temperatures can be maintained at quite low O_2 concentrations. For instance, the diffusion of O_2 in potato flesh is about 2.9×10^{-4}/cm^2 sec^{-1} (Table 2.11). If it is assumed that at 10 °C the rate of O_2 uptake is 9 μl kg^{-1} h^{-1}, and the ambient O_2 concentration is 2%, then the O_2 level at the center of a slice 2 cm thick will be about 0.4%, which will result in a 6.7 μM O_2 solution in the adjacent cells, a concentration that is unlikely to limit cytochrome oxidase. We have observed that the rate of O_2 uptake of sweet potato slices 2 mm thick at 25 °C is of zero order with respect to its external level until the latter decreases below 0.5% (Fig. 2.2).

The effect of temperature on MAP modeling must also be considered because it affects both the rate of respiration and film permeability to gases. The effects of temperature on plant respiration can be expressed as Arrhenius-type equations (James 1953). In chilling-sensitive tissues, there is an increase in the energy of activation at low temperatures (Lyons 1973). Further, in a number of tissues, low temperatures may induce a rise in respiration. A classic example is potato tubers, where storage at 1 °C evokes a rise in respiration above that observed at 10 °C (Isherwood 1973).

Changes in the permeability of gases through the film with temperature can also be expressed by an Arrhenius-type equation (Mannapperuma et al. 1991). The authors have determined the energy of activation of a number of commercially available films.

2.4.3 Steady-State Modeling

The most important aspect of MAP modeling is the design of suitable packaging for generating the requisite gas environment for long-term storage of the commodity. Usually, the establishment of the steady-state CA environment takes about 24 h, which is adequate for most commodities. In cases where the creation of the desired gas composition has to be accelerated, the package can be flushed with the appropriate gas mixture before sealing. It should be underlined that the time for the system to reach its final steady state is determined by the parameters that are used for the creation of the long-term desired gas composition. A detailed knowledge of the transient changes in the gas composition is of limited practical value, though very interesting from a theoretical point of view.

It has been noted above that under steady-state conditions, the concentrations of O_2 and CO_2 inside the package can be considered constant, although small changes occur gradually due to changes in the respiratory activity of the tissue under the new gas environment. Thus, $(d[O_2]_{in}/dt)$ and $(d[CO_2]_{in}/dt)$ are zero, and the equilibrium fluxes can be determined from Eqs. (2.16) and (2.17). It is apparent from these equations that the internal concentrations of O_2 and CO_2 will be determined from the rates

of O_2 uptake and CO_2 evolution, weight of the tissue, and area and permeability properties of the film. Combining Eqs. (2.16) and (2.17) we obtain:

$$\frac{R_{O_2}}{R_{CO_2}} = \frac{P_{O_2}}{P_{CO_2}} \frac{[O_2]_{out} - [O_2]_{in}}{[CO_2]_{in} - [CO_2]_{out}} \tag{2.19}$$

It is evident that both the RQ and the ratio of permeabilities of O_2 over CO_2 will be critical in establishing a particular CA environment. For most tissues, RQ is close to one, in particular for tissue slices of bulky plant organs such as tubers and roots (Laties 1978). Assuming a value for RQ of 1, Eq. (2.18) can be rearranged to become:

$$[CO_2]_{in} = \frac{P_{O_2}}{P_{CO_2}}[O_2]_{out} + [CO_2]_{out} - \frac{P_{O_2}}{P_{CO_2}}[O_2]_{in} \tag{2.20}$$

A plot of $[O_2]_{in}$ against $[CO_2]_{in}$ will result in a straight line with a slope equal to the permeability ratio, as $[CO_2]_{out}$ can be neglected, and the $P_{O_2} / P_{CO_2} \cdot [O_2]_{out}$ is constant. Figure 2.3 illustrates the relationship between $[O_2]_{in}$ and $[CO_2]_{out}$ for 1/2, 1/4, 1/5, and 1/6 permeability ratios of O_2 over CO_2. These ratios were chosen because they are the most common in commercially available films. For a successful MAP package, the combination of internal concentrations of O_2 and CO_2 will fall close to the line for a given permeability ratio.

Equations (2.16) and (2.17) show that the area of the film, along with the weight of the tissue, will be critical in establishing a desired MAP environment. If *W/A* is denoted by ρ, then:

$$[O_2]_{in} = [O_2]_{out} - \frac{R_{O_2}}{P_{O_2}}\rho \tag{2.21}$$

$$[CO_2]_{in} = [CO_2]_{out} + \frac{R_{CO_2}}{P_{CO_2}}\rho \tag{2.22}$$

In this way, an appropriate *W/A* ratio can be selected to move the internal CO_2 and O_2 concentrations toward the point where the lines of Fig. 2.3 intersect the right-hand *y*-axis.

Jurin and Karel (1963) determined the steady-state internal oxygen concentration from the intercept of the plot of the experimental rates of respiration and flux across the film as a function of O_2 concentration. Cameron (1989) and Cameron et al. (1989) fitted the curve showing oxygen depletion versus time to an exponential equation:

$$[O_2] = a\left[1 - e^{-(btc)^d}\right] \tag{2.23}$$

where *a*, *b*, and *c* are constants. The rate of respiration at steady state was calculated by multiplying the time derivative of Eq. (2.23) by the *V/W* ratio, where *V*, in lit, and

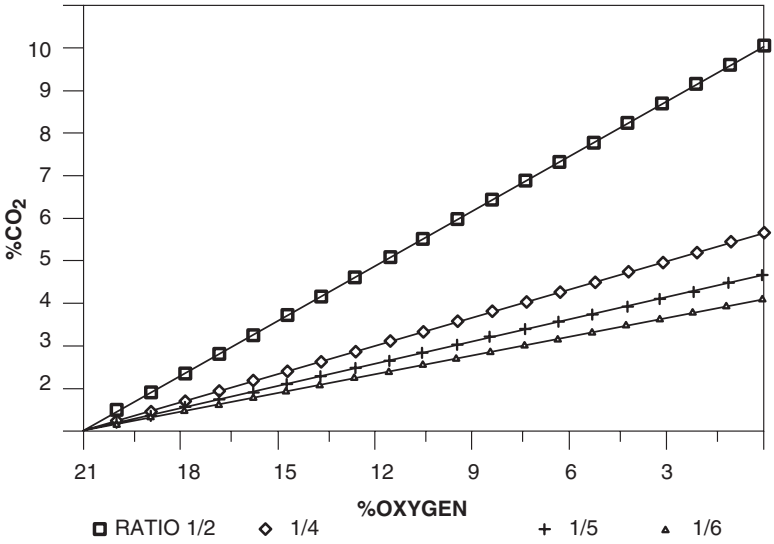

Fig. 2.3 Steady-state relationship between the internal concentrations of CO_2 and O_2

W, in kg, are the void volume of the vessel and weight of tomato fruits, respectively. The transient changes were ignored; only steady-state modeling was considered. It should be noted that Eq. (2.23) may not always be appropriate to use with plastic bags because of the changes in the void volume due to film shrinkage. Changes in V are produced by the decrease in the internal pressure due to differences in the permeabilities of O_2 and CO_2. This necessitates a decrease in volume so that the internal total gas pressure equals the ambient pressure (see later). Equations (2.16) and (2.17) are more appropriate because the volume is not a variable.

In summary, for modeling an appropriate MAP package, first the desired concentration of gases for a particular commodity can be selected from the compilation of previous data (Isenberg 1979; Kader 1985; Saltveit 1989). Then from the values of respiration under the chosen MAP environment, the appropriate film and $W/A(\rho)$ ratio can be determined.

2.4.4 Dynamic Modeling

Non-steady-state modeling can predict both the time from the start until the virtual steady-state is established, and the steady-state concentrations of O_2 and CO_2 in the package. In order to generate the appropriate expressions, the equations expressing the rate of respiration as $f(O_2, CO_2)$ must be inserted into Eqs. (2.14) and (2.15). The results in the literature differ somewhat (Chinnan 1989; Hayakawa et al. 1975; Mannapperuma et al. 1991). These differences could be partly biological in nature

because of the inherent variability in biological material, and because of the limited number of determinations that are usually used. The differences could also be due to physical considerations in assessing the rate of respiration and changes in gas concentrations inside the package during the transient stage.

In order to illustrate the latter point, we assume a solid sphere with a diffusion coefficient similar to that for O_2 in the "Russet Burbank" potato tuber (2.94×10^{-4} cm^2 sec^{-1}). Further, at zero time, the sphere contains no O_2 and is transferred to a vessel where the O_2 concentration is maintained constant at 9.1 μmoles cm^{-3} O_2 (air concentration at 10 °C). It is also assumed that oxygen is not utilized by the tissue. It can be shown that for boundary conditions $C(R,t) = C_0$, $t > 0$, and $C(0,t) = 0$ for $0 < t < t_2$, and initial conditions $C(r,0) = 0$, the solution of Eq. (2.2) is:

$$C\left(r,t\right) = C_0 + \frac{2RC_0}{\pi r}\sum_{n=1}^{\infty}\frac{\left(-1\right)^n}{n}\sin\frac{n\pi r}{R}\cdot\exp\left(-\frac{n^2\pi^2}{R^2}\cdot Dt\right) \tag{2.24}$$

where C_0, in μmoles·cm^{-3}, is the concentration of O_2 in the ambient atmosphere; R, in cm, is the radius of the sphere; t, in sec, is the time; and D, in cm^2 sec^{-1}, is the diffusion coefficient. It may be seen from Fig. 2.4a and b that even after 10 min, the concentration of O_2 at $r = 1$ is almost zero. Figure 2.4b demonstrates the distribution of O_2 along the radius after 1 h. It should be noted that the gradient would have been steeper if the utilization of O_2 had been incorporated into the solution of Eq. (2.2) and if the resistance to O_2 diffusion of the skin had also been included. Even if the initial O_2 distribution is not zero, the concentration gradient could be appreciable (Crank 1970).

It is likely that under rapid changes in the external O_2 concentration in a closed system, an appreciable concentration gradient of oxygen along the organ will develop. Under these conditions, the rate of respiration of the cells on the periphery will differ from those at the center of the organ because of the substantial differences in O_2 concentration. Further, the changes in respiration calculated from the gas isotherms may represent part of the respiration of the organ, because the distribution of the cells at the center may not be perceived.

It has been noted above that because of the differences in O_2 and CO_2 permeabilities through the film, a partial vacuum is generated inside the package which in turn produces a decrease in void volume in order that the internal pressure may equal the ambient pressure. In short, the void volume is also a function of time, and Eq. (2.14) should be written as follows:

$$\frac{d\left[O_2\right]}{dt} = \frac{P_{O_2}A\left\{\left[O_2\right]_{\text{out}} - \left[O_2\right]\right\}_{\text{in}}}{Vt} - \frac{R_{O_2}W}{V\left(t\right)} \tag{2.25}$$

A note of caution is also appropriate regarding the global validity of the rates of respiration calculated from the gas isotherms. In general, a number of interpolations produce a unique function. This, however, may not be the case for all methods of interpolation (Lancaster and Salkauskas 1986). Although the uniqueness of the local expression may be assured, this may not necessarily reflect the biochemical

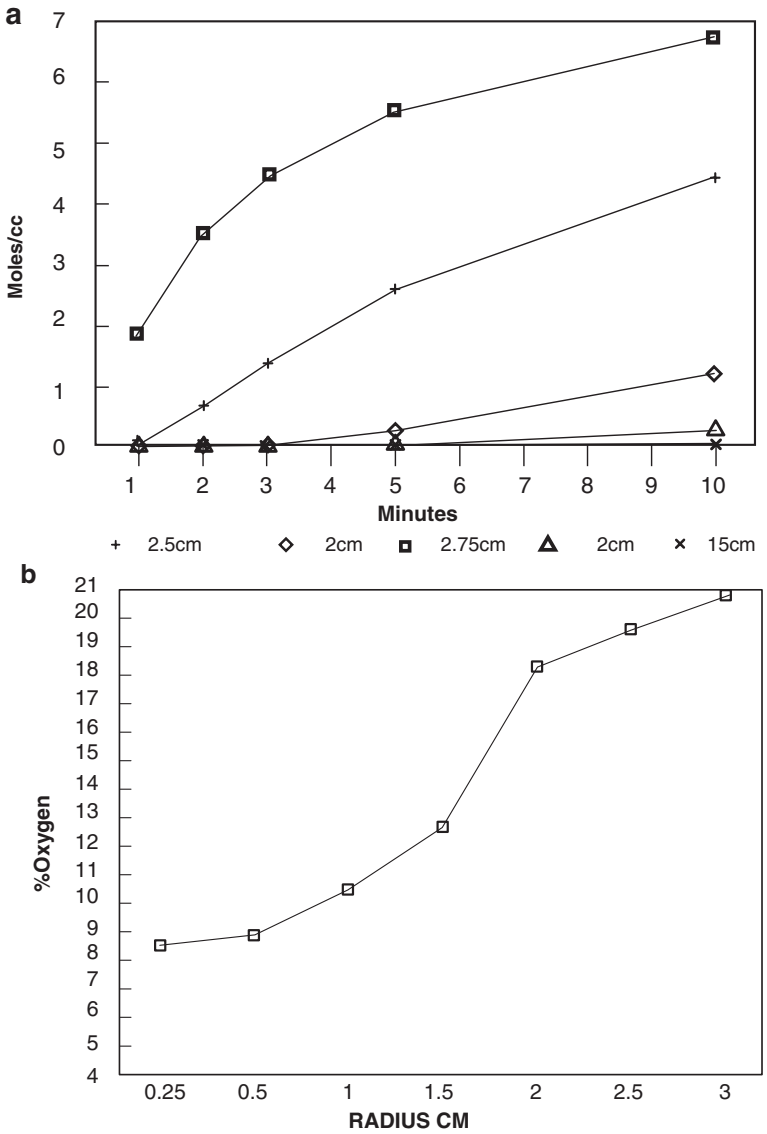

Fig. 2.4 The distribution of oxygen along the radius of a sphere with a diffusion coefficient similar to that in potato flesh. (**a**) Changes with time in O_2 concentration at a number of points along the radius. (**b**) Distribution of oxygen across the sphere after 1 h. O_2 at zero time was 0%

behavior of the system. For instance, the rate of respiration of a number of plant tissues is of zero order at concentrations of O_2 from 15% to 100% (Burton 1974; James 1953; Tucker and Laties 1985). If the local expressions reflected the biochemical events that underly plant respiration, the extension of the interpolation

to concentrations of O_2 larger than those in air will result in respiration being independent of O_2.

It should also be noted that the relationship between O_2 and respiration is enzymatic in nature and may involve more than one terminal oxidase whose affinities for oxygen may differ. Furthermore, the suppression of respiration may be the result of a metabolic depression involving alterations in the kinetic properties and/or amount of key regulatory respiratory enzymes (Storey and Storey 1990). It may thus be more appropriate to develop mathematical expressions reflecting the kinetics of multienzyme sequences (Goldbeter 1991) rather than the usual interpolating techniques that are frequently used in the literature.

2.4.4.1 Experimental Dynamic MAP Modeling

The literature on dynamic MAP modeling has been reviewed previously (Chinnan 1989; Mannapperuma et al. 1991). Here a limited amount of previous research, representing different approaches to deriving predictive mathematical expressions, will be considered.

Deily and Rizvi (1982) produced analytical formulae for predicting the gas concentration and the time necessary to reach the final dynamic equilibrium for peach fruits. They observed that the O_2 depletion isotherm consists of linear and exponential segments with an inflection point at about 5% O_2 and 20% CO_2. Further, the rate of respiration was unaffected by CO_2 concentrations in the range of 1–27%. Since the optimal MAP environment for peach storage was found to be 10–15% O_2 and 15–25% CO_2, and since the rate of respiration is constant under these conditions, the authors solved Eqs. (2.14) and (2.15) for constant R_{O_2} and R_{CO_2}. The analytical formulae derived for calculating O_2 and CO_2 are:

$$y(t) = \overline{y} + (y_a - \overline{y}) \cdot \exp\left(-AP_{O_2} t / V\right) \tag{2.26}$$

$$z(t) = \overline{z} + (z_a - z) \cdot \exp\left(-AP_{CO_2} t / v\right) \tag{2.27}$$

where $\overline{y}$ and $\overline{z}$ are the steady-state levels of O_2 and CO_2, calculated from the steady-state solution of Eqs. (2.14) and (2.15) and from limit (y(t)/t → ∞. y_a and z_a are the internal concentrations of O_2 and CO_2 at $t = 0$. The analytical formulae were tested by comparing the experimental and predicted gas concentrations using different films. Table 2.14 shows a good agreement between observed and calculated values.

Henig and Gilbert (1975) solved Eqs. (2.14) and (2.15) numerically using the experimental results of the respiration rate as a function of external O_2 and CO_2 concentrations. The authors validated the computer modeling with the experimental data. The experimental results with a FV-71 film package were in good agreement with the computer-predicted results (Fig. 2.5) (Henig and Gilbert (1975). The authors also tested the validity of the computer model by altering the variables of the inputs, for example, permeability, weight/void volume ratio, and film area. Their results

Table 2.14 Parameters and results of analytical and experimental determination of model packages of peach fruits

	Package types		Film overlaps on foam trays		
Parameters	Bags	Super-L-bags	Super firm	Barrier bag	Polyolefin
W	0.21	212.30	0.32	0.23	0.31
R_y	7.84	7.84	7.84	7.84	7.84
R_z	7.55	7.55	7.55	7.55	7.55
S	0.12	0.11	0.04	0.04	0.03
V	2.34	2.32	533.90	439.60	614.00
K_y	166.67	166.67	166.67	0.10	0.06
K_2	200.00	200.00	200.00	5.54	0.29
$\hat{y}(\%)$	12.48	11.92	–	–	–
$\hat{z}(\%)$	6.62	7.05	–	–	–
t	96.00	108.00	20.00	10.00	10.00
Analyt					
O_2%	15.99	15.55			
CO_2%	4.12	4.61			

W, S, and V are the weight, surface, and void volume of the package, respectively. R_y and R_z are the rates of O_2 uptake and CO_2 output, respectively, and K_y and K_z are the permeabilities to O_2 and CO_2, respectively, of the films. $\hat{y}$ and $\check{z}$ are the steady-state levels of O_2 and CO_2, respectively. t = time after packaging (h)
From Deily and Rizvi (1982)

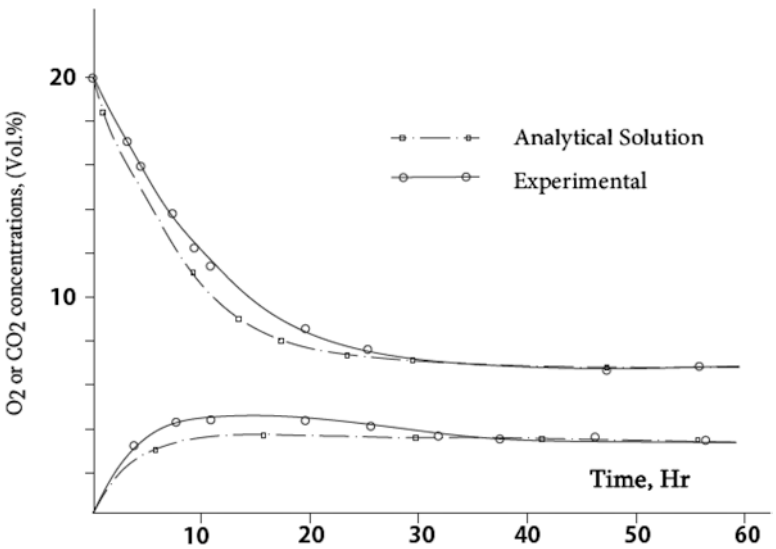

Fig. 2.5 Changes with time in O_2 and CO_2 concentrations in a RMF-61 film package of tomato fruits (From Henig and Gilbert 1975)

showed that the predicted steady-state values of CO_2 and O_2 concentrations were similar to those expected.

Hayakawa et al. (1975) derived an analytical solution of Eqs. (2.14) and (2.15) using Laplace transforms. The rate of respiration was expressed as linear segments:

$$R_{O_2} = a_i [O_2] + p_i [CO_2] + q_i \tag{2.28}$$

$$R_{CO_2} = d_i [O_2] + e_i [CO_2] + f_i \tag{2.29}$$

where a_i, p_i, q_i, d_i, e_i, and f_i are constants, and $[O_2]$ and $[CO_2]$ are the analytical expressions determining the O_2 and CO_2 levels. Because of computational complications, the authors assumed that the rate of O_2 uptake of tomato fruits was not critically affected by CO_2; hence, $p_i = 0$. Similarly it was assumed that the rate of CO_2 output was not significantly affected by the external O_2 levels. There is some uncertainty concerning the latter assumption because usually the rate of CO_2 evolution parallels that of O_2 uptake as a function of external O_2 concentrations up to the inflection point. Nevertheless, the predicted transient changes in O_2 and CO_2 concentrations were similar to those observed experimentally (Fig. 2.6).

Yang and Chinnan (1987) measured the rates of O_2 uptake and CO_2 output under 20 combinations of external O_2 and CO_2 concentrations. These data were subsequently used to develop a computer-predictive model by expressing the rates of O_2 uptake and CO_2 output as a second-degree polynomial of O_2, CO_2, and time (Yang and Chinnan 1988a):

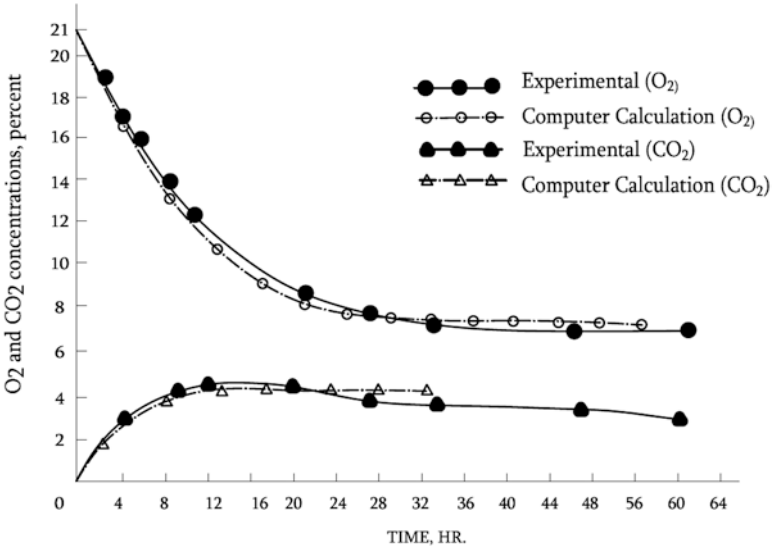

Fig. 2.6 A comparison between experimental and computed O_2 and CO_2 concentrations in a RMF-61 film package of tomato fruits (From Hayakawa et al. 1975)

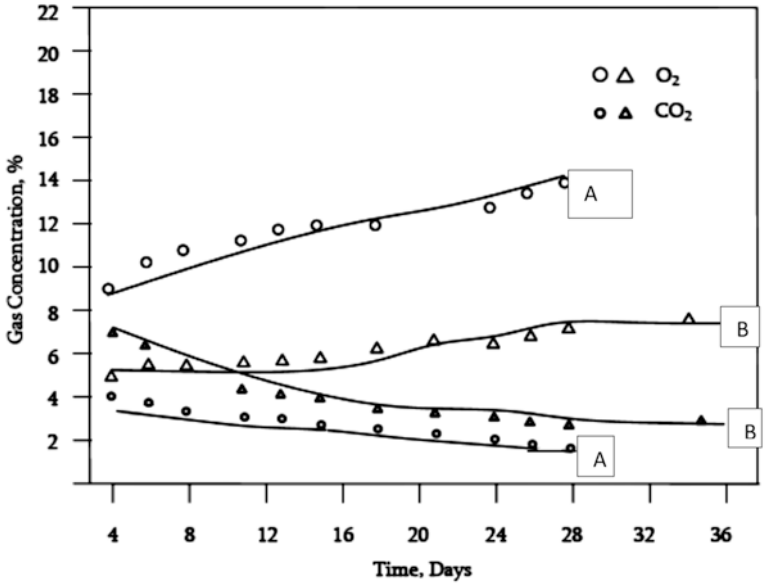

Fig. 2.7 A comparison between experimental (○△●▲) and computed (−) results of the atmosphere in two packages (*A* and *B*) made from cryo-pack E-type film. Both packages had the same surface area (1392 cm^2). Packages *A* and *B* contained two and four tomatoes, respectively (From Yang and Chinnan 1988b)

$$R_{O_2} = a_0 + a_1 C_o + a_2 C_c + a_3 t + a_4 C_o^2 + a_5 C_c^2 + a_6 t^2 + a_7 C_o C_c + a_8 C_o t + a_9 C_c t \qquad (2.30)$$

where C_o and C_c are the concentrations of O_2 and CO_2, respectively, and $a_0 \ldots a_9$ are constants. The calculated values were tested by comparing them with the experimental observations at two arbitrary combinations of O_2 and CO_2 levels (Fig. 2.7). The prediction of the steady-state concentrations of O_2 and CO_2 was achieved by iterative techniques which minimize the sum of the squares of the O_2 and CO_2 fluxes at short time intervals as the system approaches steady state (Yang and Chinnan 1988b). An innovative aspect of this work is the development of expressions to predict quality attributes, such as color, as a function of O_2 and CO_2. This is very useful for determining the apparent K_m for O_2 of the enzyme(s) whose activity is restricted by O_2, thus producing a slowing of metabolic reactions in plant senescence in general and fruit ripening in particular.

2.5 Effects of Hypoxia on Plant Tissues

Hypoxia affects a large number of metabolic activities in plant tissues, for example, the induction and suppression of gene expression (Bailey-Serres and Chang 2005; Geigenberger 2003; Giovannoni 2004; Klok et al. 2002; Liu et al. 2005; van Dongen

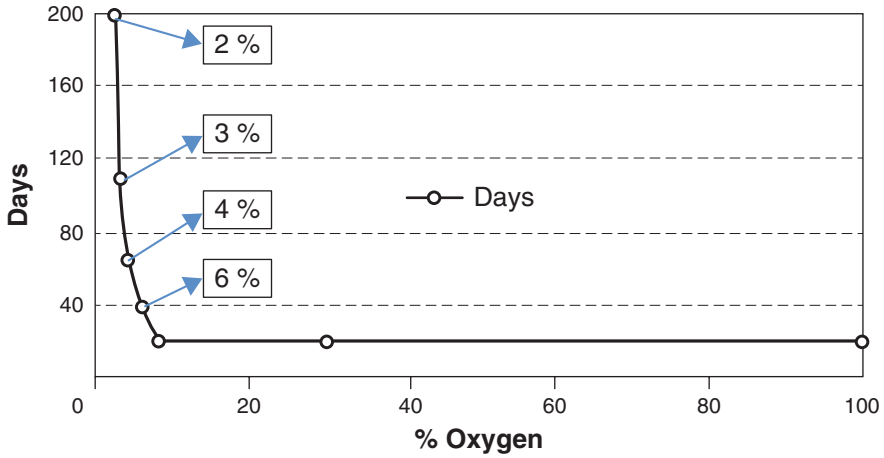

Fig. 2.8 Effect of hypoxia on the timing of the C_2H_4 climacteric onset in "Gala" apples

et al. 2009). Genes induced by hypoxia include anoxic proteins such as ADH (Kanellis et al. 1990). Conversely, hypoxia also suppresses the synthesis of existing proteins, such as those involved in cell wall synthesis, e.g., cellulase and polygalacturonase. In climacteric fruit ripening in general, its most profound effect lies in its suppression of the induction of the C_2H_4 onset. In apples, for example, the delay of the climacteric onset is initiated when the oxygen concentration falls below 8% (Fig. 2.8). Obviously, the effects of hypoxia are saturable with respect to oxygen.

2.6 Concluding Remarks

Substantial progress has been made in our understanding of the molecular aspects underlying the beneficial effects of low O_2 and/or high CO_2 on the shelf life of plant tissues. These include the induction of a number of genes as well as the suppression of the genes involved in the synthesis of existing proteins. At present, however, work still remains to be done regarding the nature of genes that are suppressed by hypoxia. This work will in turn shed light on the molecular aspects of the beneficial effects of MAP on plant tissues.

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Chapter 3
Initial Preparation, Handling, and Distribution of Minimally Processed Refrigerated Fruits and Vegetables

Fatih Yildiz

3.1 Introduction

There is a continuous demand for fresh, convenient, high-quality, and safely prepared minimally processed refrigerated (MPR) fruits and vegetables throughout the world, but consumption is concentrated in certain areas. On the other hand, most MPR food raw material production is seasonal, usually remote from consumption areas, and concentrated at certain geographical regions where yield and quality can be optimized. In addition, the raw material remains a living entity and highly perishable, bulky, price, and quantity variable commodity Anon (1990a).

New fruit and vegetable production, storage, processing/packaging, and preparation technologies made year-round availability possible for most products, except perhaps apricots, blueberries, cherries, blackberries, tangerines, carambola, and some others in a global marketing system. An optimum integrated distribution system for MPR foods will minimize energy use, environmental pollution, food waste, and cost, while maximizing the overall quality and convenience of fruits and vegetables, for optimum health of consumer.

Uneven production and processing will be equalized with new cultivars, improved storage, and MPR technologies, which will make year-round availability of almost all fruits and vegetables possible in fresh form around the world.

There are over 500,000 species of plants known on which animal and human survival depends. However, if animals and humans ceased to exist on earth, plants would continue not only to survive but also to thrive very well. There are 5000 genera of plants and 5000 cultivars (varieties) that might be used to feed the world's

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people either directly or indirectly. The species is the fundamental unit used to designate groups of plants that can be recognized as distinct kinds. In nature, individuals within one species interbreed, but they do not interbreed with other species because they are separated by some physiological, morphological, or genetic barrier that prevents the interchange of genes between the two species (Hartmann et al. 1990). A cultivar denotes individuals that are distinguished by morphological, physiological, cytological, chemical, or other characteristics significant for the purpose of horticulture and retain their distinguishing features when reproduced. An example of cultivar is the Jonathan apple. Cultivars also represent different food quality attributes such as color, flavor, texture, and nutritional value. A strain includes those plants of a given cultivar that possess the general varietal characteristics but differ in some minor characteristic or quality. A cultivar with early maturation may be considered a strain within the cultivar. An example of strain is "gray zucchini." As another example, some 70 strawberry cultivars are grown commercially in the United States; only about 10 are truly popular, but these changes from year to year as new cultivars are developed by plant breeders (Hartmann et al. 1988). Variations between species, cultivars, and strains can be due either to environmental or to genetic differences. Opportunity exists for a large number of desired transgenic cultivars or strain developments. However, some 200 species of fruits and vegetables are of major importance in world trade.

3.2 Raw Materials Characteristics and Classification for Food Purposes

Commercially fruits and vegetables are classified as follows:

Classification of fruits: Mostly fruits are tree-grown, perennial crops.

Drupes: Has one large pit or seed and grows on trees. Examples: apricot, cherry, nectarine, and peach.

Pomes: Has a core that contains seeds and grows on trees. Examples: apples, pears, and quince.

Citrus fruits: Has a leathery skin, many segments filled with juicy pellets, and grows on trees. Examples: grapefruit, lemon, lime, and orange.

Berries: Small juicy fruits that contain tiny seeds. Grows as shrub. Examples: blackberry, cranberry, blueberry, grape, raspberry, and strawberry.

Melons: Large juicy fruits that grow on vines and contain seeds and have a thick skin that may be rough and smooth. Examples: cantaloupe, casaba, honeydew, and watermelon.

Tropical and subtropical fruits: Grows only in warm sunny climate of tropics or subtropics, as trees. Examples: avocado, banana, coconut, guava, kiwi, mango, and pineapple.

Classification of vegetables: Mostly seed-grown, annual crops.

Bulbs: The underground structure where the plant's nutrient reserves are stored. Round bud with a stem and overlapping leaves. Examples: chive, garlic, onion, leek, and shallot.

Flower: The blooms or flower buds of plants eaten as vegetables. Examples: artichoke and broccoli.
Fruit: Contains the seeds of the vegetable. Examples: cucumber, eggplant, pepper, squash, zucchini, and tomato.
Leaves: Leaves of edible plants consumed as vegetables. Examples: Brussels sprouts, cabbage, lettuce, kale, and spinach.
Root: The fleshy roots of edible plants consumed as vegetables. Examples: beets, carrots, jicama, parsnips, turnip, and sweet potatoes.
Seeds: Vegetables grown and eaten from seeds. Examples: corn, green beans, and peas.
Stem: Edible stalk and leaves of plants consumed as vegetables. Examples: asparagus, bok choy, celery, and rhubarb.
Tubers: Grown underground. Examples: potatoes and yams.

Raw fruit and vegetable quality and shelf life depend to a great extent on the preharvest, harvest, and postharvest conditions (Nonnecke 1989). These include:

1. Genetically controlled factors (cultivar, strain)
2. Climatic conditions (light, temperature, percent relative humidity, wind, rain fall, etc.)
3. Soil conditions (type of soil, pH, percent moisture, microflora, mineral composition, etc.)
4. Agricultural practices (use of fertilizers, pesticides, growth regulators, irrigation, and pollination, etc.)
5. Harvesting (mechanical harvest, hand harvest, harvest temperature, etc.)

Currently, most fruits and vegetables are grown in fields, gardens, and greenhouses; however, three very different production (organic, hydroponic, and aeroponics) systems are becoming more common.

Before harvest, fruits and vegetables must meet certain minimum maturity requirements (Burton 1982). These requirements may vary from one producing area to another and from one product to another but are usually based on (1) color break, (2) minimum juice content, (3) minimum acid content, (4) minimum percentage of total soluble solids, (5) brix/acid ratio, (6) optimum flavor development, (7) abscission (detachment from parent plant), (8) development of wax on the skin, (9) softening (changes in composition of pectic substances), (10) size and shape, and (11) heat units (Pantastico 1975; Wills et al. 1989).

Fruits and vegetables can be classified in different ways for purposes of postharvest storage and processing operations. Classifications of fruits and vegetables according to the use of different plant organs were outlined by Weichmann (1987) as follows:

Root, tuber, and bulb vegetables: carrot, celeriac, garlic, horseradish, Jerusalem artichoke, onion, parsnip, potato, radish, rutabaga, salsify, scorzonera, sweet potato, beet, turnip, and yam
Leafy vegetables: Brussels sprout, cabbage, celery, chard, chicory, Chinese cabbage, collard, cress, dandelion, endive, green onion, kale, leek, chive, lettuce, spinach, and parsley

Flower vegetables: artichoke, broccoli, and cauliflower
Immature fruit vegetables: bean, cucumber, eggplant, okra, peas, pepper, squash, and sweet corn
Mature fruit vegetables: melon and tomato
Seed vegetables: shelled peas, shelled beans, corn, and lentils
Simple fleshy berry fruits: banana, grape, date, papaya, avocado, and kiwifruit
Simple fleshy hesperidium fruits: orange, lemon, lime, tangerine, and grapefruit
Simple drupe (stone) fruits: peach, plum, cherry, apricot, almond, and olive
Simple pome fruits: apple, pear, and quince
Multiple fleshy berry fruits: strawberry, blackberry, raspberry, mulberry, fig, pineapple, and pomegranate

The variation in rates of respiration and transpiration among different commodities is enormous. The various respiration and transpiration properties and temperature sensitivities of horticultural products are compiled in Appendix tables. In Table 3.1 a list of some climacteric and nonclimacteric fruits according to their respiration patterns is given (Hardenburg et al. 1986; Kays 1991).

All vegetables and fresh herbs can be considered to have a nonclimacteric type of respiratory pattern. Most climacteric fruits, and some nonclimacteric fruits such as pineapple, continue to ripen after separation from the plant Rolle and Chism (1987). The task in this case is to deliver the fruit to the consumer at an optimal level of quality. Most nonclimacteric and some climacteric products do not ripen after harvest, such as apples, berries, cherries, grapefruit, grapes, lemons, limes, oranges, strawberries, tangerines, and watermelon. In nonclimacteric commodities, quality is optimal at harvest. The task is to minimize quality loss. "Quality specifications" must be reestablished for the final product by the retailers. The following is a list of changes occurring in fruits and vegetables during harvesting, preparation, and handling:

1. Respiratory, metabolic, and enzymatic activities:

   Heat production climacteric crisis after harvest nonclimacteric metabolism
   Ethylene-induced physiological disorders – russet-spotting
   Reduced O_2- or elevated CO_2-induced physiological disorders, CO_2 injury, and blackheart in potatoes

   a. Anaerobic respiration (ethanol-acetaldehyde accumulation causing off-flavors and off-odors)
   b. Lactic acid fermentation at low O_2 concentration in cut products (Juliot et al. 1989)

   Adverse effects of polyphenol oxidases, cellulases, pectolytic enzymes, amylases, and peroxidases (discoloration, softening, off-flavors, and off-odors)

2. Transpiration (moisture loss, weight loss):

   Loss of turgidity (firmness), withering, and wilting in leafy vegetables

Table 3.1 Classification of some edible fruits according to their respiratory behavior during ripening

Climacteric fruits	Nonclimacteric fruits
Apple	Blackberry
Apricot	Cacao
Avocado	Cashew
Banana	Cherry, sour
Biriba	Cherry, sweet
Bitter melon	Cucumber
Blueberry, highbush	Grape
Blueberry, lowbush	Grapefruit
Blueberry, rabbiteye	Java plum
Breadfruit	Lemon
Cantaloupe	Litchi
Cherimoya	Mountain apple
Chinese gooseberry	Olive
Corossol fruit	Orange
Feijoa	Pepper
Fig, common	Pineapple
Guava, purple strawberry	Rose apple
Guava, strawberry	Satsuma mandarin
Guava, yellow strawberry	Star apple
Guava	Strawberry
Honeydew melon	Surinam cherry
Kiwi	Tree tomato
Mammee apple	
Mango	
Papaw	
Papaya	
Passion fruit	
Peach	
Pear	
Persimmon	
Plum	
Sapote	
Soursop	
Tomato	
Watermelon	

Source: Hardenburg et al. 1986; Kays 1991

3. Growth phenomena:

   Sprouting
   Root growth (rooting)
   Lignification (toughening)
   Ripening
   Senescence (yellowing, pithiness, feathering opening or floral buds, pink rib in lettuce)

Color changes (greening)
Elongation (asparagus)
Sloughing (skin loss)
Wound healing
Warts

4. Pest and microbial spoilage (nematode, insect, bacteria, yeast, mold, and virus attack and cause physiological disorders):

   Psyllid yellows, insect infestation, root knot, brown rot, stem-end rot, gray mold rot, rusty brown discoloration, downy mildew, blossom-end rot, and blue mold rot

5. Temperature-induced injuries:

   Chill injury (CI), freeze injury, high-temperature injury, and solar injury (sunscald, sunburn)

6. Mechanical injuries:

   Wounding
   Latent damage
   Cracking
   Broken tips
   Surface browning
   Cut or bruised products
   Vibrational cell wall and cell membrane ruptures

Temperature is a major, invisible, ever-present factor controlling respiratory metabolic and enzymatic activities, transpiration, and the growth of pests and microorganisms. Proper temperature management in the storage of MPR fruit and vegetable tissues can inactivate or retard the physiological defects. A theory developed (Parkin et al. 1989) to explain chill injury was based on low-temperature-induced membrane lipid phase transitions leading to a loss of membrane integrity and physiological dysfunction.

High-temperature and solar injury has been more a concern during the growth and development of plants than in postharvest handling. Hot-water dipping with fungicides is used in certain products to reduce microbial load without injuring the fruit (Harvey 1978; Salunkhe et al. 1991).

Mechanical injuries speed up the deterioration of fresh produce by disrupting membranes and increasing enzymatic activity which causes undesirable reactions to occur (Shewfelt 1987).

Objectionable quality changes are accelerated by the mechanical rupturing of the cells that occurs during preparation operations such as peeling and cutting, allowing enzymes to intermix with substrates. In addition, cuts and punctures allow for microbial contamination of products as well as moisture loss. Mechanical damage may take place any time a product is handled during harvesting, loading, transportation, sorting, and grading operations. Mechanical stress also stimulates peroxidase activity in cucumbers (Eckert and Ogawa 1988).

Table 3.2 Susceptibility categories of fresh fruits and vegetables to freeze injury

Group 1	Group 2	Group 3
Most susceptible	Moderately susceptible	Least susceptible
Apricots	Apples	Beets
Asparagus	Broccoli, sprouting	Brussels sprouts
Avocados	Cabbage, new	Cabbage, old
Bananas	Carrots	Savoy
Beans, snap	Cauliflower	Dates
Berries (except cranberries)	Celery	Kale
Cucumbers	Grapefruit	Parsnips
Eggplant	Grapes	Rutabagas
Lemons	Onions (dry)	Salsify
Lettuce	Oranges	Turnips
Limes	Parsley	
Okra	Pears	
Peaches	Peas	
Peppers, sweet	Radishes	
Plums	Spinach	
Potatoes	Squash, winter	
Squash, summer		
Sweet potatoes		
Tomatoes		

Cuts, punctures, and vibrations can be reduced by selecting varieties less susceptible to bruising and by proper shock-absorbing packaging. Harvested fruits and vegetables exhibit considerable resistance to pathogens and decay processes during most of their postharvest life if they are not mechanically damaged.

Most perishable crops increase in susceptibility to infection as they approach senescence, which is a progressive loss of membrane integrity. Treatments that inhibit or delay these processes reduce postharvest decay losses (Eckert and Ogawa 1988).

It is essential to understand the nature of the harvested fruits and vegetables and the effects of handling practices, to maintain optimum condition of the product at the market. Due to the diversity of products, it is impossible to suggest a single solution for all fruits and vegetables; rather, the most appropriate practices must be worked out by the individual operator for each commodity and particular situation.

Susceptibility of fresh fruits and vegetables to freezing injury was summarized by Hardenburg et al. (1986) in Table 3.2.

3.3 Optimal System Analysis

A systems approach to processing and distribution of MPR fruits and vegetables is essential to optimize storage and handling conditions for individual crops. This offers a challenge and an opportunity for food scientists to develop systems

that will attain the low overall cost for the system, as a whole, while attaining optimum overall quality of fresh horticultural products.

In a systems approach, (1) the steps of unit operations are documented within defined boundaries, (2) the system is analyzed, (3) the system is optimized, and (4) the system coordination and controls are studied. It will be necessary to, standardize some components such as container sizes, product size and shapes, labels, etc. for automation of the system.

An analysis of the system must begin with a survey of where the crops are grown and where the products are consumed for specific product and market situations. Each cultivar or closely related cultivars may be considered, a system. "For minimally processed fruits and vegetables," harvesting, processing, storage, and distribution are accomplished in a fast, highly integrated system to maintain product quality. As a result, postharvest losses have been reduced to a few percentages and manageable transportation distances have been increased up to thousands of kilometers (Meffert 1990). One of the disadvantages of the systems approach is that the understanding of the entire system is emphasized much more than the detailed understanding of each step.

Processing and distribution systems for MPR fruits and vegetables include such issues as processing at the location of production versus at the location of consumption, large versus small processing plants, and bulk transportation versus prepackaged shipment; other issues include controlled atmosphere (CA)/modified atmosphere (MA)/vacuum/air packaging and storage at the location of production or in the region of consumption of a single commodity versus a multiple fruit and vegetable processing plant.

The alternative processing and distribution systems of minimally processed products affect the kind of initial preparation and distribution that may be used. Once the postharvest handling system has been diagrammed for a specific product, quality attributes are measured at each step. Where the greatest quality losses are occurring, they can then be subjected to more intense investigation under controlled conditions. An improved technique can then be evaluated within the context of the entire system and assessed for economic feasibility. In general, quality deterioration occurring in MPR food systems is cumulative (Bogh-Sorensen 1990).

The location of the storage facility will depend greatly on the type of marketing operation and the location of the orchard or field. It is desirable to have wholesale bulk refrigerated storages as close to the production area as possible to dispose of or utilize the waste at nearby areas. On the other hand, retail storage and displays should be in the consumption area (Figs. 3.1 and 3.2).

The following type of information should be developed for each product in a systems approach. An example of systems approach to a fresh sliced strawberry processing operation should include at least the following parameters (Rosen and Kader 1989):

1. Cultivar selection: Allstar is a June-bearing strawberry cultivar, ideal for fresh market.
2. Harvest: Hand or mechanical harvesting at full bright red or pink, in shallow tray.

Fig. 3.1 A retail display shelf for MPR fruits and vegetables

Fig. 3.2 Living potted culinary herbs and sprouts

3. Precooling: Rapid forced air or hydro precooling to below 7 °C (44.6 °F) within 8 min or maximum 2 h.
4. Field processing: Dump-wash tank to remove sand, trash eliminator to separate berry from plant, and mechanical stemming to remove calyx.
5. Transportation: To packing house with dry ice in shallow tray around 0 °C (32 °F).
6. Sorting and grading: Defectives and color sorting, separation into two sizes (small, large) by a tapered-finger sizing device.
7. Processing: Slicing into quarters, water washing to remove all exudates, dipping into $CaCl_2$ solution, and spin drying.
8. Packaging: Controlled atmosphere (CA) packaging at 12% CO_2, 2% O_2, and 95% relative humidity and 0 °C (32 °F) packaging into portion 1-lb, 2-lb, and 11-lb (5 kg) size re-sealable plastic pail packaging for fresh or strawberry shortcake.

9. Storage at warehouse: 95% relative humidity, 0 °C (32 °F) wholesale warehousing on standard pallets; maximum 7 days.
10. Storage and display at retail: Display cabinets 1 day at 18–20 °C (64–68 °F); the first noticeable quality defect is loss of volatiles.

The ideal system will be a completely integrated, computerized, and automated one.

3.4 Major Initial Unit Operation of MPR Fruits and Vegetables

The industrial harvesting, handling, processing, preparation, and distribution of fruits and vegetables require a number of steps that are primarily physical in nature, although their effects may contribute to biological, chemical, and physical changes in the products. Many individual processes are required to change or separate horticultural products into various MPR foods. By systematically studying these operations, all processes are unified, simplified, and speeded up. Batch operation requires that theory and equipment be considered together. The understanding of the basic physical principles of an operation and the formulation of these principles into a mathematical expression are the first requirements for the application of the unit operation concept. The design and operation of the equipment and the material and energy balance calculations are based on unit operation principles. Major unit operations involved in MPR processing of fruits and vegetables are given in Table 3.3. Packaging and preservation operations are given in other chapters in this book.

3.4.1 Raw Material Handling Operations

Materials handling is movement of MPR foods from the field to the retail display cabinets. It involves the conveying (in all directions) and storage of materials. Hydraulic flow, pneumatic or air flotation methods, conveyors, and forklift trucks are basic to many materials handling systems. An understanding of the characteristics of produce, such as shape, size, density, and hardness, is necessary in designing process and equipment. Rapid handling, along with precooling and without damage to the product, preserves quality. The product should not be transferred from different containers to the field or in storage that will increase the chance of damage to the product. Palletized unit loads, mobile racking, and lift trucks reduce the time and labor requirements in handling operations. Therefore, unit loads should be maintained until the final sales point. *Unitizing* refers to various methods of grouping together shipping containers, whereby they can be mechanically handled as a unit load. The most common unitizing method is palletizing; that is, containers are stacked on a pallet. The standard pallet size most commonly used is 1.0 m × 1.2 m and has a thickness of 15 cm. Some of the methods of increasing efficiency in material handling are (1) to minimize movement, (2) handle in bulk or unit loads, (3) concentrate the

Table 3.3 Major unit operations of MPR fruit and vegetable processing

A. Materials handling operations	
1. Harvesting	
2. Field processing	
3. Transportation	
4. Receiving	
B. Preparation operations	
1. Separation and multiphase contacting operations	
a. Separating operations:	
Grading	Cleaning
Sorting	Husking
Screening	Heading
İnspection	Topping
Brine separation	Shelling
Culling	Snipping
Dewatering	Silking
Draining	Trimming
Cluster separation	Stemming
Flotation	Skinning
Centrifugation	Peeling
De-stoning	Pitting
Dusting	Coring
b. Mixing operations:	
Blending	Mixing with solids
Emulsification	Mixing with liquids
2. Size reduction operations:	
Chopping	Slicing
Cutting	Dicing
Strip cut	Segmenting
V cut	Shredding
Flat cut	Pulping
Crinkle cut	Mashing
Halving	Juicing
C. Distribution and utilization operations	
1. Wholesaling: storage and control	
CA/MA/air/vacuum storage (O_2, CO_2, N_2, CO, C_2H_4, H_2O controls)	
Computer-controlled warehousing	
Wholesale storage	
Retail storage	
Labeling	
2. Physical distribution or movement	
3. Retailing and foodservice	
4. Communications network	

products to minimize the quantity of material to be moved, (4) make the operation continuous and mechanized if possible, and (5) make units in the proper size. Perishable MPR foods may be damaged by excessive temperature fluctuations, severe vibration, or microbial contamination during the handling stage.

3.4.2 Harvesting

Fruit and vegetable harvesting and handling operations are varied and highly dependent on the particular commodity. Lack of uniform ripening can make a one-time-mode harvest difficult but quite manageable. Harvesting at the proper stage of maturity is an extremely exacting operation. Harvest dates may be estimated in advance by crop scheduling systems or the heat unit system. Harvesting at the lowest possible temperature (night or early morning) is advantageous for maintaining fruit quality during handling and storage. Morris (1990) reported that grapes harvested when fruit temperature was high (above 30 °C) had a poor color and produced high levels of alcohol and acetic acid, indicating microbial spoilage. The delicate nature of many fruits and vegetables requires careful handling, and many products for the fresh and MPR processed market are hand harvested. Frequently, mechanical harvesting aids are used. Hydraulic platforms or ladders enable workers to be lifted while harvesting tree fruits. Bulk collection containers or conveyors are used to transfer the harvested products rapidly from fields to the processing unit. Machine harvesting may improve quality over that obtained by hand harvesting because it is faster and reduces holding time in the fields. In one study, mechanical and hand-harvesting systems bruised 11–40% and 0–18% of apples, respectively (Tennes et al. 1969).

3.4.3 Field Processing

Shelling and threshing of peas, beans, and lentils are done in the field by combines. Beets and carrots are harvested and topped mechanically at the field. Large boom conveyors have been used to carry the harvested pineapple from the pickers to the loading trucks. Potato harvesters dig, lift, clean, and load the product. Field processing includes inspecting for size, defects, maturity, and precooling in the field. Dry sorting in the field removes gross contamination and defective fruit which would otherwise contaminate wash waters. Insects in machine-harvested fruits can be removed by a tank washing technique in which infested fruits pass through water containing a 0.1% nonalkaline anionic wetting agent (Crandall et al. 1966). A water spray is then used to remove insects, debris, and wetting agents. Ninety-five percent of the chemical residues such as those arising from the use of pesticides can be removed by this method with no loss of quality. Precooling may be performed in the field or at the packing house on bulk loads, pallets, bin boxes, or shipping containers. Rapid precooling of fruits and vegetables to remove the field heat and the heat of respiration can be achieved by (1) forced air cooling, (2) hydrocooling, (3) hydro-air cooling (fine-mist spray combined with forced air cooling), and (4) vacuum cooling.

Significant losses in market life of fresh broccoli were noted (Brennen and Shewfelt 1989) within the 3-h cooling delay after harvest. Some MPR products,

such as oranges, strawberries, and honeydew melons, may be hydro-cooled in the field, but green pepper will fill with water if hydro-cooled. However, efficient immediate handling techniques need to be established for chill-sensitive products at the field. Curing or preconditioning, which is holding produce at moderate temperature for a period, prior to low-temperature storage, is effective to prevent chill injury for some fruits such as grapefruit. All harvesting equipment should be maintained in clean condition to prevent deterioration caused by fungi and bacteria. Knives, belts, and other surfaces should be cleaned daily to remove accumulated dirt and soil. Boxes, trays, sacks, and other receptacles for harvested produce should be cleaned daily to reduce microbial load. Metal and plastic receptacles are more easily cleaned than wooden boxes. There are distinct advantages to do as much processing, such as cleaning, trimming, and coring, as possible at the production field as can be done without greatly increasing perishability. This prevents costly disposal problems at metropolitan consumption areas.

3.4.4 Transportation

It is obvious that perishable MPR fruits and vegetables must be quickly and carefully handled during transportation. The choice of shipping in packages or in bulk depends on the product and on market requirements and economics. Bulk transport of some vegetables, such as peas, beans, and sweet corn, presents problems with self-heating due to respiration, and cooling may be required before transport. In bulk packaging of leafy and stem vegetables, application of ice slush lowers the temperature while maintaining high relative humidity in transportation. The containers used in the transport of horticultural products must be so used to avoid any mechanical damage to their contents, both through particle-to-particle or particle-to-container contacts, by load shifting, shock, overhead weight, and vibration. Fleshy berry fruits are placed in shallow boxes to prevent crushing by packing under their own weight. Fast, reliable transportation by air, sea, truck, and rail is an important element in the distribution of minimally processed foods. Mechanically refrigerated CA/MA/air/vacuum intermodal containers can be transported by truck, rail, ship, or air. Internationally standardized, dry, and refrigerated intermodal containers of truck load capacity (20,000–30,000 kg) are now available. Mechanical refrigeration systems consist of a compressor- condenser-evaporator unit that is separated from the load compartment by an insulated bulkhead. The machine section also contains a thermostat for temperature control and an air temperature indicator.

The air distribution system in a rail car uses a fan to draw air through the evaporator coil and discharge it into a ceiling duct above the load. Air distribution in all refrigerated trailers is normally from front to back. Liquid nitrogen and solid CO_2 have been used for transit refrigeration and modified atmospheres of fresh produce. However, a prepared atmosphere with a specified mixture of N_2, CO_2, O_2, C_2H_2,

H_2O, and CO should be tailored to meet the requirements and tolerances of the product treated in airtight transport vehicles. It is sometimes desirable to initiate the ripening of pears, apples, plums, and tomatoes with a low concentration of ethylene at a controlled temperature during the transit period so that the product is ready for retail sale when it arrives at the market. Regardless of the method of loading, provision should be made for refrigerated, controlled, or modified air to circulate uniformly to all parts of the load. Kays (1991) reported the specific requirements of commodities being transported. The refrigeration requirement is higher during transport than in static storage due to infiltration of air through the container walls, floor, and ceiling. Highly perishable commodities, such as strawberries, apricots, figs, cherries, grapes, lettuce, and mushrooms, may be transported by air in refrigerated cargo containers. Less perishable commodities, such as citrus fruits, potatoes, pears, apples, bananas, tomatoes, and cabbage, may be shipped by sea in refrigerated holds of insulated ships. Fruits and vegetables that require different temperatures, relative humidity (RH) conditions, fumigation, and those that are ethylene producing and non-ethylene producing, odor absorbing, odor projecting, and possessing different chemical properties should not be loaded into the same container. Grouping of compatible loads is essential in mixed load transportation and storage. Containerization, modulization, unitization, and metrication allow delivery of MPR foods with a minimum of handling and physical injury to the product and a short transit time.

3.4.5 Receiving

At the receiving of MPR product, the cold chain is interrupted; consequently, proper care must be exercised not to lose the quality that has been retained in harvesting and transporting. The efficiency of the receiving operation will be increased by the use of palletized loads. In receiving, produce must be properly segregated to permit adequate grading. Materials should be moved rapidly through the shortest distance possible from the unloading to the storage point to reduce costs. Batch and continuous automatic digital weighing have replaced manual weighing, saving time and labor. Accurate weighing is necessary for proper cost accounting, product formulation, planning, and quality control. Contracts between the supplier and the MPR product processing plant include the required standards. In some cases, contract growing may be necessary as in canning. There is a need to be able to rapidly and nondestructively evaluate the quality of fresh produce at the receiving (Dull 1986) for such safety aspects as pesticide residues, heavy microbial loads, toxic metals, naturally present undesirable compounds, and plant growth regulators.

After products have been received, they should be transferred immediately to the proper (−1 °C to +6 °C, +6 °C to 13 °C, or +13 °C to 18 °C) storage areas depending on the chill characteristics of the product.

Table 3.4 Raw or cooked yield of edible portion (EP) per pound of selected fruits and vegetables as purchased (AP)

Food items	Yield (pounds of EP)
Apples, fresh peeled	0.92
Bananas, with peel	0.65
Beans, green (cooked)	0.88
Broccoli	0.81
Cabbage	0.87
Cantaloupe	0.52
Carrots	0.70
Carrots (cooked)	0.60
Lettuce (head)	0.55
Mushrooms	0.76
Onions	0.98
Peaches	0.88
Pears, pared	0.76
Pineapple	0.78
Plums	0.54
Potato, baked with skin (cooked)	0.94
Potato, mashed (cooked)	0.81
Spinach (cooked)	0.81
Sweet potato, baked with skin (cooked)	0.81
Tomato	0.61
Watermelon	0.99

Source: USDA 1984

3.4.6 Preparation Operations

Ready-to-cook, ready-to-eat, and ready-to-use type convenience fruits and vegetables require many preparation operations. Most of these involve physical changes, but chemical reactions also take place. The weight of the prepared cooked final product may be 50–99% of the raw material as shown in Table 3.4 (USDA 1984). Vegetables in the following food groups are prepared for ready-to-use, ready-to-cook, MPR products (Figs. 3.3, 3.4, and 3.5).

Snack vegetables: whole and sliced onion, celery strip, cut carrots, sliced cucumbers, and whole lettuce

Stew vegetables: cut green beans, sliced onions, diced potatoes, corn, diced tomatoes, asparagus, riba, diced peppers, peas, diced broccoli stalks, diced mushrooms, Brussels sprouts, crinkle-sliced eggplants, and whole okra

Salad vegetables: shredded carrots, shredded cabbage for coleslaw, halved and cored pepper, diced onions, sliced red cabbage, shredded lettuce, whole parsley, sliced tomatoes, endive, and chicory

Grill vegetables: zucchini, red pepper, and eggplant

Fig. 3.3 Minimally processed fresh-cut salads in a retail display

Fig. 3.4 Fresh-cut ready-to-cook vegetables: carrots, green beans, zucchinis, and pumpkins

Soup vegetables: diced peppers, diced mushrooms, diced onions, strip-cut parsley, crinkle-cut celery, diced garlic, and crosscut leeks
Sandwich vegetables: sliced tomatoes and shredded lettuce
Ready-to-cook vegetables: sliced potatoes, strip cuts for French fries, and stir-fry vegetables
Souve and gravy vegetables: diced peppers, diced mushrooms, diced onions and garlic, strip-cut peppermint, diced tomatoes, etc.
Puree and juice vegetables: shredded potatoes, mashed potatoes, diced tomatoes, diced carrots, diced celery, diced beets, and diced eggplants
Pizza topping vegetables: strip-cut peppers, sliced mushrooms, and sliced tomatoes

Fruits in the following groups are prepared for MPR products in the end-use form (Figs. 3.5 and 3.6).

Fruit cocktail: diced peaches, diced pears, whole seedless grapes, diced pineapples, pitted cherries, and diced apples

Fruit pies: apple slices, peeled peach halves, and peeled apricot halves

Fig. 3.5 Ready-to-eat mixed snack vegetables with dip

Fig. 3.6 Ready-to-eat fruit salad; watermelon, grape, and honeydew mix and honeydew chunks on a display cabinet

Fruit salads: diced peaches, diced pears, seedless grapes, diced pineapple, pitted cherry halves, sliced bananas in syrup, citrus salads, segmented oranges, segmented mandarins, halved grapefruits, etc.
Fruit soups: pitted prunes, pitted cherries, sliced peaches, sliced apricots, etc.
Fruit cakes: sliced strawberries, peeled bananas, pitted cherries, and sliced apricots
Fruit jellos: whole strawberries, sliced bananas, pitted cherries, seedless grapes, and sliced oranges
Fruit puddings: cut strawberries, sliced bananas, and pitted cherry halves
Snack fruits: sliced melons, pitted plums, and peeled oranges
Fruit sauce/puree/juice: shredded apples for puree or sauce, prune sauce, whole or cut oranges, lemons, and grapefruits for juice

The preparation functions may be simplified by using assembly line concepts and related equipment to enable mass production. The primary objective of industrial food preparation is to ensure ultimate consumer safety (nutritional and health), quality, convenience, and innovation at minimum cost. Preparation of MPR fruits and vegetables for ready-to-use form involves washing, cutting, rinsing, conditioning, packaging, and storage operations as illustrated in Fig. 3.7 (Anon. 1988d).

In addition to general methods of doing these operations for some products, specialized equipment is required such as green bean snippers and centrifugal spin driers, special cutters, and aseptic assembly and filling rooms. In the design of a processing line for MPR products, the major economic consideration is the cost of operation and amount of the time the equipment is being utilized. The initial investment cost is usually secondary to the costs of operation. Operating costs on a per unit of product processed basis take into account the type and use of energy, the labor employed, the amount and type of water used, and the cost of effluent disposal and innovation at minimum cost.

3.4.7 Separation and Multiphase Contacting Operations

In MPR food preparation, solid-solid, solid-liquid, and solid-gas contacting systems are utilized for separation and mixing operations. Process equipment for such systems is designed to achieve the appropriate transfer operations with a minimum expenditure of energy and capital investment. Screening is the solid-solid separation of a mixture of various sizes of fruits. Blending of ingredients may be the main objective of a solid-solid mixing. Leaching or simple washing is a separation of solid-liquid system which consists of the displacement of dirt by a liquid in which it is soluble. Separation and multiphase contacting operations of this kind are carried out in single or multiple steps or stages. A stage may be defined as a unit of equipment in which two dissimilar phases are brought into contact with each other and then are mechanically separated. Gravity sedimentation operations are also solid-liquid separations. Solid-liquid mixing is involved in fruit juice and puree preparations. Modified and controlled atmosphere storage and packaging involves

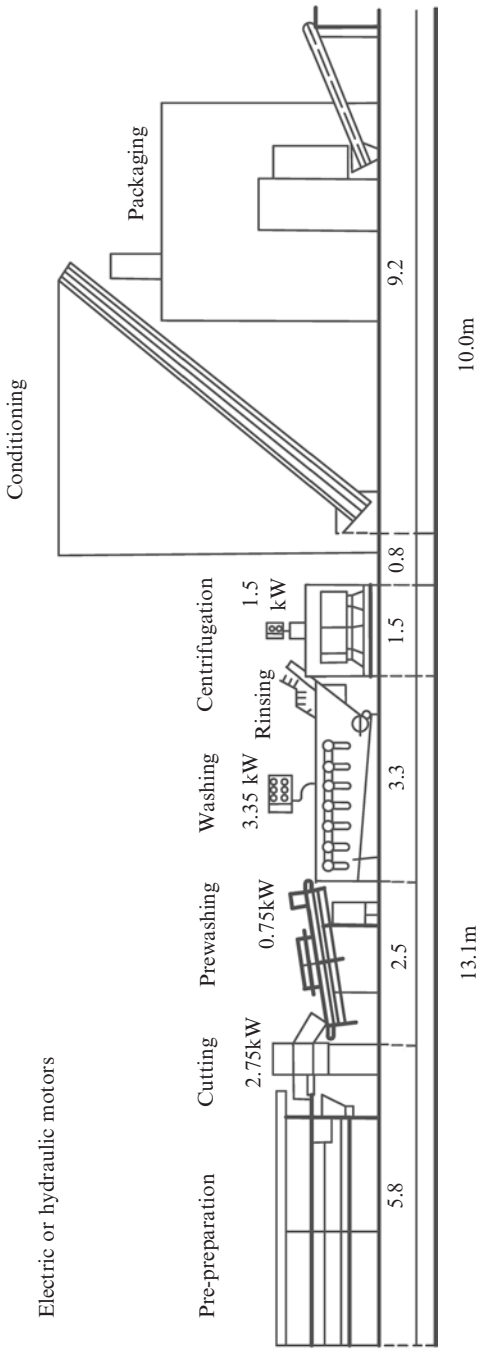

Fig. 3.7 Diagram of an MPR salad production line

gas-solid mixing operations. The solid phase is usually in a static condition. The gas phase flows or circulates more or less freely around the solid particles. Gas-solid separation is involved in dust collection, aspiration, dewatering, O_2 scavenging, CO_2 scavenging, and ethylene emitting.

Solid materials such as horticultural produce may be separated by virtue of differences in density, shape, size, color, surface characteristics (surface area, electrostatic charge), and solubility. In general, separating includes the following operations: grading, cleaning, washing, screening, sorting, peeling, coring, draining, paring, pitting, stemming, sedimenting, trimming, and centrifuging. The MPR products processing industry uses separators of various kinds. Solid-solid separators include screens, sizers, classifiers, magnetic separators, and cluster separators. Solid-liquid separators are exemplified by the commonly used clarifiers, basket centrifuges, strainers, and percolators. Also, solid-gas separators such as driers, dehumidifiers, aspirators, cyclone separators, air filters, electrical precipitators, dust collectors, and ethylene-removing catalyzers are used.

3.4.8 Sorting, Sizing, and Grading

Figures 3.8 and 3.9 show separation of raw materials into size and weight quality groups. This provides uniformity and standardization of the finished products for buying and selling. The most important grade factors are size, shape, color, firmness, flavor, friability, bruises, cut surfaces, chemical composition, disease, and soundness. Overripe, undersized, and blemished products are separated from those of acceptable quality. Grading and sorting comprise the last separation stage before processing. Damage and spoilage therefore are likely to be transmitted to the

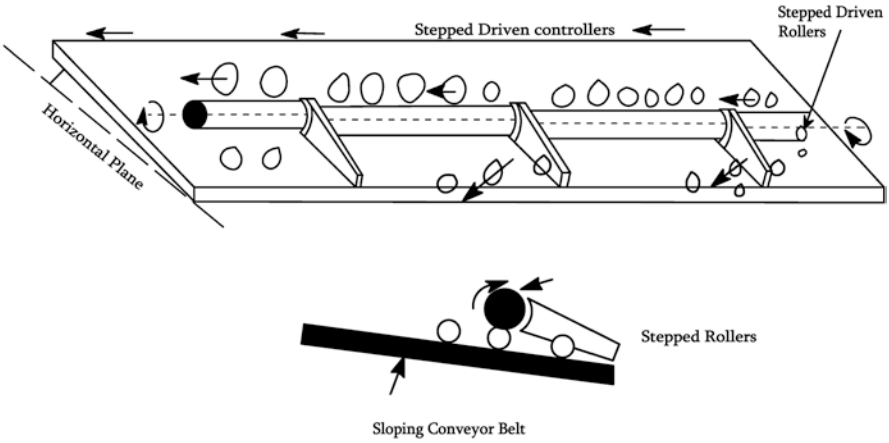

Fig. 3.8 The operating principle of a belt and roller sorter: **a** oblique view, **b** section across conveyor belt (From Brennan et al. 1990)

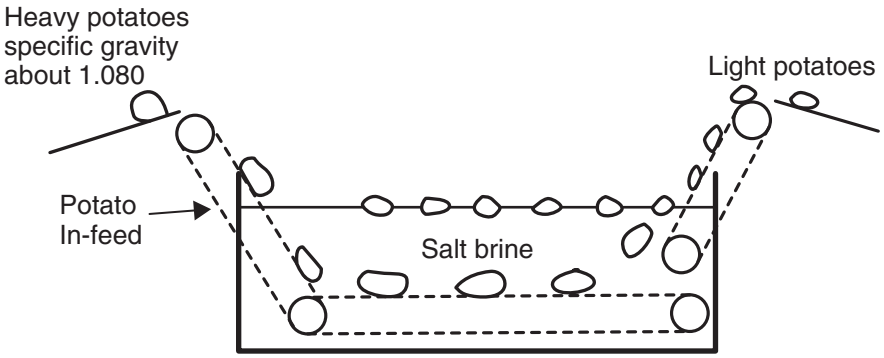

Fig. 3.9 Potato handling system (From Farrel 1976)

finished product if the bad products are not removed. The emptying of field containers onto sorting belts and dropping of product units from sorters can cause extensive damage if it is not controlled. Screening is the separation of a mixture of various sizes of produce such as peaches, strawberries, apricots, or oranges into two or more portions by means of a screening surface. Material that remains on a given screening surface is the normal size and that passing through the screening surface is the undersize material. Vibrating bar screens are used for coarse size separation and dewatering at 4 mesh (4.76 mm) and larger screening operations. Smaller than 4 mesh and larger than 48 mesh (0.29 mm or 297 μm) is referred to as fine separation. Ultrafine separation screens are smaller than 48-mesh size (Perry et al. 1989). In the grading and sorting of fruits and vegetables, various devices and types of apparatus are used to facilitate and mechanize grading operations. Screens of various designs are used. Flatbeds, drums, rollers, vibrating screens, and belt and roller sorters are a few examples of industrial operations. Light reflectance and transmittance sorting are used for nondestructively sorting and internal examination of foods (Dull 1986). Some grading is carried out manually by trained personnel who are able to assess a number of grading factors simultaneously. Automated grading has the advantages of speed, reliability, and low labor cost. The consumer recognizes, in descending order of preference, fancy, choice, standard, and seconds for most fruits and vegetables. A fancy product is normally one that can be secured only from the most nearly perfectly grown crop in a given season.

3.4.9 Cleaning, Washing, and Disinfection

Cleaning and washing may be the only preservation treatments in most of the MPR fruits and vegetables. Cleaning refers to the removal of foreign materials. As a unit operation, cleaning is a form separation concerned with the removal of twigs, stalks, dirt, sand, soil, insects, pesticides, and fertilizers residues from fruits and

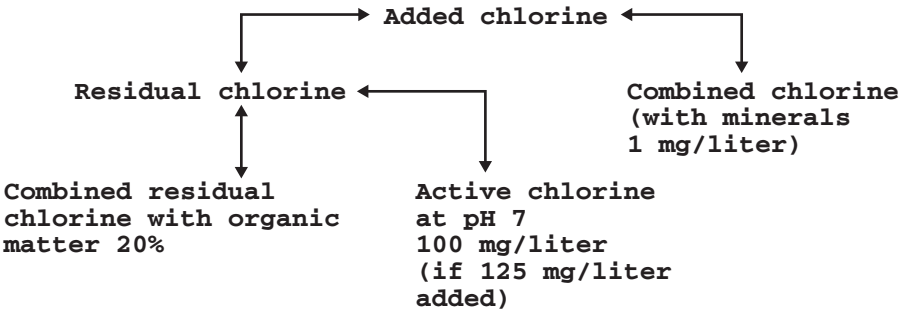

Fig. 3.10 Chlorination of wash water for MPR fruits and vegetables (Anon. 1988d)

vegetables, as well as containers and equipment, as the first step in processing. The cleaning process also involves separation of light from heavy materials by gravity, de-stoning, flotation, picking, screening, dewatering, and others Robinson and Hills (1959).

In an MPR fruit and vegetable process line, washing is generally done in an isolated chamber with a restricted number of entrances; human contact to product is limited which is in sharp contrast with the previous operations. At this stage the product becomes ready-to-eat and ready for preservation. To this end, the product is washed and freed of the majority of microorganisms by chlorine treatment up to 200 ppm allowed in the United States. The MPR product is immersed in a bath in which bubbling is maintained by a jet of air. This turbulence permits one to eliminate practically all traces of earth and foreign matter without bruising the product. The addition of various forms of chlorine to wash waters helps to prevent microbial contamination. Chlorine is the only technological washing aid permitted (Anon. 1988d); it is eliminated from the product by a final step (Fig. 3.10).

Water is one of the key elements in the quality of the MPR products. The source and quality of water must be considered. Three parameters are controlled in washing MPR fruits and vegetables (Anon 1990b): mushrooms, potatoes, and sweet potatoes are never washed or are washed after storage since added moisture is undesirable. Dry cleaning methods such as screening, brushing, aspiration, abrasion, and magnetic separation can be applied. The automatic aeroseparator for spinach and leafy vegetables removes foreign matter such as worms, insects, stones, wood pieces, etc. (Femia Industries, S. A., France, Anon. 1990b).

The washing operation has been studied for specific products (Gould 1974), and such steps as the soak period, spray pressure, and use and concentration of detergents added to the soak tank have been optimized. Rotary drum washers are used for cleaning apples, pears, peaches, potatoes, turnips, and beets; high-pressure water is sprayed over the product and it never comes in contact with dirty water. In wire-cylinder leafy vegetable washers, medium-pressure sprays of fresh water are used for washing spinach, lettuce, parsley, and leeks.

Fig. 3.11 Continuous spin drier for salads and vegetables

Because free moisture and cellular exudates on the surface of horticultural products tend to stimulate the growth of yeasts, molds, and bacteria, many types of driers (dewaterers, centrifuges, screens, dehumidifiers) have been used to remove water after washing (Fig. 3.11).

3.4.10 Peeling

The removal of the outer layer of a fruit or vegetable is referred to as peeling, paring, skinning, husking, shelling, etc. Peeling may be done (1) by hand, (2) with steam or boiling water, (3) with lye or alkalies (NaOH, KOH), (4) by dry caustic peeling with infrared heat, (5) by flame, (6) by mechanical means, (7) by high-pressure steam, (8) by freezing, and (9) with acids (Lopez 1987). Industrial peeling of large volumes of products can be accomplished mechanically, chemically, or in high-pressure steam peelers. Root vegetables such as potatoes, beets, carrots, turnips, and onions may be peeled mechanically or lye peeled. Lye peeling of peaches, pears, apricots, and tomatoes causes less loss of fruit and permits rapid handling but requires a large water supply, NaOH, and a source of heat. Hand peeling is slow, costly, and wasteful of the product. Husking of corn, shelling of peas, and snipping of beans may be done by high-speed machines. Silking is an operation applied exclusively to corn. An apple preparation system has been developed by FMC (Anon. 1988a) that automatically peels, cores, and slices apples in a high-speed continuous operation (Fig. 3.12).

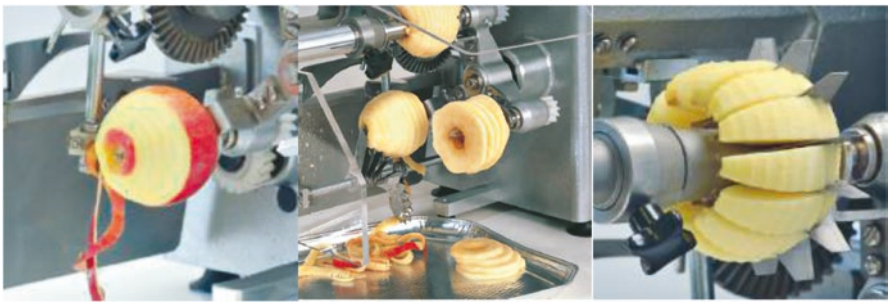

Fig. 3.12 Automatic apple washing, peeling, coring, and slicing machine

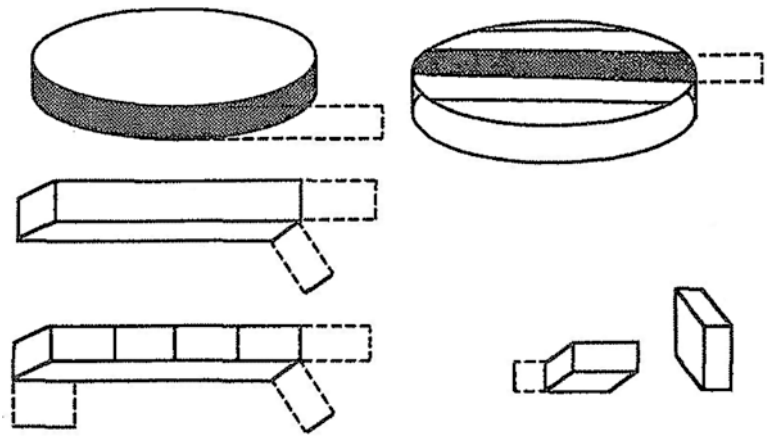

Fig. 3.13 Cutting, giving a definite size and shape to the product

3.4.11 Size Reduction Operation

Size reduction describes all means by which fruits and vegetables are cut or broken into smaller and uniform pieces of definite shape and size (Fig. 3.13). Size reduction may be an essential step to improve taste, digestibility, ease of handling, and effective heat transfer, but it has accompanying disadvantages.

3.4.12 Cutting

Cutting accelerates respiration, causes mechanical damage, and softens plant tissue. Cut tissues have lower barriers to gas diffusion, and they tolerate higher concentrations of CO_2 and lower O_2 levels than intact commodities. Therefore, the products

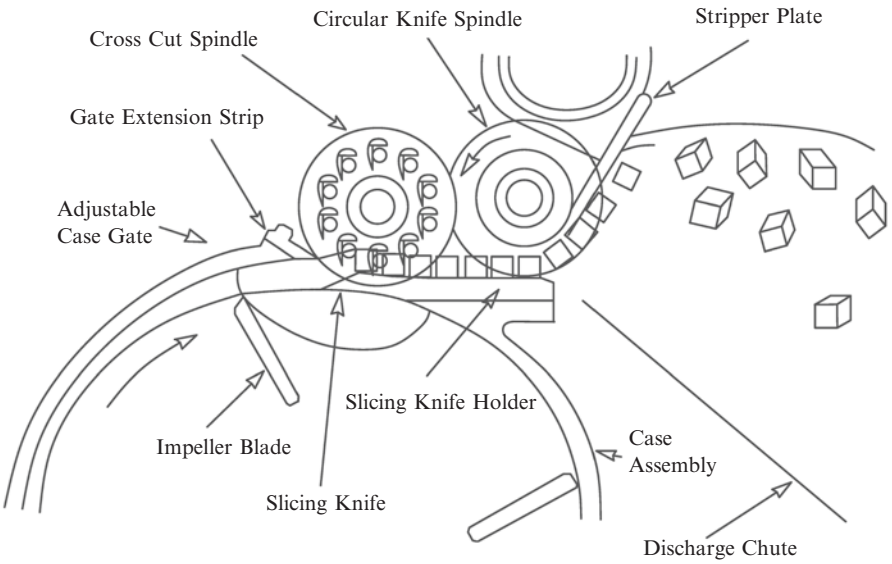

Fig. 3.14 Automatic high-speed centrifugal slicing, dicing, and strip cutting machine (Urschel Laboratories, Inc.; Anon. 1988b)

must be taken to a 4 °C room immediately after cutting. Four types of force are generally recognized in cutting machines.

They are (1) compression, (2) impact, (3) attrition, and (4) cutting. The performance of a machine for reducing the size of material is characterized by the capacity, the power required per unit of material, shape and surface characteristics of the product, and the optimum size (Perry et al. 1989).

MPR fruits and vegetables are moved on a belt or centrifugally to vertical or horizontal cutting blades (Fig. 3.14). Size reduction equipment is divided into grinders for grinding, pulping, mashing, and juicing. Cutting machines are divided for chopping, slicing, dicing, and shredding of horticultural products. Grinders break large pieces, then the products pass through a 200-mesh screen that cuts them into particles 1–50 μm in size. The smallest size attainable by wet grinding with suitable surfactants is 0.5 μm, but in dry grinding, it is 1 μm (Perry et al. 1989).

Fresh horticultural products are automatically sliced, diced, and strip cut with high-speed centrifugal machines. A slicing knife, circular knife, spindle, and crosscut knife spindle are used for dicing. Changing the size of the cubes is done by using the required cutting spindles and adjusting the slice thickness. Strip cuts of any product can be made by removing the crosscut knife from the machine. The length of the strips will depend on the size of the original product (Fig. 3.14, Anon. 1988b).

The use of power is a major expense in size reduction operations. Cutters give products of definite size and shape (fixed dimensions). The most satisfactory cutting device is a knife of extreme sharpness and as thin as structurally possible. In general, impact and shearing forces applied via a cutting edge are used in the disintegration

Fig. 3.15 A various size and shape cutting machine for fruits and vegetable

of fibrous materials. Cutting machines are constructed of 18–8 stainless steel that contacts the product. The slicing knives are made of high-carbon stainless steel alloys. Most knives are ground to one of the three shapes: fully tapered, partially tapered, or hollow ground. The knives of a cutter must be kept sharp and usually must be sharpened after each 8-h operation. The effects of cutting angle, cutting speed, and core diameter on energy requirement have been studied. It was concluded that the energy and the peak force are not affected by the cutting speed (Kulshreshtha et al. 1988). The energy requirement is minimum for a cutting angle of about 21°. The cutting angle corresponding to the minimum peak force is dependent on the diameter. Cutting and grinding equipment must be thoroughly washed after each operation. It is possible to produce aseptically diced or sliced products by using sterile knives and aseptic conditions. Thorough washing with water alone to remove the free cellular contents that are released by cutting was found to be important in prolonging the shelf life of cut carrots (Bolin and Huxsoll 1991) (Fig. 3.15).

Water knives are a new innovation where the fruits and vegetables are cut by a fine jet of high-pressure water (3000 kPa). Heiland et al. (1990) investigated the use of water knives as a high-capacity, high-speed, accurate, and automatically controlled cutting equipment for fruits and vegetables. Cell exudates were washed away by the very stream that produced them. The cost study showed that, at the high capacity, water knives may be economical.

3.4.13 Mixing and Assembling

Combined foods such as salads and ready-to-eat meals all require mixing and assembling before packaging. The object of mixing in fruit and vegetable processing is to ensure that a homogeneous mixture is formed and maintained with as low as energy input as possible at the lowest overall cost. Blending, coating, and dipping operations all require solid-solid mixing. Salad dressings are emulsions that are a mixture of liquids. Stable emulsions are formed by homogenizing. The three basic mechanisms by which solid particles are mixed are diffusive mixing, convective mixing, and shearing. There are several types of solid mixing machines. Tumblers are suitable for gentle blending of solids. Ribbon mixers are effective blenders for thin pastes and for solids that do not flow readily. The power they require is moderate. Agitators are used for slow-speed mixing with a number of paddles and baffles. The mixing efficiency of an industrial mixer is judged by the time required (mixing time) and power load (power consumption) and the properties of the product. If two or more gases are brought together, complete blending is achieved instantly. In gas-solid mixtures, the diffusion of gas molecules and the convective currents will cause slow but certain mixing. The simplest approach to mixing gases with liquid foods is to introduce the gas with a sparger at the bottom of the tank containing the liquid food and to permit it to bubble up through the liquid. Whippers and beaters are used for the mixing of gases with low-viscosity liquids. The colloid mill is a special mixing device for mixing extremely fine suspensions of either solids or liquids in a liquid. Mayonnaise, salad dressing, seasoning blends, fruit cocktail, and fruit and vegetable sauces all require mixing and emulsification.

The final operation in the processing of MPR foods takes place in the assembly and packaging room. Shredded lettuce, sliced carrots, peeled oranges, or mixtures of fresh vegetable or fruit ingredients are combined to produce a palatable mixture with dressing, mayonnaise, and other ingredients. Cook-chill meals and pizza mixes are prepared, portioned, plated, and filled in consumer packaging containers. The assembly room is the most critical zone in the processing chain and aseptic techniques are employed. A schematic diagram of an assembly and packaging room is given in Fig. 3.16. Operators working in the assembly room wear special dress, mouth masks, hair caps, and gloves. Inside the assembly room, a positive air pressure is maintained with filtered air; ambient temperature is controlled at 10–12 °C and humidity is 60–70% RH.

3.5 Distribution and Utilization of MPR Fruits and Vegetables

Distribution, in general, may be defined as the fast and efficient movement and handling of fruits and vegetables from the farm gate to the point of consumption. This involves collecting 46 million tons of produce from 350,000 farms and distributing into 37,459 food stores and 1 million foodservice establishments in the United

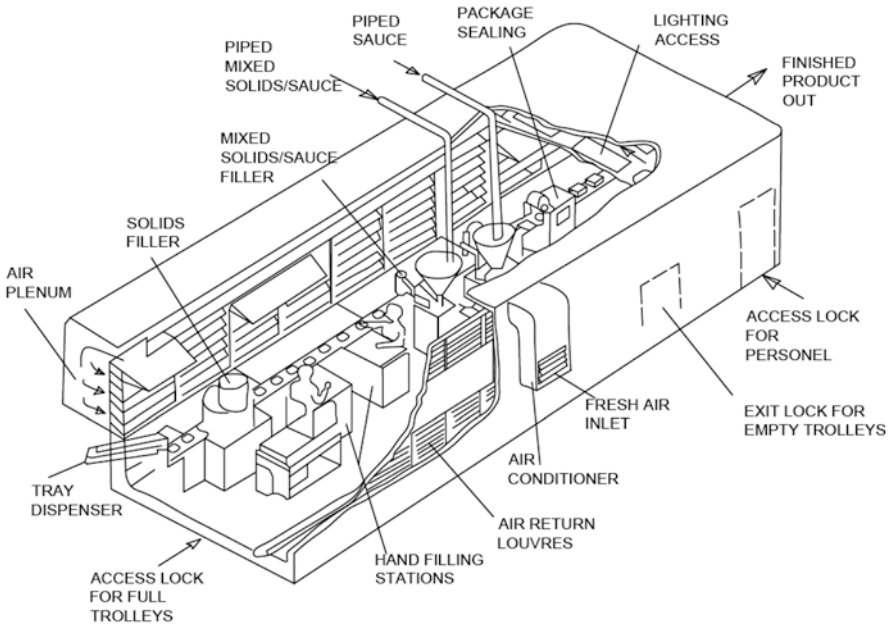

Fig. 3.16 An aseptic assembly and packaging room for MPR foods (Source: Knight 1991)

States (Fig. 3.17; Cook 2011). This involves 26.8 billion USD farm gate value to 150 billion USD consumer value (Cook 2011; News and Facts 2017). Distribution and utilization of MPR foods include the following operations:

1. Production center operations: raw and processed fruit and vegetable storage and control, central processing operations
2. Physical distribution: intra- and intercity transportation
3. Consumption center operations: food distribution centers, wholesaling, retailing, and foodservice operations
4. Communication network

MPR food distribution systems seek to maximize the time and place utility or economic value of products by getting and having the products where they are wanted, at the time they are wanted, and at a reasonable cost. The exact marketing channels differ with each commodity and change in pattern over the years. Quality and quantity of MPR food losses occur in the field, at the processing plants, in shipment to warehouses, and in the retail stores. Pilferage and tampering losses occur primarily in retail outlets and, to some extent, in truck and rail shipments. It has been reported (Kays 1991) that 3.62% of durable products such as dried fruits, nuts, and potatoes, 5.42% of fruits, and 10.3% of fresh vegetables are lost during transportation. Total distribution systems should reduce food losses and standardize the product in wholesale, retail, and consumer packaging. This would also increase the speed of distribution.

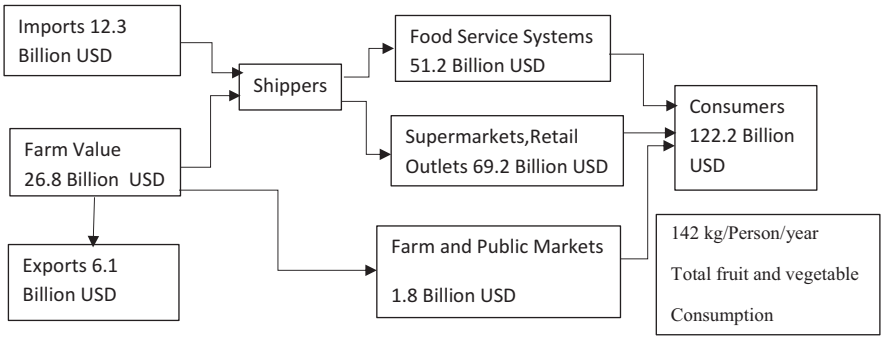

Fig. 3.17 US fresh fruit and vegetable value chain, estimated dollar sales, billions, in 2010 (Roberta Cook 2011)

The selection and establishment of a distribution system is a key decision area as it usually binds the firm long term, involves heavy investment, and can be the deciding factor in determining the success or failure of a marketing strategy. If a product is to sell, it must be made readily available to target segments such as fast-food chains, supermarkets, restaurants, hotels, institutional cafeterias, and caterers. The extensiveness of the distribution system is the foundation of marketing in the industrialized countries. The system must be able to adjust the supply of commodities to market demands quickly and easily as either supply or demand changes. During the 3–10-day distribution time, the product is often handled four to six times in loading, warehousing, and unloading at the retail outlet (Anon. 1978).

Quality maintenance is aided by the following procedures in distribution channels:

1. Minimize handling frequency.
2. Provide continued control of temperature, % RH, modified atmosphere (MA)/controlled atmosphere (CA) conditions (total environmental control) during storage and transportation.
3. Always transfer product from truck to refrigerated storage immediately.
4. Always rotate product on a first-in/first-out basis; rotate the complete inventory on a weekly basis.
5. Never stack individual cases more than five cases high.

Proper control of storage and transportation will extend the shelf life of MPR fruits and vegetables. Extended shelf life MPR foods can reduce number and magnitude of returned products, extend the distribution range of products, and will reduce the frequency of deliveries to retail from two or three per week to one per week.

Currently, the MPR food section offers at least 300–400 items in retail display cabinets. These items include:

Ready-to-eat fruits and vegetables
Ready-to-cook fruits and vegetables
Ready-to-cook mixed meals
Fresh ready-to-use herbs and sprouts
Fresh ready-to-eat specialty fruits and vegetables (tropical plants)

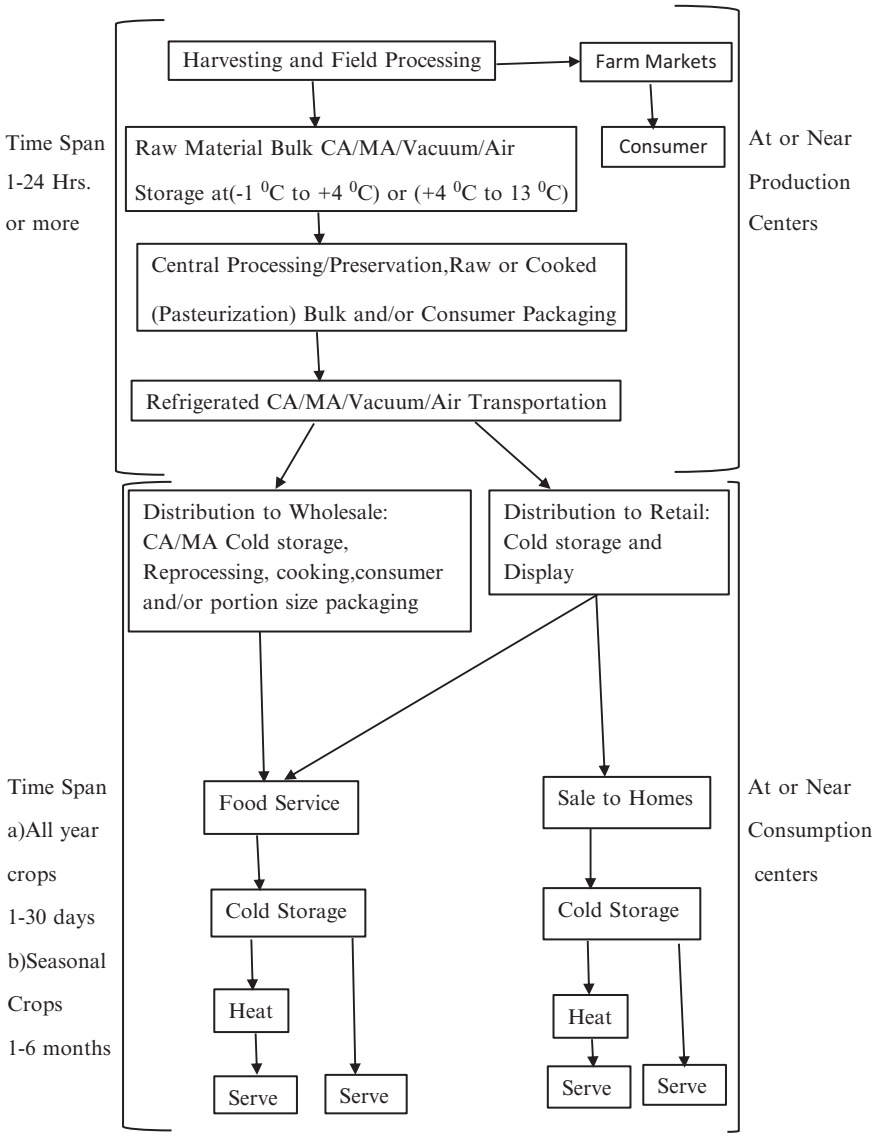

Fig. 3.18 An alternative processing and distribution system for MPR fruits and vegetables

An alternative processing and distribution system for MPR foods is given in Fig. 3.18. A single item or a few items may be more efficiently handled, but the more the produce items, the more complex the problems of distribution. The MPR foods are of low unit value and purchased frequently as convenience foods, which are usually eaten within 2 days of purchase. An intensive distribution system seeks maximum market penetration to increase, attract, or retain more customers.

However, the adoption of this system reduces the incentive for a retailer. On the other hand, an exclusive or selective distribution system may be used for some high-priced items to push the product to retail outlets. High-image products, for example, some tropical fruits, vegetables, or fresh herbs, may not be distributed to every retailer who is willing to buy but only to upscale retailers and restaurants. There may be a conflict of interest between fresh produce distribution channels and MPR foods distribution channels in local, regional, national, or even international markets.

3.6 Production Center Operations

Functions performed at production centers can be divided into two categories: first, physical operations which include harvesting, cooling, sizing, ripening, cutting, preservation, packaging, storing, and shipping and, second, related service operations which include buying, selling, financing, market finding, inspecting, and regulating. Production center operations will vary from product to product and from place to place. In the processing of MPR foods, two important functions may be distinguished: (1) raw material or processed products CA/MA/air/vacuum-packaged storage operations (storage and control) and (2) central processing and preservation operations.

3.7 Storage and Control Operations

Fresh fruits and vegetables are stored in bulk or in prepackaged forms in conventional refrigerated storage. In addition to refrigeration at certain relative humidity, CA/MA/air/vacuum and low-pressure or hypobaric storage methods are used for improving quality and shelf life. The latter methods involve a combination of lowering the O_2 level from the usual 21% level in air, raising the CO_2 level to 20% in a gas mixture, and controlling the level of ethylene (Wills et al. 1989, Robinson et al. 1975). Newer storage methods include the low-oxygen (LO) storage method which reduces the O_2 content to 2.0–1.5% by volume and maintains the CO_2 content at 0.1–0.2% below the O_2 value. The ultra-low-oxygen (ULO) storage method reduces O_2 content to the biological compatibility level of 1.2–0.8% by volume. The CO_2 content should be held at a lower level than O_2 concentration (Anon. 1989). All of these methods are practiced in completely airtight warehouses, in bulk containers, or in individual consumer packages. All storage methods also involve humidity control to prevent dehydration and maintain turgor. Stacking of unit loads must provide for free passage of gases surrounding the product during storage. Optimum levels of O_2, CO_2, % RH, and temperature must be established for each precut fruit or vegetable variety. Because CA/MA storage leaves no undesirable residues on the products, its use for minimally processed products will increase. Much research is needed to establish optimum conditions for each product.

The marketing of minimally processed fruits and vegetables requires storages for the long and short term and for bulk or packaged products at the wholesale and retail levels. The CA/MA storage can affect all forms of postharvest deterioration of fresh produce directly or indirectly and, consequently, its quality and postharvest shelf life. The composition of the gases in a closed space may be altered by increasing the concentration of some gases while reducing the concentration of others. This can be accomplished by scrubbing the atmosphere of CO_2 or O_2 by controlled venting and, if only O_2 is lowered, by continuous evacuation of the storage space. Individual gases can be added from pressurized cylinders or insulated tanks or by catalytic burners that consume O_2 and produce CO_2. Membrane air separation systems may be employed for lowering the O_2 level or increasing CO_2 level in CA storage. Air filtration is used in storage rooms to prevent microbial contamination.

The CA/MA storage rooms must be constructed similarly to conventional refrigerated storages with adequate insulation and vapor barriers and enough cooling surface to ensure high humidities and air circulation. The most reliable method of making a room gastight is to line the walls and ceiling with 28-gauge galvanized steel and connect it to the floor. The metal sheets must be fastened tightly to the walls and ceiling and the joints between sheets must be well sealed. A method of testing for gas tightness is to build up a pressure in the room 1 inch of water (gauge) and to observe the rate of pressure drop. If at the end of 1 h, 0.10–0.2 inch of water (gauge) remains, the room is tight enough (Anon. 1990b).

In cold storage rooms with ceiling evaporators, the following clearances should be respected: (1) between rows of pallets in the direction of air flow, 5–10 cm (2–4 inches); (2) wall to evaporator, 40 cm (15.7 inches); (3) under evaporator, 30 cm (11.8 inches); and (4) side walls, 40 cm (15.7 inches). Commodities should never be stacked higher than the lower edge of the evaporator.

Control systems are used to regulate mass, volume, temperature, pressure, time, % RH, % O_2, % CO_2, % C_2H_2 concentrations, and other controllable variables to minimize total cost and maintain product quality. The refrigeration system should be designed such that the thermostats work with very small switching differences if possible. Because new storage methods require drastic reduction in the O_2 level, as in the ULO process, while at the same time maintaining CO_2 at certain levels, highly accurate measuring and control systems are needed to ensure efficient control and to prevent damage to the stored products.

Microprocessor-controlled systems have been designed for the refrigeration CA/MA system to monitor the entire cooling installation and regulate cooling, ventilation, defrosting, and compressor utilization, balancing all of these different factors for maximum efficiency, which results in significant energy savings. The levels of C_2H_4, CO_2, and O_2 are measured by special analyzers and displayed digitally. At the same time, the stored data are compared with target set points for CO_2, C_2H_4, and O_2 levels for the relevant storeroom. Any differences between set points and actual levels are recorded, and the system sends instructions to the CO_2 adsorption, O_2 reduction, or ethylene removing units to make the necessary corrections. So far, no suitable sensors have been designed for measuring the relative humidity. The data recorded by the microcontrollers are continuously displayed on the terminal monitor. Besides temperature, all analytical data pertaining to the CA/MA refrigeration

system, total defrosting time, temperature readings, set point values, adsorbers running times, and O_2 supply times are printed out automatically on the printer. The electronic analyzer should be checked once a week with portable electronic devices as a manual emergency control for a fully automatic system (Ryan and Lipton 1979).

The quality of minimally processed food products is dependent on their temperature history, from production through distribution and storage to consumption. Therefore, time-temperature indicator labels are an integral part of the storage and packaging; they monitor changes in food quality arising from poor temperature maintenance during storage and distribution of MPR foods (Labuza and Breene 1989).

3.8 Central Processing Operations

The processing and preservation center at the raw material production areas will expand or replace conventional packing house operation in the MPR food system. Certain economic scale of operation is required for the establishment of a central processing plant for single or multiple commodities. A minimum efficient scale plant is the smallest sized plant at which minimum unit cost is achieved (Marion 1986). At the processing center, MPR foods will go through the processing stages of handling, cleaning, and washing and many of the following operations:

Grading and inspection: Size, shape, and quality grading can be done mechanically or by hand.

Pesticide residue and other contaminants are tested. Preservation operations: Chemical preservatives, mild heat treatments, pasteurization, pH modification, water activity reduction, ionizing radiation, or other minimal treatments (National Food Processors Assoc. 1968).

CA/MA/vacuum packaging and storage: A few commodities, such as winter apples and potatoes, can be stored for long periods. However, most items must be shipped and sold as soon as possible.

3.8.1 Physical Distribution

Physical distribution refers to the portion of the total distribution system concerned with long- or short-distance transportation of MPR products from the producer to the consumer under the cold chain including local delivery. Knowledge of the available transportation systems and the ability to select the most appropriate mode of transportation for each product and destination are essential to minimize cost while optimizing quality and shelf life. Over 80% of produce is shipped by truck; the remainder travels mostly by rail, water, or air (Imming 1985). Sanitation is a major factor in food transportation. Inadequate cleaning of trucks or rail cars may lead to

food spoilage and waste. Freight cars designed specifically for MPR foods need to be used. These are called "reefer containers." The temperature range is adjustable, between −25 °C and +25 °C (−15 °F to +75 °F ± 1 °F). This degree of temperature control may be done by the use of platinum sensors and integrated circuits. The microbial risks in CA/MA/vacuum-packaged products are much greater in the absence of proper sanitation and temperature control (Jay 1992).

New multicomponent trailers and containers offer the possibility of combined, cold (−1 °C to +4 °C), chilled (+4 °C to +13 °C), and ambient (+13 °C to +18 °C) transportation. Radio-linked computers installed in all trucks give operators a direct link to the stock control computer telling them which pallets are required for each dispatch (Goad 1989).

When the transport vehicle travels over land, sea, or air at a constant speed, some degree of vibration and sudden compression impact (shock) is always present. Transportation shocks and vibrations are transmitted from the vehicle through the packaging to the product inside, causing injury during long journeys from production areas to markets. Therefore, optimum package design is essential for maximum protection of MPR products in transportation.

3.9 Consumption Center Operations

Fresh, minimally processed products arrive at terminal market facilities which include food distribution centers, wholesalers, retailers, and foodservices.

3.9.1 Food Distribution Center Operations

National or international food distribution centers are important for MPR foods to extend the season and to facilitate the distribution. The typical distribution center operations include receiving, storing, order picking, wholesale packaging, and final shipment. The intermodal terminal food distribution centers are large facilities designed to receive unit trains of produce or MPR food containers, truck lots, and shipment by nearby air or water ports. The operation would require short-time storage and standardized or containerized shipment allowing for easy and efficient intermodal transfer for local distribution.

3.9.2 Wholesaling

The wholesaling function consists of procurement, ripening of some products, warehousing, repacking, and reselling smaller amounts to many different types of customers. Local warehouses stock items close to the consumers to provide immediate

availability. Automating warehouse operations allows for faster handling of larger volumes of products with less labor. Repacking or some reprocessing of MPR products in consumer units is done at all marketing levels including warehousing.

3.9.3 Retailing and Foodservice

Retail stores and foodservice establishments are the final link in the distribution of MPR foods. A small portion (3–6%) of fresh produce are directly marketed by farmers to consumers. Retailing is a very specialized operation involving all business activities that are concerned with selling the MPR products to final consumers for at-home use. In general, MPR products are priced 10% above conventional produce at the retail level, but products are 100% useable and there is no waste (Swientek 1991). A retail storewide computer system that uses data derived from an automated checkout system and controls physical facilities for heating, lighting, refrigeration, and interfaces electronically with suppliers will increase efficiency and speed of operation. Standardization of retail packages, cases, and pallets has been advocated (Kadoya 1990) as a means for improving efficiency in handling products moving through the market channels by reducing the number of different sizes and shapes, improving modularity, and making palletizing more efficient. There is a need for a better design of the location and layout of the MPR produce section within the retail outlets. Figure 3.19 gives an alternative layout for an MPR produce section in the supermarkets.

Foodservice operation can be divided into commercial or noncommercial eating places. Commercial operations consist of cafeterias, catering, fast-food outlets, restaurants, and other places. Noncommercial places include schools, hospitals, military establishments, and other facilities. Sales of food to eat away from home are increasing (How 1991). Fast-food chains are good examples of mass production and service of certain MPR products, especially salad bar technology (Fig. 3.20). For example, McDonalds requires that its lettuce suppliers harvest a week earlier than normal to prevent the core from becoming too crunchy. A company grows lettuce year-round exclusively for McDonalds. About 175 acres of lettuce is cut each week, leaving about 25% in the field that failed to meet the quality standards. Following harvest the lettuce is promptly cooled and then shipped to McDonalds' ten central salad processing plants strategically located around the country. Lettuce and other salad vegetables are processed. Processing involves chopping, washing, drying, and packaging in 3 1/2-lb bags, each holding the equivalent of four heads. The product is then shipped to 1500 stores, some as far as 300 miles from the processing plant. To meet the quality standards, the lettuce must be used within 10 days. The lettuce is harvested and delivered to the processor within 5 days. Processing takes about 1 day, so the store must sell the product within 4 days.

Delivery of complete meals to the home is a possible extension of changing lifestyles and MPR technology. The sous-vide concept has been tried with several variations for feeding elderly or handicapped individuals and children in special programs (Schafheifte 1990).

BACK ROOM PREPARATION AND STORAGE,PACKAGING AREA		
SLIDING MIRROR WINDOWS AND LIGHTING MIST SPRAYING		
Cold Section (Ice-slush or mechanical) 0 ^{0}C to 3 ^{0}C % RH 85-95	Chilled section 6 ^{0}C to 10 ^{0}C % RH	Dry-cool section 13 ^{0}C to 18 ^{0}C % RH 55-
Fresh fruits/Vegetables,Juices Precut Fruits and Vegetables Fresh-cut fruits and vegetables Ready-to-cook meals Green salads	Cut Pepper, Tomatoes Cucumbers Fresh herbs	Bananas Avocado Tropical fruits Subtropicals Sweet potatoes

Seasonal ambient temperature display area

Potaoes Onions Cut Pumpkins	Oranges Limons Limes	Apples Pears Melons

Fig. 3.19 An alternative MPR foods display section layout within the store

Sous-vide processing involves vacuum packaging of prepared raw or par-cooked meals or meal components, pasteurizing them under controlled time-temperature conditions (closed-pack pasteurization), chilling, then storage under 3 °C until reheating and serving.

3.10 Communication Network

Communications provide the exchange of information among the distribution channel members. Information as accurate and up to date as possible on supply, demand, and price is essential for anyone directly or indirectly involved in the production, processing, and consumption of MPR foods. The ability to identify what information is important and how to use it will be the key for success of managers.

The National Agricultural Statistics Service (NASS) gathers and disseminates official information on the production, storage stocks, and seasonal average price of major commodities. Government grades and international standards provide impartial general information about the individual product.

MPR food prices fluctuate widely from day to day and between places due to many factors. However, knowledge of differences in price elasticities of demand for a commodity in different markets may be used to raise total returns in those markets.

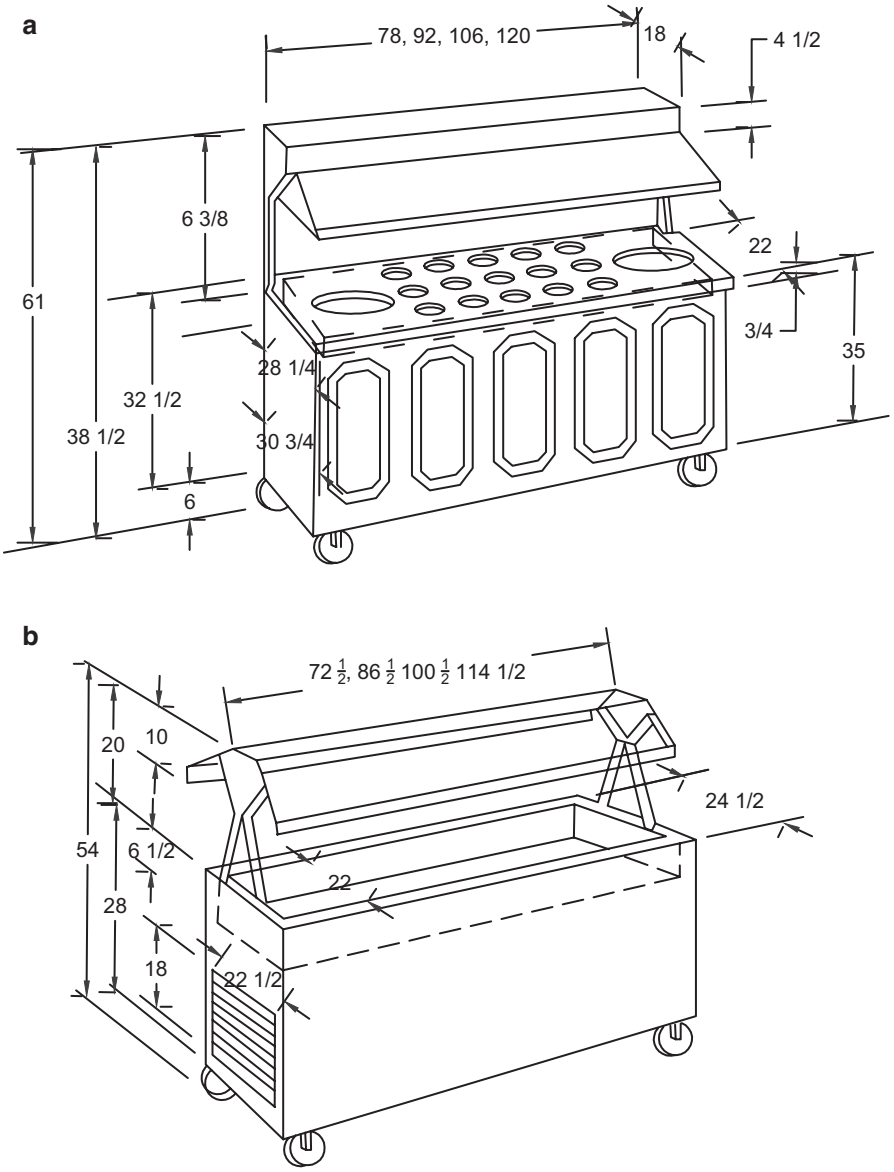

Fig. 3.20 Two types of mobile mechanically cooled salad serving equipment: **a** mirrored back salad bar, **b** two-sided salad bar (Source: Duke Manufacturing Co.; Anon. 1988c)

Information between firms needs to be exchanged for compatibility, transport type, scheduling, unit load requirements, warehousing, product availability, package size and type, delivery dates, and order quantity. Inventories exist as a necessary device to coordinate supply and demand. It is information that adjusts the relationship between orders, inventory, and production output (O'Shaughnessy 1988).

Advertising and promotion provides consumers with information and an incentive to purchase the advertised product. There are three major types of advertising programs directed specifically at buyers of MPR products. First, generic advertising is directed at expanding the market for a commodity or group of commodities grown in a specific area or countrywide, such as Washington State hazelnuts and the national potato program. Second, brand advertising seeks to increase sales and prices for a commodity or commodities sold by a specific company, such as Sunkist oranges. Third, private label advertising is used to expand sales of product under the label or brand of a chain retailer or foodservice wholesaler. The use of a private label does not bind retailers to purchase branded products from any particular seller, and so they can shop around for the best buy. The private label package does not have to support as heavy an advertising budget as branded products and so can be sold at a lower price.

The MPR fruit and vegetable processing company deals with a host of environmental elements that influence physical distribution planning and operations. Technological changes can have a great effect on distribution systems. Changes can be received through an appropriate information channel. The gross national product, population, inflation, economic growth, changing lifestyles, competition, changes in labor rates, and political changes all affect distribution in a variety of ways. Radio Frequency Identification (RFID): RFID tags are an advanced form of information carrier that can identify and trace a product. In a typical system, a reader emits a radio signal to capture data from an RFID tag. The data is then passed to a computer for analysis. RFID tags contain a microchip connected to a tiny antenna. This allows for the tags to be read for a range of 100 feet(30 m) or more in more expensive tags, to 15 feet(4 m) in less expensive tags. In contrast to a barcode, RFID does not need to be in a direct line of sight to be recognized by a scanner. Many RFID tags can be read simultaneously at a rapid rate. RFID tags could also store information such as temperature and relative humidity data, nutritional information and cooking instructions. They could be integrated with a time temperature indicator or a biosensor to carry time temperature information or microbiological and pesticide information. RFID technology in the food system is still evolving to be used in many applications.

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Chapter 4
Enzymes in Minimally Processed Fruits and Vegetables

Ayhan Temiz and Dilay K. Ayhan

4.1 Introduction

The term "minimally processed fruits and vegetables" is also referred to as "fresh-cut fruits and vegetables", "lightly processed fruits and vegetables", "fresh-processed fruits and vegetables", "partially processed fruits and vegetables", "ready-to-use fruits and vegetables", or "preprepared fruits and vegetables" (Yildiz 1994; Garcia and Barrett 2004). Minimally processed fruits and vegetables is defined by the International Fresh-Cut Produce Association (IFPA) as any fresh fruit or vegetable or any combination thereof that has been physically altered from its original form but remains in a fresh state (Garcia and Barrett 2004). Minimal processing of fruits and vegetables includes all operations such as washing, peeling, slicing, shredding, trimming, sorting, dicing, chopping, coring, paring, mashing, abrasion, sanitization, rinsing, and drying before packaging and storage at low temperature, in order to extend shelf life and preserve nutritive and sensorial properties of the products (Brecht 1995; Paviath and Orts 2009; Dea et al. 2011; Oliveira et al. 2011; Yemenicioğlu 2015). Minimal processing uses in order to prepare an original product or commodity for consumption, without affecting the original, "fresh-like" quality of the product (Shewfelt 1987). All these minimal processing steps have an effect on the shelf life, nutritional value, and quality of the prepared product (Perera 2007).

Enzymes are essential in the physiology and metabolism of plants. There are various endogenous enzymes in fruits and vegetables. There is also microbial enzymatic activity in the plants. Both endogenous and microbial enzymes play important roles in the development of desired or undesired changes in the sensorial and nutritive quality of the fruits and vegetables. Most enzymes remain active at the postharvest stage (Terefe et al. 2014).

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Endogenous enzymes may play roles in desirable events where ripening takes place during postharvest storage. It is well known that thousands of primary and secondary metabolites are produced during fruit ripening, which results from many metabolic pathways involving many endogenous enzymes (Song 2010). Different endogenous enzymes play a vital role in the development of desired changes affecting the sensorial and nutritive quality such as desired color, texture, flavor, nutritive value, and bioactivity of edible fruits and vegetable parts.

Endogenous enzyme activities are controlled by "natural mechanisms" in whole fruits and vegetables. The natural control mechanisms over the enzymatic reactions are lost mainly during the various minimal processing. Minimally processed fruits and vegetables are complex and more active systems than whole fruits and vegetables. Preparation steps remove the natural protection (peel or skin) of fruits and vegetables and cause bruises, rendering them susceptible to desiccation and wilting. Fresh-cut product tissues deteriorate more rapidly, and their physiology significantly differs from that of intact fruits and vegetables (Garcia and Barrett 2002; Yemenicioğlu 2015).

Although minimal processing steps are relatively unobtrusive, nature and rate of respiration changes immediately after most minimal processing, with the product becoming immediately more perishable (Paviath and Orts 2009). In most cases, minimal processing causes disruption of cell tissues and breakdown, releasing its liquid content at the sites of wounding (Ayala-Zavala et al. 2009; Paviath and Orts 2009). Subcellular compartmentalization is disrupted at the wound surfaces, favoring contact of normally separated substrates and endogenous enzymes. The contact of substrates and enzymes initiates deteriorative reactions that normally are absent in the whole product (Yemenicioğlu 2015). Some important responses to physical damages, i.e., wounding, in the plant tissue occur. One of the most common responses to wounding in plant tissue is an increase in both respiration rate and ethylene production (Dea et al. 2011). Responses to wounding also expose internal tissues to microorganism growth and deleterious microbial enzyme activities as well as potentially deleterious endogenous enzymes of fruits and vegetables (Garcia and Barrett 2002). The main enzymes responsible for quality degradation may also be of microbial origin. Whole fruits and vegetables have a characteristic natural microflora on their surfaces. However, fresh-cut products are very sensitive to microbial contamination, favoring increased microorganism growth and activities in the wounds.

The undesirable enzymatic reactions due to both endogenous and microbial enzymes then progress very rapidly, causing deteriorative changes in the sensorial and nutritive quality attributes of fruits and vegetables such as color, flavor, texture, and nutritional value. Discoloration, loss of texture, off-flavor formation, lipid oxidation, and loss of nutritional value are the important detrimental changes in the quality of minimally processed fruits and vegetables (Terefe et al. 2014; Yemenicioğlu 2015). Respiration and ethylene production rates, color, aroma, and texture may change as a response to physical damages produced during cutting operations as well as to the activity of undesirable microorganisms (Montero-Calderón and Cerdas-Araya 2010).

Processing of vegetables induces a rapid physiological deterioration, biochemical changes, and microbial degradation of the product even when only slight processing operations can be used (Sonti 2003). Minimal processing such as removing the skin from the surface or altering the size leads to "leakage of nutrients", "accelerated enzymatic reactions", "rapid microbial growth", "color change", "texture change", and "weight losses", resulting in decreased shelf life of the product (Jiang and Joyce 2002). As a result, the activity of endogenous deteriorative enzymes together with microbial enzymatic activities and/or other nonenzymatic reactions (usually oxidative) considerably shorten the shelf life of horticultural products (Terefe et al. 2014).

Among the limitations to shelf life of fresh-cut products are enzymatic spoilage due to natural endogenous enzymes of fruits and vegetables and microbial spoilage due to mainly enzymes of microorganisms. The shelf life of fruits and vegetables after harvest is strongly influenced by intrinsic factors such as respiration rate, ethylene production and sensitivity, transpiration, surface microbial flora and compositional changes, as well as the extrinsic factors such as microbial contaminations, packaging, and storage (Arvanitoyannis and Bouletis 2012). Such deleterious changes in the quality of fresh-cut fruits and vegetables can be diminished with several preserving technologies and techniques at suitable processing steps. Several chemical preservative additives can be used in order to reduce decay rate and loss of quality of fresh-cut fruits and vegetables. However, there may be a residue problem with them, which could affect human health and cause environmental pollution (Ayala-Zavala et al. 2009; Yemenicioğlu 2015). Uses of controlled and modified atmosphere packaging and edible films are other important physical and chemical treatments to increase quality and safety of these fresh products. Many other techniques have been studied in order to overcome the quality problems and extend the shelf life of fresh produce, for example, ionizing radiation, high-pressure processing, and the use of biopreservative cultures and/or their metabolites (Garcia and Barrett 2002; Dea et al. 2011; Oliveira et al. 2011).

This chapter is a review of the occurrence, distribution, function, and properties of some endogenous and microbial enzymes of minimally processed fruits and vegetables. These naturally occurring enzymes could significantly affect the sensorial and nutritional quality of minimally processed fruits and vegetable products. Major endogenous enzymes related to desired and deteriorative changes in the texture, flavor, color, and nutritive value of the fresh products and major microbial enzymes involved into the microbiological spoilages will be discussed in this chapter.

4.2 Endogenous Enzymes in Fruits and Vegetables

Enzymes are biocatalysts that are essential in the physiology and metabolism of plants. All physiological changes and catabolic and anabolic reactions of plants are performed by the catalytic action of enzymes.

There are two types of enzyme activity in plant tissues, endogenous enzyme activity and microbial enzyme activity. Endogenous enzymes are naturally occurring

enzymes in fruits and vegetable tissue. There is also the existence of microbial enzymes in fruits and vegetables. Endogenous and microbial enzymes may affect the quality of fruits and vegetables and minimally processed fruits and vegetables.

Endogenous enzymes in fruits and vegetables play a vital role in the development of desired color, texture, flavor, nutritive value, and bioactivity of edible vegetable parts, that is, leafs, seeds, stems, roots, flowers, and fruits (Yemenicioğlu 2015). On the contrary, some endogenous enzymes and microbial enzymes may cause deteriorative changes in fruits and vegetables and especially in minimally processed fruits and vegetables at the postharvest stage. Endogenous deteriorative enzymes may cause several enzymatic spoilages in minimally processed fruits and vegetables, resulting in short shelf life of products.

At postharvest stage, fruits and vegetables remain biologically and physiologically active, in that the plant tissues are living and respiring (Dea et al. 2011; Terefe et al. 2014). However, endogenous enzyme activities are controlled by "natural mechanisms" in whole fruits and vegetables unless the plant is exposed to an unusual stress originating from different factors such as diseases, infection, drought, and wrong agricultural practices (Yemenicioğlu 2015). "Compartmentation", "latency", "solubility control", and "inhibition by endogenous enzyme inhibitors" are some examples for the important natural mechanisms responsible for the control of enzyme-substrate interaction and enzyme activity (Whitaker 1996). The compartmentation is an effective mechanism that maintains enzymes and their substrates in different organelles or locations in the cell, while latency works by preventing the synthesized proenzyme's full transformation into a final active form, and solubility control works by regulating release or binding of enzymes and/or their substrates on cell walls, membranes, or organelles (Şimşek and Yemenicioğlu 2010).

A plant cell contains many compounds that are kept in separate compartments by semipermeable membranes. Enzymes and substrates are normally located in different cellular compartments, and their transfer is actively regulated. The cell membrane that surrounds the living cytoplasm of the cell establishes a boundary between it and its external environment. The membrane surrounding the largest compartment of a mature cell, the vacuole, separates the cytoplasm, with its many enzymes, from stored various substrates such as organic acids and phenolic compounds (Lamikanra 2002; Saltveit 2002).

Transportation, cold storage, and processing of fruits and vegetables may cause some severe changes in the natural mechanisms at postharvest stage. The natural control mechanisms over the enzymatic reactions are lost mainly during the various minimal processing. Minimally processed fruits and vegetables are complex and more active systems than whole fruits and vegetables. Minimal processing such as peeling, slicing, dicing, and shredding removes the natural protection of the epidermis and destroys the internal compartmentalization that separates enzymes from substrates. Biochemical and physiological consequences of fresh-cut processing are related to the wounding of tissue that occurs (Lamikanra 2002; Dea et al. 2011; Yemenicioğlu 2015). Wounding not only physically damages the membranes in the injured cells but also disrupts membrane function in adjacent cells, so that incompatible compounds mix and produce unwanted and uncontrolled reactions (Saltveit 2002).

Subcellular compartmentation is disrupted at the wound surfaces, favoring contact and interaction of normally separated endogenous enzymes with their substrates. Tissue disruption increases permeability and mixing of enzymes and substrates that are otherwise sequestered within vacuoles (Lamikanra 2002). Moreover, minimal processing results in a stress response by the produce characterized by an increased respiration rate (wound respiration) and ethylene production, leading to faster metabolic rates (Ragaert et al. 2010). The contact of solubilized, released, or activated endogenous enzymes with their substrates initiates deteriorative reactions that do not normally occur in the intact fruit or vegetable. The consequent increase in enzymatic activity may cause sensory deteriorations such as off-flavor, discoloration, and loss of firmness. Therefore, minimal processing increases the degree of perishability of the processed materials (Lamikanra 2002; Dea et al. 2011; Yemenicioğlu 2015). Enzymatic browning due to enzymatic oxidation of phenolic compounds and cell wall degradation due to enzymatic hydrolysis of the cell wall pectin substances are two examples for the undesirable enzyme-catalyzed changes in minimally processed fruits and vegetables.

Major endogenous enzymes affecting the quality of fruits and vegetables and minimally processed fruits and vegetables are summarized in Table 4.1.

4.2.1 Endogenous Enzymes Related to Physiology of Minimally Processed Fruits and Vegetables

Physiology of minimally processed fruits and vegetables products differs from that of intact fruits and vegetables. Minimally processed fruits and vegetables products are essentially wounded tissues that are susceptible to rapid deterioration (Dea et al. 2011). The physiology of minimally processed fruits and vegetables is the physiology of wounded tissue. The behavior of the minimally processed fruits and vegetables tissue is generally typical of that observed in plant tissues that have been wounded or exposed to stress conditions. Minimal processing as well as physical damages may cause a wound formation in the cut surfaces of fruits and vegetables. The physical damage and injury associated with the minimal processing of fruits and vegetables induce some of the naturally occurring stresses. In minimally processed fruits and vegetables, these responses are usually detrimental to the overall quality of the product. In some cases, there is induction of wound healing processes (Brecht 1995; Saltveit 2002).

4.2.1.1 Physiological Changes as Responses to Wounding

Biochemical and physiological consequences of fresh-cut processing are related to the wounding of tissue that occurs (Lamikanra 2002). Loss of cell integrity and destroying of subcellular compartmentation, leading to the reactions between endogenous enzymes and their substrates, are the main physiological changes in the

Table 4.1 Major endogenous enzymes and their importance for fruits and vegetables quality

Enzyme/enzyme group	Fruits/vegetables	Effect type/reaction products/spoilage type
Enzymes related to responses to wounding	Wounding tissues of fruits and vegetables	Increase in respiration rate Increase in wound-induced ethylene production Detrimental to the overall quality (loss of firmness and loss of flavor, discoloration of cut surfaces, possible decrease in vitamins, and increase in water activity at the cut surface)
Enzymes related to membrane lipid degradation:		
Lipid acyl hydrolases and phospholipase D	Damaged membrane systems of fruits and vegetables	Free fatty acids production from the membrane lipids
Lipoxygenase (LOX)	Damaged membrane systems of fruits and vegetables	Further membrane disruption to the production of traumatin (wound hormone)
Enzymes related to secondary metabolites	Wounding tissues of fruits and vegetables	Accumulation of unusual metabolites such as phenylpropanoid phenolic compounds, polyketide phenolic compounds, and flavonoids
Enzymes related to ethylene production and respiration:		
ACC synthase	Wounding tissues of fruits and vegetables	Conversion of ACC into ethylene
Various catabolic pathway enzymes		Increase in senescence and in respiratory activity Performing various types of fermentations
Enzymes related to senescence and ripening:		
Phospholipase and lipoxygenase (LOX)	Tissues of a number of vegetables	Tissue breakdown Plant tissue senescence (due to the metabolites of unsaturated fatty acids by LOX activity)
Peroxidases (POD)	Tissues of fruits and vegetables	Participation in growth regulation ripening and senescence
Enzymes related to defense responses against pathogens and insects		
Shikimate intermediate pathway enzymes:		

Peroxidases (POD)	Fruits and vegetables	Plant defense mechanisms against pathogens
Polyphenol oxidase (PPO)		Restriction of pathogens in various fruits and vegetables
Lipoxygenase (LOX)		Plant defense mechanisms against pathogens
Phenylalanine ammonia-lyase Chalcone isomerase Shikimate dehydrogenase		Plant defense mechanisms or senescence
β-glucosidases		Activation of plant defense chemicals (phytoanticipins) and conjugates of plant growth regulators Role in the release of many volatile compounds from their glycosidic precursors in fruits and vegetables
Enzymes related to textural degradation and softening:		
Pectin methylesterase (PME)	Fruits and vegetables	Fruit growth
Polygalacturonases (PG)		Ripening Softening of cell wall components
Peroxidases (POD)		Softening of cell wall components
Amylases		Ripening and softening, desired changes in texture and flavor in curing
Polyphenol oxidases (PPO)		Slight softening of the plant tissue
Enzymes related to flavor changes:		
Lipoxygenase (LOX) and hydroperoxide lyase (HPL)	Fruits and vegetables	Desired flavor development (by oxidation of polyunsaturated fatty acids into the corresponding hydroperoxides by LOX activity and then formation of C6 and C9 carbon aldehydes, the major aromatic volatile flavor compounds, from 13-hydroperoxy lipids by HPL activity)
	Fruits and vegetables	Off-flavor development (by formation of undesirable and odorous carbonyl compounds by further degradation of hydroperoxides produced by LOX activity)
Peroxidase (POD)	Fruits and vegetables	Off-flavor and off-odor formation

(continued)

Table 4.1 (continued)

Enzyme/enzyme group	Fruits/vegetables	Effect type/reaction products/spoilage type
Alcohol dehydrogenase	Fruits and vegetables	Flavor compound formation: Conversion of C6 and C9 carbon aldehyde volatiles into their corresponding alcohols Conversion of aldehydes and alcohols back and forth depending on the condition of the tissue
Pyruvate decarboxylase (PDC)	Fruits and vegetables	Flavor compounds formation: Formation of acetaldehyde from pyruvic acid. Acetaldehyde is required for ethanol formation through fermentation
Acyl-CoA alcohol transferase (AAT)	Fruits and vegetables	Flavor compounds formation: Formation of esters by combining of alcohols and acyl-CoAs
Cystine lyase	Broccoli and cauliflower	Off-flavor development Production of thiocysteine (cysteine persulfide), pyruvate, and ammonia through β-elimination reaction of *L*-cystine
	Brassica genus vegetables such as cabbage and broccoli	Formation of undesirable sulfur compounds such as methanethiol, dimethyl disulfide, and dimethyl trisulfide in disrupted tissues
Alliinase	Garlic and onion	Beneficial flavor compound (allicin) in garlic (hydrolysis of alliin into pyruvate, ammonia, and allicin)
		Flavor compound in onion (hydrolysis of isoalliin into di(1-propenyl) thiosulfinate
Myrosinase	*Brassica* genus vegetables	Characteristic flavor (by formation of secondary degradation products (isothiocyanates, thiocyanates, nitriles, thiones) of the glucosinolate hydrolysis) Rise to undesirable bitterness and too strong taste and flavor (by formation of high concentrations of above secondary degradation products)
Polyphenol oxidases (PPO)	Fruits and vegetables	Bring along unpleasant flavors and/or taste
β-glucosidases	Fruits and vegetables	Role in the release of many volatile compounds from their glycosidic precursors

Various enzymes such as deaminases, decarboxylases, transferases, dehydrogenases, oxidases, hydratases, thiolases, synthases, and acyl carrier proteins with substrate specificity in various metabolic pathways	Fruits and vegetables	Involving in volatile compound production
Endogenous enzymes related to color changes:		
Polyphenol oxidases (PPO) (tyrosinase, cresolase, catecholase or catechol oxidase, diphenolase, laccase, phloroglucinoloxidase, *p*-diphenol oxygen oxidoreductase, diphenol oxygen oxidoreductase, and phenolase)	Fruits and vegetables	Enzymatic browning (formation of *o*-quinones by PPO activity and then formation of melanins, heterogeneous brown pigments, by nonenzymatic polymerization)
	Fruits and vegetables (general)	Color deterioration
	Some fruits and vegetables such as tea and cocoa	Desirable change in color
Lipoxygenase (LOX)	Fruits and vegetables	Discoloration of plant pigments indirectly (the hydroperoxides and the free radicals formed by LOX reactions can damage pigments such as chlorophyll, carotene, and xanthophyll)
Peroxidase (POD)	Fruits and vegetables	Browning and pigment bleaching (by oxidation of phenolic compounds in the presence of hydrogen peroxide leading to the formation of brown degradation products)
Anthocyanase	Fruits and vegetables	Involving in browning indirectly by anthocyanin degradation (anthocyanidins formed from anthocyanins by anthocyanase activity are further oxidized by PPO and POD or react with *o*-quinones, the highly reactive PPO oxidation products, to form melanins)
Chlorophyllase	Fruits and vegetables	Chlorophyll degradation (change in color from brilliant green to olive brown in processed foods and to yellow, brown, or colorless in senescent tissue)
Alliinase	Processed garlic products Onion	Green color discoloration Pink discoloration

(continued)

Table 4.1 (continued)

Enzyme/enzyme group	Fruits/vegetables	Effect type/reaction products/spoilage type
Endogenous enzymes related to nutritional quality:		
Polyphenol oxidases (PPO)	Fruits and vegetables	Decrease in nutritional value (quinones formed by PPO activity can react with amino acids, peptides and proteins or mediate oxidation of amino acids cystine, methionine, and tryptophan)
	Fruits and vegetables	Decrease in biological value and digestibility of proteins (*o*-benzoquinone formed by PPO activity may combine with the *ε*-amino groups of lysine residue in the plant proteins)
	Fruits and vegetables	Involving in the oxidative degradation of ascorbic acid
Anthocyanase	Fruits and vegetables	Degradation of anthocyanins (important phytochemicals)
Lipoxygenase (LOX)	Fruits and vegetables	Degradation of vitamins (the free radicals formed by LOX activity cause oxidation of carotenoids (vitamin A precursors as well as potent antioxidants), tocopherols (vitamin E), ascorbic acid, and folate)
	Fruits and vegetables	Damage of cysteine, tyrosine, tryptophan, and histidine residues of proteins
	Fruits and vegetables	Lowering the protein quality and functionality
	Fruits and vegetables	Decrease in the amounts of essential fatty acids (linoleic acid and linolenic acid) or arachidonic acid, a conditionally essential fatty acid
Ascorbic acid oxidase	Fruits and vegetables	Degradation of ascorbic acid
Myrosinase	*Brassica* genus vegetables	Enhancement in nutritional quality (by conversion of glucosinolates into the biologically active forms such as thiocyanates and isothiocyanates)
Thiaminase	Certain plants	Degradation of thiamine
Digestive enzymes: Proteases, amylases, and lipases	Fruits and vegetables	Supporting the digestion of proteins, starch, and lipids in human body

wounding tissues of minimally processed fruits and vegetables products. There are some important physiological changes as responses to wounding in the plant tissue.

One of the most common responses to wounding in plant tissue is an increase in both respiration rate and wound-induced ethylene production. Other consequences of wounding in plant tissue are chemical or physical changes in nature, such as increased ion leakage, loss of components, alteration in flux potential, and loss of turgor. It is indicated that these physiological changes are also accompanied by loss of firmness and loss of flavor, discoloration of cut surfaces, possible decrease in vitamins, and increase in water activity at the cut surface which accelerates water loss. In addition to these, cut fruit surfaces provide a favorable environment for microbial growth, due to increased moisture, sugars, and analytes leaking from open cells (Brecht 1995; Dea et al. 2011). All chemical reactions during these physiological changes are carried out by the catalytic action of the specific endogenous enzymes in fruits and vegetables.

The wound response in fresh-cut tissue is influenced by the factors of species and variety, cultural practices, physiological maturity, postharvest handling, severity and extend of wounding, ripeness stage, storage conditions (i.e., temperature, humidity, and atmosphere) and storage duration, and various inhibitors (Brecht 1995; Dea et al. 2011).

Endogenous Enzymes Related to Membrane Lipid Degradation

Wounding plant tissues in the course of preparation of minimally processed fruits and vegetables may cause membrane lipid degradation (Rolle and Chism 1987). Extensive enzymatic degradation occurs in damaged membrane systems, causing loss of lipid components and loss of compartmentalization of enzymes and substrates. The ethylene produced upon wounding may play a role in this process by increasing the permeability of membranes and reducing phospholipid biosynthesis (Watada et al. 1990).

The enzymatic reactions catalyzed by lipid acyl hydrolases and phospholipase D produce free fatty acids from the membrane lipids. These free fatty acids are toxic to many cellular processes and are capable of causing organelle lysis and binding to and inactivating proteins. Fatty acids are the substrates of the endogenous enzyme lipoxygenase (LOX). Free radicals generated by LOX activity from fatty acids can attack intact membranes and thus cause further membrane disruption (Brecht 1995). Hexenal produced by Lipoxygenase (LOX) activity is then isomerized into the more stable 12-oxo*trans* -10-dodecenoic acid, also known as Traumatin or as "wound hormone". Traumatin is known to mimic some physiological effects associated with wounding in plant tissues, inducing cell division and subsequent callus formation. It can also be converted to the acid derivative by a nonenzymatic oxidation of the aldehyde moiety. Traumatic acid also appears to be involved in plant responses to wounding (Lamikanra 2002). The protective mechanism implicated is the further catabolism of oxidation products to jasmonic acid and methyl jasmonate. They trigger gene activation during wound response in plants (Lamikanra 2002; Baysal and Demirdöven 2007).

Cell wall metabolism in wounded tissues differs in some aspects from that of whole fruit. For example, the activity of pectic enzymes in minimally processed fruits is altered in relation to their normal levels in whole fruit. It is indicated in the literature that the levels of polygalacturonases (PG) and β-galactosidase (β-gal) in papaya are increased after cutting and remain higher than intact fruit for the remaining storage period (Goulao et al. 2010). Wounded tissues undergo accelerated deterioration and senescence (Brecht 1995). Minimal processing leads to "accelerated enzymatic reactions" as well as "leakage of nutrients", "rapid microbial growth", "color change", "texture change", and "weight losses", resulting in decreased shelf life of the product (Jiang and Joyce 2002; Terefe et al. 2014). Minimizing the negative consequences of wounding in minimally processed fruits and vegetables will result in increased shelf life and greater maintenance of nutritional, appearance, and flavor quality in these products (Brecht 1995).

Endogenous Enzymes Related to Secondary Metabolites

In response to wounding, plants synthesize an array of secondary compounds, many of which appear to be related to "wound healing" or "defense against attack by microorganisms and insects". Stress due to wounding or infection often induces plant tissues to accumulate unusual metabolites, such as phenylpropanoid phenolic compounds, polyketide phenolic compounds, flavonoids, terpenoids, glycoalkaloids, tannins, glucosinolates, and long-chain fatty acids and alcohols (Brecht 1995; Dea et al. 2011). The term "wound healing" generally is used to refer to suberin and lignin production and deposition in cell walls at the wound site, possibly followed by cell division beneath the suberized layer to form a wound periderm. In certain cases, these compounds may affect the aroma, flavor, appearance, nutritive value, or safety of minimally processed fruits and vegetables. Some aroma and flavor compounds may be evanescent, resulting in poor flavor after a short storage period compared to freshly prepared items, while some off-odors and off-flavors may be persistent. The specific complement of secondary compounds formed depends on the plant species and tissue involved (Brecht 1995).

4.2.1.2 Ethylene Production and Respiration

Wounding plant tissues induces increased ethylene production rates, usually within 1 h, with peak rates achieved usually within 6–12 h. It is indicated that it may be performed sometimes within a few minutes. In the wound-induced ethylene production, firstly the increased amount of 1-aminocyclopropane- 1-carboxylic acid (ACC) is formed. ACC is subsequently converted into ethylene by an endogenous enzyme named as ACC synthase. The increased formation of ACC causes increased ethylene production (Brecht 1995; Dea et al. 2011).

Ethylene level increases in proportion to the amount of wounding in several fruits and vegetables. It is indicated in the literature that levels of ACC and ACC synthase activity increase along with ethylene in wounded tomato, winter squash,

and cantaloupe muskmelon (Brecht 1995). Wound ethylene may accelerate deterioration and senescence in vegetative tissues and promote ripening of climacteric fruit. Some specific examples include accumulation of phenolic compounds in carrots, sprouting in potatoes, lignification in asparagus, brown spot (russet spotting) in lettuce, and general softening (Reid 1992). Wounding climacteric fruits may cause increased ethylene production, which can speed up the onset of the climacteric, resulting in a difference in physiological age between intact and sliced tissue (Watada et al. 1990). Slicing breaker-stage tomato fruit increased ethylene production three- to fourfold and increased ripening compared to whole fruit (Mencarelli et al. 1989).

The increased rate of ethylene production induces certain physiological changes such as "increased cell permeability", "loss of compartmentation", "increased senescence and respiratory activity", and "increased activity of endogenous enzymes" (Hyodo et al. 1983). During respiration, plant cells use atmospheric oxygen (O_2) and nutrients in the plant cell cytoplasm in order to make catabolic reactions. Energy and CO_2 as well as certain catabolic by-products are produced by various catabolic pathways such as glycolysis, Krebs cycle, and pentose phosphate. If the O_2 concentration within the tissue falls below about 2% or if the CO_2 concentration rises about 5%, the predominant respiratory reactions within the tissue could change from aerobic to anaerobic (Saltveit 2002). Then, various types of fermentations are carried out in the cells of the plant tissue with the production of fermentation compounds. These fermentation compounds may give the plant product undesirable flavor and aroma. All these catabolic reactions including respiration and fermentations are carried out by the catalytic action of endogenous catabolic enzymes of plant cells.

The increased rate of ethylene production varies depending on the type of "commodity", "cultivar", "ripeness stage", and "storage temperature". Storage temperature has a significant effect on wound-induced ethylene production as well (Dea et al. 2011).

4.2.1.3 Senescence and Ripening

Generally, the activity of lipolytic enzymes, including phospholipase and lipoxygenase (LOX), increases during senescence, and they have been implicated as one of the major causes of tissue breakdown in a number of vegetables (Dea et al. 2011). LOX catalyzes the oxidation of polyunsaturated fatty acids into the corresponding hydroperoxides in the presence of molecular oxygen. The metabolites of unsaturated fatty acids by the catalytic action of LOX have been implicated in plant senescence. An increase in LOX activity is a common feature in senescent plant tissues. The catalysis of *cis,cis*-1,4-pentadiene structures is related to the critical role of LOX in plant tissue senescence (Ludikhuyze et al. 2003). Peroxidases (POD) are considered to have important effects on ripening and senescence. An important function of POD is related to its role in indole acetic acid (IAA) oxidation action. POD participates in growth regulation by this action. There are various POD isoenzymes related to the ripening and senescence. Changes in the activity of three POD isozymes appear to be related to the ripening process (Lamikanra 2002).

4.2.1.4 Defense Responses Against Pathogens and Insects

There are various endogenous enzymes involved in plant defense mechanism against pathogenic microorganisms and insects. Some of them are discussed in the following sections.

Shikimate Intermediate Pathway Enzymes

Peroxidase (POD)
Peroxidase (POD; EC 1.11.1.7) is a group of iron-porphyrin-containing haloenzymes. The PODs are the members of major enzyme class "oxidoreductases" (Lamikanra 2002; Yemenicioğlu 2015).

POD is thought to be important in a variety of plant defense responses against pathogens. The involvement in POD plant defense mechanism is related to its role in the "shikimate intermediate pathway" in the synthesis of aromatic amino acids, indole acetic acid (auxins), cinnamic acids (precursors of phenylpropanoid phytoalexins), coumarins, and lignins (Biles and Martyn 1993). Pathogen- and wound-induced POD activities have been demonstrated in fruits and vegetables (Lamikanra 2002).

Polyphenol Oxidase (PPO)
Polyphenol oxidase (PPO; EC 1.14.18.1) is a group of copper-containing haloenzymes. PPO enzymes are the members of major enzyme class "oxidoreductases".

Active PPO appears to be present in all photosynthetic organisms. PPO performs some essential functions in plants, including deterrence of insects and fungal pathogens. There are evidences of PPO involvement in the restriction of pathogens in various fruits and vegetables (Lamikanra 2002). The antisense downregulation of constitutive and induced PPO expression results in hypersusceptibility to pathogens in tomato, suggesting a critical role for PPO-mediated phenolic oxidation in plant defense (Thipyapong and Steffens 1997).

Lipoxygenase (LOX)
Lipoxygenase (LOX) in vegetative tissues provide hydroperoxide substrates that can be metabolized to compounds that play important roles in plant defense (Baysal and Demirdöven 2007).

Other Enzymes Involved in the Shikimate Pathway

Phenylalanine ammonia lyase (PAL), chalcone isomerase, and shikimate dehydrogenase are the other enzymes involved in the shikimate pathway. These enzymes are also associated with plant defense mechanisms or senescence (Lamikanra 2002).

***β*-Glucosidases**
β-Glucosidases (*β*-glucosidase glucohydrolase, EC 3.2.1.21) catalyze the hydrolysis of aryl and alkyl *β*-D-glucosides releasing *β*-D-glucose. These enzymes have a broad specificity for *β*-D-glucosides. *β*-Glucosidases are mainly involved in the

activation of plant defense chemicals (phytoanticipins) and conjugates of plant growth regulators by hydrolyzing β-glycosidic bonds.

They also play a significant role in the release of many volatile compounds from their glycosidic precursors in fruits and vegetables (Terefe et al. 2014).

4.2.2 Endogenous Enzymes Related to Quality of Minimally Processed Fruits and Vegetables Products

Endogenous enzymes in minimally processed fruits and vegetables may cause desired and deteriorative changes in fruit and vegetable quality. However, endogenous enzymes are usually responsible for a specific quality loss in minimally processed fruits and vegetables. Polyphenol oxidases (PPO), lipoxygenases (LOX), pectinases, and peroxidases (POD) are the major enzymes involved in fruit and vegetable quality. The enzymes lipase, cellulase and hemicellulose, chlorophyllase, ascorbic acid oxidase, and thiaminase are also capable of catalyzing complex reactions related to fruit and vegetable quality.

PPOs are involved mainly in enzymatic browning. LOX is involved in flavor and off-flavor formation and discoloration of plant pigments, and pectinases are involved in textural changes like softening and firming. POD is capable of catalyzing complex reactions related to browning, off-flavor formation, textural degradation, and pigment bleaching (Yemenicioğlu 2015). It is known that PPO, chlorophyllase, POD, LOX, and lipases may be responsible for color and flavor changes. Pectinases, cellulase, and hemicellulase may cause textural degradation. LOX, ascorbic acid oxidase, PPO, POD, and thiaminase may cause loss of nutritional value (Terefe et al. 2014).

The key enzyme responsible for a specific quality loss in minimally processed fruits and vegetables may vary from one product to another. For instance, LOX is the key enzyme in the development of off-flavor in green peas, green beans, and corn, while cystine lyase is the key enzyme responsible for the development of off-flavor in broccoli (with some contribution from lipase) and cauliflower (Whitaker 1991).

4.2.2.1 Endogenous Enzymes Related to Textural Degradation and Softening

Texture and Consistency of Fruits and Vegetables

Texture is an important and complex quality attribute of fruits and vegetables. The structural integrity and texture of fruits and vegetables can be attributed mainly to the primary cell wall, the middle lamella, and the turgor generated within cells by osmosis (Jackman and Stanley 1995). Plant cell walls consist of a complex and highly variable combination of cellulose, pectins, and structural proteins. The undisrupted surface of a fruit or vegetable is covered with a protective layer called cuticle consisting of cutins. The middle lamella that is primarily pectin holds plant cells together. Inside a cell wall, cell membrane consists of phospholipids and proteins (Chen 2002; Goulao et al. 2010).

The basic structure of the primary plant cell wall consists of a cellulose–hemicellulose network with pectin polymers. Structural glycoproteins, phenolic esters, minerals, and enzymes are also present in the plant cell wall (Lamikanra 2002; Goulao et al. 2010). It is indicated in the literature that the plant cell wall of dicotyledonous species is composed of approximately 90% polysaccharides. The polysaccharides of plant cell wall can be classified into three main groups: cellulose, hemicellulose, and pectin, representing, respectively, 35%, 15%, and 40% of the cell wall mass of fruits and vegetables (Goulao et al. 2010). Pectic substances are one of the most abundant polysaccharides in cell walls of higher plants, particularly in young and fruit tissues (Lamikanra 2002). Pectin is the main constituent of the middle lamella of the cell wall. Pectins serve as cementing material between cellulose and hemicellulose fibers. Pectin cements cell walls and gives firmness and elasticity to tissues (Lamikanra 2002; Warriner and Zivanovic 2005). The firmness generated by the cellulose–hemicellulose domain of plant cell wall is not significantly affected by processing or storage. However, the pectin component is affected by both enzymatic and nonenzymatic reactions (Terefe et al. 2014).

Pectic Substances

The important pectic substances are pectin and pectic acid. Pectin, one of a group of colloidal carbohydrates, consists of a chain of D-galacturonic acid units linked together by α-(1–4) glycosidic bonds, in which around two-thirds of the carboxylic acid groups are esterified by methyl group. Therefore, the units of pectin are galacturonic acid and methyl-esterified galacturonic acid. Pectins are high-molecular-weight molecule with numerous branches (Lamikanra 2002; Warriner and Zivanovic 2005). Branching of the galacturonan typically occurs more in the cell walls than in the middle lamella. Free carboxylic acids in galacturonic acid are involved in intermolecular linkages that act as cross-bridges that influence cell wall strength. Calcium appears to be involved in forming intermolecular bridges by interaction with free carboxyl groups of pectic acid polymers to form insoluble salts that form ionic linkages between pectin molecules. Pectin molecules are involved in cross-linking other polysaccharides and proteins in the cell wall (Lamikanra 2002). Pectic acid contains galacturonic acid units linked together by α-1,4-glycosidic bonds. The units of pectic acid are galacturonic acid only. Pectic substances, mainly pectins, form the middle lamella in plant tissues, which keeps cells together (Warriner and Zivanovic 2005).

Changes in Cell Wall Structure and Composition

Changes in the structure of the cell wall are associated with dissolution of the middle lamella and modifications of the primary cell wall. Modifications in pectin, hemicellulose, and cellulose together are assumed to be responsible for the alteration of cell wall structure during ripening-related loss of firmness (Goulao et al. 2010). Many of the textural changes occurring on fruits and vegetables are a continuation

of the normal ripening events that lead to softening. In whole fruit, cell walls undergo a natural degradation during fruit ripening, reducing cell wall firmness and intercellular adhesion. Structural changes common to all fleshy fruit involve loosening of the primary cell walls and loss of cell cohesion, which can be or not accompanied by actual cell wall degradation (Goulao et al. 2010; Dea et al. 2011).

Softening

Softening is mainly attributed to changes in turgor pressure and in the structure and composition of cell walls (Dea et al. 2011). Calcium ions and pectic enzymes play important roles in the softening process. One of the most obvious changes that occurs during the softening of fruits and vegetables is the progressive solubilization and depolymerization of pectic substances. Pectin degradation results in liquefaction of the pectic substances leading to tissue maceration or softening (Chen 2002). Although biochemical changes in the cell wall polysaccharides are a major mechanism underlying textural changes in fleshy fruit, turgor pressure also accounts for texture of plant organs, especially when texture is accessed as resistance to compression. In addition, softening may be attributable to the accumulation of osmotic solutes in the intercellular space and partly to postharvest water loss from ripening fruit. The loss of textural quality is related to aging processes and senescence, water loss, reduced turgor, and wounding effects, including the leakage of osmotic solutes. Wilting is the major cause of loss of visual appearance and texture in delicate leafy produce such as lettuce and spinach. Ripening-related changes in texture are not alike in the various species (Goulao et al. 2010; Dea et al. 2011). The texture of fruits and vegetables can also be affected by starch degradation. Starch degradation is carried out by amylases.

Enzymes Related to Texture and Consistency

No single enzyme is responsible for the textural changes in fruit ripening and fruit softening. Three enzymes are generally believed to be involved in evolution of the textural properties of plant materials, namely, pectin methylesterase (PME), polygalacturonases (PG), and peroxidases (POD). Amylases and polyphenol oxidases (PPO) may also affect the texture of fruits and vegetables.

Major endogenous enzyme examples attributed to texture and consistency of minimally processed fruits and vegetables are discussed in the following sections.

Pectic Enzymes (Pectinases)

Pectin-degrading enzymes are widespread in nature. Pectic enzymes have received considerable attention regarding their involvement in ripening and softening of cell wall components. Firmness retention is an important quality parameter in fresh-cut fruit and vegetable products. Endogenous plant pectinases have an important role in

fruit growth and ripening (Lamikanra 2002; Warriner and Zivanovic 2005). Depending on the reaction they catalyze, pectin-degrading enzymes (pectinases) are categorized as hydrolyses (e.g., polygalacturonase, polygalacturonosidase), lyases (e.g., pectin lyase, pectate lyase), and esterases (e.g., pectin methylesterase, pectin acetylesterase). Hydrolyses and lyases are also called as depolymerases. Hydrolases require water as a reactant.

Pectin Methylesterase (PME)

Pectin methylesterase (PME; EC 3.1.1.11) is a deesterification enzyme. PME catalyzes the hydrolysis of methyl ester bonds in pectin molecules, liberating pectic acid and a lower degree of methanol. This is an enzymatic deesterification (demethylation) process of pectin to pectic acid and methanol. PME removes short branches of the pectin chains. These enzymes have no effect on the overall chain length, but they may alter the solubility of the pectins (Chen 2002; Warriner and Zivanovic 2005).

All higher plants can produce PME. PME is also produced by numerous phytopathogenic fungi and bacteria. The physiological role of PME is well established. PME is involved in fruit ripening as well as cell wall extension during cell growth. Activity of PME found in the different plants varies considerably with species and variety, part of the plant or fruit, and stage of growth and season. PMEs from different sources exist in several isoforms, which may be distinguished from one another by their molecular weight, isoelectric point, biochemical activity, and/or stability. It is well known that many enzymes are found in the same tissue in different molecular forms and concentrations (Ludikhuyze et al. 2003; Terefe et al. 2014).

The activity of PME affects pectin and the firmness of plant tissue in different ways. PME plays a crucial role in the degradation of cell walls in higher plants. The synergistic activity of PME and polygalacturonase (PG) results in pectin modification and subsequent change in texture (Warriner and Zivanovic 2005). The texture of fruit and vegetable products is modified by the activity of PME. PME hydrolyses highly polymerized pectin into demethoxylated pectin which is susceptible to further degradation by PG (Lamikanra 2002; Ludikhuyze et al. 2003; Terefe et al. 2014). PG attacks the glycosidic linkages between adjacent demethoxylated galacturonic acid units of pectin resulting in pectin degradation and consequent decrease in firmness. Plant PME demethoxylates pectin blockwise, which increases the probability that two adjacent polygalacturonic polymer chains form an "egg box" structure in the presence of divalent cations such as calcium leading to an apparent increase in firmness. The synergistic action of PME and PG results in the modification of pectin leading to degradation of textural quality of plant materials. A similar phenomenon is responsible for the softening of plant tissues during ripening and senescence (Terefe et al. 2014). PME can also prepare a substrate for pectate lyase activity. Demethoxylated pectin resists β-eliminative degradation. Pectate lyase is specific for polygalacturonide esters and will not hydrolyze nongalacturonide methyl esters or those in short-chain galacturonans to a large extent (Lamikanra 2002).

In intact plant tissues, PME is inactive, but it becomes active when the tissue is damaged (by heating to 50–80 °C, bruising, or freezing) and the cation concentration is increased (Van Buren et al. 1962).

Food industry can also benefit from a controlled activation of PME in the context of texture improvement of fruits and vegetables. It is assumed that deesterification of the pectin substances in the cell wall promotes firming. After attaining the desired degree of deesterification, PME should be inactivated to avoid the production of uncookable products (Ludikhuyze et al. 2003). For root vegetables like potatoes, the increased firmness originating from cross-linked pectate formation is highly desirable when potatoes are processed into French fries. In contrast, the hardening of the pre-peeled cold-stored potatoes due to Ca- and Mg-cross-linked pectate formation is an important industrial problem since these minimally processed products are mostly designed for cooking. Thus, it is clear that the PME is a key enzyme that affects not only vegetable softening but also vegetable firmness (Yemenicioğlu 2015).

Polygalacturonase (PG)

There are two subgroups of polygalacturonase (PG): endo-PG (EC 3.2.1.15; poly(1,4)-α-D-galacturonide glycanohydrolase) and exo-PG (EC 3.2.1.67; poly(1,4)-α-D-galacturonide galacturonohydrolase). Endo-PG catalyzes the cleavage of α-(1–4) glycosidic bonds between two galacturonic acid residues within the pectin molecule resulting in pectin depolymerization. The preferred substrate for the action of PG is pectic acid, i.e., demethoxylated pectin, produced by the action of PME (Terefe et al. 2014). The rate and extent of hydrolysis are dependent on the degree of pectin esterification (Lamikanra 2002). Exo-PG catalyzes stepwise hydrolysis of galacturonic acid from the nonreducing end of the chain, liberating one galacturonic acid molecule at a time (Lamikanra 2002; Warriner and Zivanovic 2005).

PG is a cell wall-bound enzyme, which is present in many fruits and vegetables. It is also produced by pathogenic fungi and bacteria and plays a major role in plant pathogenesis (Terefe et al. 2014). Endo-PGs occur in fruit and filamentous fungi but not in yeast or bacteria. Exo-PGs occur in fungi, bacteria, and plants. In general, endo-PGs account for most of the polygalacturonase activity in ripe fruit, although both endo- and exo-PGs are active in some ripening fruits (Lamikanra 2002). There are many PG isoforms with variable substrate preference, specific activity, optimal pH, and stability. For example, PG in tomato juice exists as a mixture of two isoenzymes: PG1 and PG2 (Terefe et al. 2014). Endo-PGs appear to catalyze solubilization of pectins within the cells followed by further hydrolysis by exo-PGs. Some fruits such as pears and freestone peaches that soften markedly during ripening contain not only endo-PG but also exo-PG. Other fruits (e.g., apples and clingstone peaches) contain only exo-PG, consistent with slow softening characteristics. Low levels of exo-PG are found in many vegetative and storage tissues (Lamikanra 2002).

Most of the studies on the activity of PG are focused on the role of PG in ripening, postharvest senescence, and pathogenesis. PG is abundantly present in tomatoes. The majority of studies with respect to the effect of processing in general and emerging technologies in particular are on tomato PG, probably due to its commercial significance and the substantial impact of PG on the rheological characteristics of tomato-based products. The consistency of tomato products is highly dependent on pectic substances, which form a matrix in which other particles are suspended.

Following tomato crushing during processing, degradation of pectin by the synergistic action of PME and PG ensues, resulting in large decrease in viscosity over a short period of time (Terefe et al. 2014). PG activity results in a dramatic decrease of consistency of tomato products. To prevent these quality defects, PME and PG can be inactivated (Ludikhuyze et al. 2003). Early ripening tomato varieties also show higher PG and PME activity at all the stages, as compared to the late ripening varieties, where PG and PME activity increases during ripening. Unlike PG, PME is more commonly present in large amounts in unripe fruit (Lamikanra 2002).

Pectin Lyases and Pectate Lyases

There are two types of lyases: pectin lyase and pectate lyase. Pectin lyase (*trans*-eliminases) (EC 4.2.2.10) and pectate lyase (EC 4.2.2.2) cleave α-(1–4) glycosidic bonds in pectin and pectic acid, respectively. Both of them cleave α-(1–4) glycosidic bonds in pectin or pectic acid molecules, non-hydrolytically. This cleavage is carried out by *trans* elimination of hydrogen on fifth C of the galacturonic acid with the oxygen on the glycosidic bond. An unsaturated C=C bond is created between the 4- and 5-positions of the galacturonic acid residue at the nonreducing end of the fragment released. Pectin lyase depolymerizes highly esterified carboxyl pectin by splitting glycosidic linkages next to methyl-esterified carboxyl groups by β-elimination, while pectate lyase attacks glycosidic linkages next to a free carboxyl group (Lamikanra 2002). Pectin lyases act as endoenzymes, preferentially splitting highly esterified pectin, while pectate lyases act on nonesterified or low esterified pectate and exist in both endo and exo forms (Warriner and Zivanovic 2005). Lyases produce unsaturated monomers that rearrange to the 2-keto-uronic acid (Lamikanra 2002).

Lyases are almost exclusively from microorganisms (Lamikanra 2002). In general, these enzymes have not been found in plants. However, it is indicated in the literature that there are indications of their natural occurrence in some fruits (Lamikanra 2002; Warriner and Zivanovic 2005).

Peroxidases (POD)

Peroxidases (POD) catalyze the oxidation of cinnamic acids and tyrosine-containing cell wall proteins, which are involved in the oxidative cross-linking of cell wall polysaccharides (Terefe et al. 2014).

Amylases

The texture of fruits and vegetables can also be affected by starch degradation. Starch degradation is carried out by amylases. Starch is a storage polymer with no structural function. However, starch is accumulated in certain plant organs with high levels. Soluble sugars are accumulated as a result of starch hydrolysis, and

soluble sugars increase the osmotic pressure within the cells, therefore contributing to the turgor pressure. Most fruits such as banana, kiwifruit, and apple can accumulate significant levels of starch during development and undergo extensive starch hydrolysis during ripening. In contrast, starch levels in starchy vegetables such as potato remain high during postharvest storage although starch reserves undergo mobilization (Goulao et al. 2010).

Amylases are the starch degradation enzymes. They are the members of major enzyme class "hydrolases". Most of the plants produce amylases endogenously. There are four types of amylases which hydrolyze starch in different manner: α-amylase, β-amylase, glucoamylase, and pullulanase. α-Amylase and pullulanase are endoenzymes, while β-amylase and glucoamylase are exoenzymes. Generally, endoenzymes cause rapid loss in viscosity, while exoenzymes do not affect viscosity but increase sweetness (Warriner and Zivanovic 2005). α-Amylase is often referred to as "liquefying enzyme" due to the rapid loss in viscosity. As a result, α-amylase may participate in softening of fruits and vegetables in a way. Amylases may also cause desired changes in plants. For example, amylases in sweet potatoes assist in curing to give desirable texture and flavor (Underkofler 1975).

Polyphenol Oxidases (PPO)

Polyphenol oxidases (PPO) cause mainly enzymatic browning in fruits and vegetables in the presence of oxygen. Apart from color deterioration, browning reactions by PPO activity may bring along a slight softening of the plant tissue (Ludikhuyze et al. 2003).

4.2.2.2 Endogenous Enzymes Related to Flavor Changes

Flavor is an important quality parameter that determines the suitability of a food product for consumption. It is usually difficult to characterize the critical combination of compounds that contribute to the characteristic flavor and aroma of food products and identify the enzymes responsible for the biosynthesis of flavor compounds and off-flavor development (Whitaker 1996). Cut fruit and vegetable products rapidly lose their typical flavor, even when stored under refrigerated conditions (Dea et al. 2011).

Aroma Volatile Compounds in Fruits and Vegetables

Volatile compounds of fruits and vegetables are comprised of diverse classes of chemicals, including esters, alcohols, aldehydes, ketones, and terpenes. Some volatile compounds are only produced in certain fruit. It is indicated in the literature that more than 80% of the volatiles produced by ripe apple, strawberry, banana, and melon are esters. Most esters have sweet and fruity odors and relative low aroma

thresholds. Presently it is believed that the metabolism of fatty acids and branched amino acids may serve as precursors for the biosynthesis of aroma volatiles in most fruit. In contrast to most fruits, vegetables contain a large group of terpenes; these are important volatile compounds that play an important role in influencing the sensory qualities that are measured as taste and flavor. Production of flavor volatile compounds in fruits and vegetables is the result of complex metabolic networks involving many pathways and control mechanisms. The metabolomic diversity is also caused by low enzyme specificity and is directly related to the availability of substrates (Song 2010).

Major enzymes of flavor volatile production and off-flavor development in fresh fruits and vegetables are discussed in the following sections.

Lipoxygenase (LOX) and Hydroperoxide Lyase (HPL)

Activity of lipoxygenase (LOX) and hydroperoxide lyase (HPL) in fruits and vegetables is the most important feature in the development of flavor and off-flavor. LOX and HPL are involved in the "LOX pathway". Both of them are the key enzymes in this pathway. LOX pathway is a serial reaction including mainly LOX and HPL catalytic actions (Terefe et al. 2014).

Lipoxygenase (linoleate: oxygen reductase, E.C. 1.13.11.12) catalyzes the oxidation of polyunsaturated fatty acids into the corresponding hydroperoxides in the presence of molecular oxygen (Baysal and Demirdöven 2007; Song 2010). LOX catalyzes the addition of molecular oxygen at either the C9 or C13 residue of unsaturated fatty acids with a 1,4-pentadiene structure. Linoleic acid and linolenic acid are the most abundant fatty acids in the lipid fraction of plant membranes and are the major substrates for LOX to form fatty acid hydroperoxides (Lamikanra 2002; Baysal and Demirdöven 2007; Song 2010). Fatty acids are quantitatively the major precursors of volatile compounds responsible for the aroma of plant products. The phospholipase activity releases unsaturated fatty acids from the membrane that can serve as substrates for LOX. The fate of fatty acids in this enzyme system (LOX pathway) is determined by the substrates and specificity of LOX and HPL (Song 2010).

HPL catalyzes the cleavage of 13-hydroperoxy lipids into six carbon aldehydes. The 13-hydroperoxides of linoleic and linolenic acids selectively cleavage by HPL lyase to the formation of hexanal and hexenel, respectively. These C6 and C9 carbon aldehydes are considered to be the major volatile compounds in many fruits and vegetables when tissues are mechanically damaged, homogenized, or chewed. They contribute to the "green" and "fresh" flavor of fruits and vegetables. In many fruit tissues, however, those C6 and C9 volatiles cannot be detected in intact tissues, and they become the substrates for further flavor metabolism. Therefore, LOX can be seen as an enzyme responsible for secondary volatile generation that directly influences human flavor perception (Song 2010; Terefe et al. 2014).

LOX, an iron-containing dioxygenase, is found in a wide variety of plant and animal tissues (Ludikhuyze et al. 2003; Baysal and Demirdöven 2007). Highest activity is

found in leguminosae family plants such as soybean, peas, and green beans. Lower activities are observed in solanaceae (e.g., tomato, potato), cruciferae (e.g., cauliflower), and compositae (e.g., artichoke) (Pinsky et al. 1971). There are many isoenzymes of LOXs in plant tissue with different pH optimum, isoelectric point, and other properties (Terefe et al. 2014). LOX I converts linoleic acid preferentially to 13-hydroperoxide derivatives, and LOX 2 forms 9-hydroperoxide compounds, while LOX III yields a mixture of hydroperoxides. It is indicated in the literature that LOX types II and III, in the presence of fatty acids and dissolved oxygen, co-oxidize carotenoids and chlorophyll. Appearance of new LOX isoenzymes (LOX IV, V, and VI) in germinated soybean cotyledons was also reported (Lamikanra 2002).

HPL is a membrane-based/membrane-bound enzyme present in small amounts in plant tissue. HPL has been classified into three types on the basis of substrate specificity: 9-HPL, 13-HPL, and nonspecific HPL. The substrate specificity determines aroma composition of many plant products, despite the specific action of LOX (Lamikanra 2002).

Physiological Role of LOX Pathway (LOX and HPL Activities)

LOX pathway is one of the most studied enzyme system in fruits and vegetables. The physiological role of LOX pathway is not sufficiently understood although metabolites of unsaturated fatty acids have been implicated in growth and development, plant senescence, and responses to disease and wounding (Terefe et al. 2014).

Desirable Effects of LOX Pathway on the Quality of Fruits and Vegetables

It is well known that thousands of primary and secondary metabolites are produced during fruit ripening, which results from many metabolic pathways involving many enzymes. There are many enzymatic flavor changes occurring during ripening, senescence, and postharvest handling. LOX plays a major role in the formation of aromatic volatile flavor compounds in fruits and vegetables responsible for the "fresh" and "green" sensorial characteristic. Different fruits and vegetables have different volatile profiles resulting from the LOX pathway (Ludikhuyze et al. 2003; Song 2010; Terefe et al. 2014).

Detrimental Effects of LOX Pathway on the Quality of Fruits and Vegetables

Besides their contribution to the formation of many desirable fresh fruit and vegetable flavors, LOX pathway may cause some detrimental effects on minimally processed fruit and vegetable products especially during storage. Development of off-flavor, loss of nutritional quality, and loss of color are three important detrimental effects of LOX pathway activity.

Off-Flavor Development

Hydroperoxides produced by LOX activity may undergo further degradation to produce undesirable and odorous carbonyl compounds (Yemenicioğlu 2015). Vegetables such as green beans, green peas, corn, broccoli, and cauliflower develop off-flavor during frozen storage if they are not properly blanched. LOX is the main enzyme responsible for off-flavor development in green beans, green peas, and corn. Development of off-flavor often characterized as hay-like, which is due to further degradation of the hydroperoxides into volatile compounds such as aldehydes, ketones, and alcohols (Terefe et al. 2014). Such changes are mainly due to the loss of the principal flavor-related volatiles and the synthesis of stress-related off-flavor volatiles such as ethanol grassy, hay, painty, stale, and oxidized flavors have been detected as off-flavor due to LOX action (Ludikhuyze et al. 2003; Dea et al. 2011). Though the flavor compounds produced through the LOX pathway are essential, their concentration above the normal level results in off-flavor development (Terefe et al. 2014).

Peroxidase (POD)

As discussed in the previous sections, peroxidase (POD) activity may cause detrimental and desired effects on fruit and vegetable quality. The main detrimental effect of POD in fruit and vegetable quality is contribution to off-flavor formation, browning, and pigment bleaching. Most flavor changes in raw and unblanched fruits and vegetables could be correlated to POD activity, and there is an empirical relationship between residual peroxidase activity, off-flavors, and off-odors in foods. Changes that occur in POD activity in wounded and fresh-cut fruits and vegetables significantly contribute to their product quality (Lamikanra 2002; Yemenicioğlu 2015).

Alcohol Dehydrogenase (ADH)

Alcohol dehydrogenase (ADH; EC 1.1.1.1) is responsible for formation of aldehydes and alcohols (Song 2010).

As it is previously discussed, LOX produces C6 and C9 carbon aldehydes in fruit volatile production systems. These aldehyde volatiles are converted into their corresponding alcohols through the action of ADH (Terefe et al. 2014). It is well known that ADH converts aldehydes and alcohols back and forth depending on the condition of the tissue. The predominant role that ADH plays in the production of aldehydes and ethanol has been studied in fruits and vegetables under stress conditions such as high CO_2 or low oxygen (Song 2010).

Pyruvate Decarboxylase (PDC)

Pyruvate decarboxylase (PDC; EC 4.1.1.1) catalyzes the decarboxylation of pyruvic acid to acetaldehyde. It is one of the enzymes specially required for ethanol formation through fermentation (Song 2010).

It has been reported that acetaldehyde in fruit can be formed from pyruvic acid through the action of PDC, from fatty acids via the LOX pathway, or from ethanol through enzyme oxidation by ADH. Observation of volatile production and enzyme activity of LOX, PDC, and ADH from apple fruit maturing on the tree indicated that both LOX and HPL may act together to control emission of volatile compounds. The decrease of ethanol production throughout maturation was believed to be due to the diversion to acetaldehyde rather than ethyl acetate. It was reported that ADH activity increased at the beginning of fruit ripening and then decreased gradually in peel tissue and remained constant in flesh tissue. High concentrations of acetaldehyde found in fruit of advanced maturation was found to be related to ADH rather than PDC. Preliminary sensory evaluations indicated that fruit with elevated ADH activity and higher levels of alcohols were found to have a more intense "ripe fruit" flavor (Song 2010).

Alcohol Acetyl Transferase (AAT)

Acyl-CoA alcohol transferase (AAT; EC 2.3.1.84) catalyzes the combining of alcohols and acyl-CoAs to form esters. This enzyme is responsible for the final step of ester formation. AAT is a common endogenous enzyme existing in many fruits. Plants contain a large number of acyl transferases with approximately 88 found in *Arabidopsis* (a plant genus in Brassicaceae family) and more than 40 in rice. Physiology and biochemistry studies revealed that AAT is under control of fruit development and is regulated during fruit ripening. Other enzymes such as LOX, ADH, and PDC are believed to be involved in the pathways to provide aldehydes and alcohols for ester synthesis. However, the source of alcohols and aldehydes for ester synthesis in fruit is not fully understood (Song 2010).

Cystine Lyase and Alliinase

Cystine lyase and alliinase are included in the group of enzymes collectively called "C-S lyases". C-S lyases cleave alkylcysteines and alkyl-cysteine sulfoxides, which are the major constituents of the free amino acid pool of many vegetables of the family Cruciferae, Leguminosae, and Liliaceae. Cystine lyase (cystathionine *L*-homocysteine-lyase; EC 4.4.1.8) catalyzes the cleavage of *L*-cystine producing thiocysteine (cysteine persulfide), pyruvate, and ammonia through β-elimination reaction. Alliinase (cysteine sulfoxide lyase; C-S lyase, EC 4.4.1.4) hydrolyzes the nonprotein amino acid *S*-allyl-*L*-cysteine sulfoxide (alliin) into pyruvate, ammonia, and diallyl thiosulphinate (allicin) (Terefe et al. 2014).

Cystine lyase has been identified in several members of the *Brassica* genus vegetables such as cabbage, cauliflower, kale, mustard, turnip, and rutabaga (Ramirez and Whitaker 1999). This enzyme has been determined to be the principal causative agent for development of off-flavor in broccoli and cauliflower (Whitaker 1991). The activities of cystine lyase and C-S lyases were also reported to be responsible

for the formation of undesirable sulfur compounds such as methanethiol, dimethyl disulfide, and dimethyl trisulfide in disrupted tissues of *Brassica* genus vegetables such as cabbage and broccoli (Terefe et al. 2014).

Allicin, a product of alliinase activity, is the most important beneficial compound in garlic, which is responsible for its characteristic pungent flavor and its therapeutic and antimicrobial effects. Alliinase also catalyzes the hydrolysis of *S*-(1-propenyl)-*L*-cysteine sulfoxide (isoalliin) into di(1-propenyl) thiosulfinate, which gives onion its flavor (Terefe et al. 2014).

Myrosinase

Myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) catalyzes the hydrolysis of glucosinolates into β-glucose, sulfate, and a series of aglycons, which are further nonenzymatically degraded into nitrogen- or sulfur-containing compounds such as isothiocyanates, nitriles, and thiocyanates. Although the exact reaction mechanism has not yet been elucidated, the hydrolysis is suggested to occur due to cleavage of a β-thioglucosidic bond (Terefe et al. 2014).

Myrosinase is found in all glucosinolate-containing plants including cabbage, brussels sprout, radish, turnip, watercress, and mustard. They are important in human diets as vegetables or condiments. Several isozymes of myrosinases have been observed. Myrosinase has also been found in bacteria, fungi, insects, and mammals. However, less information is available regarding these non-plant myrosinase (Ludikhuyze et al. 2003; Terefe et al. 2014).

The secondary degradation products (isothiocyanates, thiocyanates, nitriles, thiones) of the glucosinolate hydrolysis reaction can contribute either positively or negatively to the characteristic properties of many plant species, especially cruciferae (Ludikhuyze et al. 2003). For example, the characteristic flavor of *Brassica* genus vegetables is due, in part, to the presence of volatile. Depending on the pH, the presence of ferrous ions, double bond in the side chains, and proteins such as epithiospecifier protein, further conversion of isothiocyanates and indoles into epthionitriles, nitriles, thiocyanates, and other compounds occur. On the other hand, high concentrations of these secondary degradation products can give rise to undesirable bitterness and too strong taste and flavor. Moreover, high intake levels of certain glucosinolate hydrolysis products are associated with toxic or goitrogenic effects (Ludikhuyze et al. 2003; Terefe et al. 2014).

Polyphenol Oxidases (PPO)

Polyphenol oxidase (PPO) activity cause mainly browning reactions in fruits and vegetables in the presence of oxygen. Apart from color deterioration, browning reactions by PPO activity may bring along unpleasant flavors and/or taste (Vámos-Vigyázó 1981; Martinez and Whitaker 1995).

β-Glucosidases

These enzymes are mainly involved in the activation of plant defense chemicals (phytoanticipins) and conjugates of plant growth regulators by hydrolyzing *β*-glycosidic bonds.

They also play a significant role in the release of many volatile compounds from their glycosidic precursors in fruits and vegetables. Therefore, they are also increasingly found in various food processing applications (Terefe et al. 2014).

Other Enzymes Related to Flavor

In addition to AAT, LOX, ADH, and PDC, there are some other enzymes with substrate specificity in various metabolic pathways, which are involved in fruit and vegetable volatile compound production. For example, various enzymes in the biosynthesis pathways of fatty acid, branched amino acid (isoleucine), sesquiterpene, and phenylpropanoid are responsible for ethylene-induced aroma volatile production. Deaminases, decarboxylases, transferases, dehydrogenases, oxidases, hydratases, thiolases, synthases, and acyl carrier proteins with different substrates are important enzyme groups involved in these biosynthesis pathways (Song 2010).

4.2.2.3 Endogenous Enzymes Related to Color Changes

Color is one of the main physical attributes of food products. It plays perhaps a more important role than any other quality parameter in consumer decision of initial purchase. Carotenes and carotenoids, anthocyanins, chlorophylls, and phenolic compounds are the main groups of pigments that are responsible for the characteristic colors of fruits and vegetables. In addition to imparting color to horticultural products, carotenoids, anthocyanins, and other phenolic compounds are potent antioxidants, which are thought to be beneficial to health (Terefe et al. 2014).

The main enzymes that are involved in biochemical degradation of plant pigments are polyphenol oxidase (PPO), chlorophyllase, peroxidase (POD), lipoxygenase (LOX), anthocyanase (anthocyanin-*β*-glucosidase), and alliinase. These enzymes can cause color change problems in minimally processed fruit and vegetable products. Especially, oxidative browning at the cut surface is the limiting factor in storage of many minimally processed fruits and vegetables (Brecht 1995; Terefe et al. 2014).

PPO, POD, anthocyanase, LOX, chlorophyllase, and alliinase are discussed in this section.

Polyphenol Oxidase (PPO)

Polyphenol oxidase (PPO; EC 1.14.18.1) is a group of copper-containing haloenzymes. PPO enzymes are the members of major enzyme class “oxidoreductases”. PPO catalyzes the oxidation of phenolic compounds into brown pigments at cut or

damaged surfaces of fruits and vegetables. PPO mainly causes the enzymatic browning of fruits and vegetables (Lamikanra 2002; Ludikhuyze et al. 2003).

These enzymes are found in many plants, animals including arthropods and mammals, fungi, and bacteria. Plant PPO is localized intracellularly as an active soluble form or inactive membrane-bound form. Soluble and active PPO fraction exists in the majority of the fruits and vegetables (Ludikhuyze et al. 2003; Terefe et al. 2014; Yemenicioğlu 2015). However, some products like mushroom, carrot, broad bean, pepper, spinach, and iceberg lettuce might contain also a considerable fraction of inactive latent form. This inactive PPO is located on the thylakoid membrane of chloroplasts as a membrane-bound protein, which could be activated by specific proteases or activators (Yemenicioğlu 2015).

It is indicated in the literature that the distribution of PPO in different parts of fruits and vegetables and ratios of particle-bound and soluble enzymes with maturity vary considerably. During ripening, the concentration of particulate enzymes decreased with the concurrent appearance of a soluble fraction. In cells of immature fruits and tissue culture, PPO was detected in organelles other than the vacuoles, presumably in plastids. In green leaves, PPO is predominantly located in the chloroplasts (Lamikanra 2002). Discoloration occurs at the cut surface of fruits and vegetables as a result of the disruption of compartmentation that occurs when cells are broken, allowing substrates and oxidases to come in contact. Wounding also induces synthesis of some enzymes involved in browning reactions or substrate biosynthesis (Brecht 1995).

There are various PPOs known by different names including tyrosinase, cresolase, catecholase or catechol oxidase, diphenolase, laccase, phloroglucinoloxidase, *p*-diphenol oxygen oxidoreductase (EC 1.10.3.1), diphenol oxygen oxidoreductase (EC 1.10.3.2), and phenolase (Lamikanra 2002; Terefe et al. 2014). The substrate of PPO may be monophenol or *o*-dihydroxyphenol derivatives such as tyrosine, cresol, catechol, caffeic acid, hydroxygallic acid, and protocatechuic acid. PPO acts on phenols in the presence of molecular oxygen. Enzymatic browning is a complex process that can be subdivided in two parts. The first part is mediated by PPO. After the enzymatic oxidation of phenolic compounds by PPO, *o*-quinones are produced. The *o*-quinones, which are yellow in color, are highly unstable and undergo nonenzymatic polymerization reactions in the second part. This nonenzymatic polymerization results in the formation of heterogeneous brown pigments or reddish-brown, blue-gray, and even black pigments commonly called melanins (Dea et al. 2011; Terefe et al. 2014; Yemenicioğlu 2015).

This reaction initiated by PPO is called enzymatic browning. Enzymatic browning causes loss of quality during processing of vegetables, fruits, mushrooms, and some crustacean species (Şimşek and Yemenicioğlu 2007). An example for enzymatic browning is illustrated in Fig. 4.1.

PPO catalyzes two quite different types of reactions involving phenolic compounds and molecular oxygen. Some PPOs such as cresolase and tyrosinase (EC 1.14.18.1) have monophenolase activity (monophenol monooxygenase). Some others such as catechol oxidase or diphenol oxygen oxidoreductase have diphenolase activity (diphenol oxidase). Monophenolase activity is the hydroxylation of monophenols to

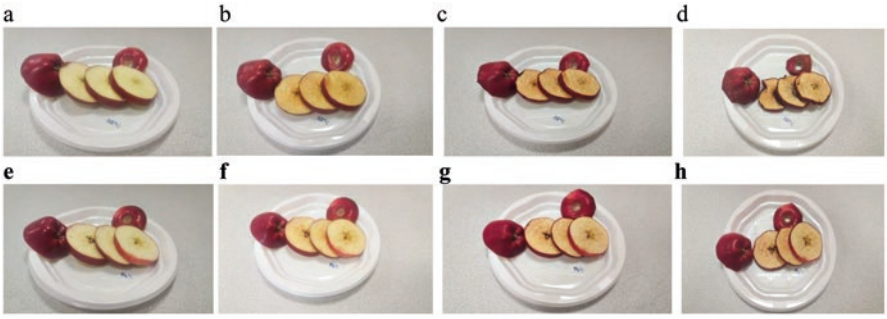

Fig. 4.1 Enzymatic browning of cut apple surface by PPO. (**a**) Initial time of storage at 25 °C. (**b**) After 1 h of storage at 25 °C. (**c**) After 1 day of storage at 25 °C. (**d**) After 2 days of storage at 25 °C. (**e**) Initial time of storage at 4 °C. (**f**) After 1 h of storage at 4 °C. (**g**) After 1 day of storage at 4 °C. (**h**) After 2 days of storage at 4 °C photographed by Ayhan Temiz and Dilay K. Ayhan in 2016

o-diphenols (Ludikhuyze et al. 2003; Terefe et al. 2014). Diphenolase activity is the oxidation of *o*-diphenols to *o*-quinones. Tyrosinase catalyzes hydroxylation of monophenols to *o*-diphenols (monophenolase activity) and oxidation of *o*-diphenols to *o*-quinones (diphenolase activity). Tyrosinase exhibits diphenolase activity as well as monophenolase activity. Cresolase, a monophenolase, catalyzes hydroxylation of monophenol to *o*-diphenols. This monophenolase activity is followed by catecholase activity. Catecholase, a diphenolase, catalyzes oxidation of *o*-phenols to *o*-quinones (Lamikanra 2002; Yemenicioğlu 2015). Laccase catalyzes oxidation of both *o*-diphenols and *p*-diphenols to corresponding quinones. Phloroglucinol oxidase catalyzes the oxidation of three hydroxyl phenols but does not act on diphenols (Yemenicioğlu 2015).

The PPO in vegetables is frequently a catechol oxidase. Tyrosinase is also found in some important vegetables. For example, the PPO in mushrooms and potatoes acts as a tyrosinase and shows both cresolase and catecholase activities. It is indicated in the literature that the PPO in some vegetables such as lettuce, artichoke, eggplant, green bean, and broccoli florets shows mainly catecholase activity and acts as a catechol oxidase. The vegetables might sometimes contain a labile bound fraction of PPO with cresolase activity. Laccase and phloroglucinol oxidase are not abundant in vegetables. However, it is indicated in the literature that the PPO from cauliflower was identified mainly as a phloroglucinol oxidase and laccase activity is detected in some mushrooms and tomatoes (Yemenicioğlu 2015).

Other Effects of Browning Reactions over Discoloration

Enzymatic browning is one of the most limiting factors on the shelf life of fresh-cut products. However, consequences of enzymatic browning are not restricted to discoloration. In addition to color deterioration, enzymatic browning may also cause undesirable flavors and nutrient loss in minimally processed fruit and vegetable products.

PPO action usually results in the formation of highly reactive quinones that can then react with amino and sulfhydryl groups of proteins and enzymes as well as with other substrates, such as chlorogenic acid derivatives and flavonoids (catechins, anthocyanins, leucoanthocyanidins, flavonols, and cinnamic acid derivatives). These secondary reactions may bring about changes in physical, chemical, and nutritional characteristics and may also affect the sensory properties of fruits and vegetables (Lamikanra 2002). Apart from color deterioration, the browning reaction may bring along unpleasant flavors and/or taste and a slight softening of the tissue (Vámos-Vigyázó 1981; Martinez and Whitaker 1995).

Desirable Changes of Enzymatic Browning

Enzymatic browning is not always deleterious to foods. In fact, enzymatic browning may be desirable for some fruits and vegetables. In some cases, such as the manufacture of tea and cocoa, enzymatic browning contributes to desirable color, flavor, and/or taste and forms an essential step in the production process (Ludikhuyze et al. 2003). In tea processing, the activity of oxidative enzymes is desired for enhancing a brown-black color by increasing the formation of theaflavin and thearubigin compounds and sometimes the processing of dried fruits such as dates and raisins. The activity of PPO also has an impact on the flavor and aroma of horticultural products, since phenolic compounds play a role in bitter, sweet, pungent, or astringent taste of products such as apple, grape, olives, paprika, ginger, and turmeric (Terefe et al. 2014).

Lipoxygenase (LOX)

LOX activity may be responsible for discoloration of plant pigments indirectly. The hydroperoxides and the free radicals are formed by LOX reactions. These radicals, intermediately formed in the LOX reaction, can damage pigments (e.g., chlorophyll, carotene and xanthophyll) or vitamins, resulting, respectively, in color changes and decreased nutritional value (Ludikhuyze et al. 2003; Terefe et al. 2014; Yemenicioğlu 2015). It is indicated that bleaching of plant pigments and loss of β-carotene and chlorophyll a have been shown to occur during LOX-mediated reactions (Dea et al. 2011).

Carotenoids are generally stable in their natural environment. Enzymes such as LOX cause the co-oxidation of carotenoids in the presence of free fatty acids through a free radical-mediated mechanism, which significantly affects the color intensity of foodstuffs. Besides LOX, PPO is also thought to be involved in the co-oxidation of carotenoids in the presence of polyphenols through a similar mechanism. It is indicated that these two reactions lead to the formation of different aroma compounds including ionones, which are found in many fruit flavors including blackberry, peach, and apricots (Terefe et al. 2014).

Peroxidase (POD)

In addition to off-flavor formation, POD is believed to be involved in browning and pigment bleaching of horticultural products (Terefe et al. 2014; Yemenicioğlu 2015).

Peroxidase (POD; EC 1.11.1.7) catalyzes the oxidation (single-electron oxidation) of phenolic compounds in the presence of hydrogen peroxide leading to the formation of brown degradation products. Hydrogen peroxide is essential in POD-catalyzed reactions. The low internal concentration of hydrogen peroxide in plants limits POD activity. Hydrogen peroxide is generated during the PPO-catalyzed oxidation of phenolic compounds. Therefore, the possible role of PPO as a promoter of POD activity is suggested (Terefe et al. 2014). The POD has a very low substrate specificity, and it can catalyze the oxidation of a wide range of organic compounds including fatty acids and phenolic compounds in the presence of hydrogen peroxide (Yemenicioğlu 2015).

PODs are widespread enzymes found in plants, animals, and microorganisms. The principal physiological function of POD is to control the level of peroxides to avoid excessive formation of radicals that are harmful to all living organisms. Therefore, POD is found in almost all living organisms. They are widely distributed in plants and seem to be normal components of most plant cells. Plant PODs contain ferriprotoporphyrin III (hematin) as the prosthetic group. Plant POD consists of a complex spectrum of isoenzymes occurring in both soluble and bound forms in fruits and vegetables (Lamikanra 2002; Terefe et al. 2014; Yemenicioğlu 2015).

Peroxidase-Catalyzed Browning

Although the PPO is the primary enzyme responsible from the enzymatic browning in fruits and vegetables, the roles of POD in enzymatic browning are still questioned. The ability of POD to contribute to enzymatic browning is related to its affinity to accept a wide range of hydrogen donors, such as polyphenols. Two possible mechanisms are proposed for POD-catalyzed browning reactions. One involves the generation of H_2O_2 during the oxidation of some phenolic compounds that is used as in a normal peroxidatic action to further oxidize the phenol, while the second involves the use of quinonic forms as substrate by POD. Both mechanisms indicate that the presence of polyphenol oxidase enzyme would enhance POD-mediated browning reactions (Lamikanra 2002; Yemenicioğlu 2015).

Phenylalanine Ammonia-Lyase (PAL) and Its Importance for PPO and POD Activities

Phenylalanine ammonia lyase (PAL) activity may be important for PPO and POD activities. Some metabolic products including various phenolic compounds and possibly other substrates (e.g., anthocyanins) are obtained by "phenylpropanoid metabolism". PAL catalyzes the rate-limiting step in phenylpropanoid metabolism that produces phenolase-catalyzed oxidizable substrates. Wounding apparently first induces an increase in PAL activity and, consequently, an increase in oxidizable substrates. Both wounding and ethylene production induce PAL activity in many plant tissues. Browning occurs when the phenolics are oxidized in the reactions catalyzed by PPO and peroxidases POD (Brecht 1995; Lamikanra 2002). The phenolics can be oxidized by PPO and POD to quinones that ultimately polymerize to produce the

browning appearance common to wounded lettuce. Such increase in enzymatic activity as a result of the fresh-cutting process has been reported for several vegetables (Dea et al. 2011).

Anthocyanase (Anthocyanin-β-Glucosidase)

The enzyme anthocyanase (anthocyanin-β-glucosidase) is a specific β-glucosidase. Anthocyanase catalyzes the hydrolysis of sugar moieties from anthocyanins yielding highly unstable anthocyanidins. Anthocyanidins are further oxidized by PPO and POD or react with *o*-quinones, the highly reactive PPO oxidation products, to form melanins. Anthocyanins are not good substrates of PPO due to their structure. The sugar moiety of anthocyanins is thought to be a steric hindrance against PPO attack. The main agent responsible for enzymatic browning in fruits and vegetables is PPO. However, it is indicated that a possible synergistic effect between PPO and POD cannot be excluded. The studies on enzymatic browning showed that there is a high correlation between anthocyanin degradation and browning discoloration during postharvest storage with increased POD activity. The activity of anthocyanase needs to be controlled during postharvest storage and processing of anthocyanin-rich products such as berry fruits (Terefe et al. 2014).

Chlorophyllase

Chlorophyllase (Chlase, E.C. 3.1.1.14) catalyzes the hydrolysis of chlorophyll into chlorophyllide and phytol, which is the first step in the biochemical degradation of chlorophyll. Chlorophyllase is a glycoprotein, which is located in the plastid envelope of green plants. Chlorophyllase has been found to be the rate-limiting enzyme in chlorophyll catabolism during ripening, senescence, seasonal changes, as well as natural turnover. Chlorophyll degradation during processing and postharvest storage of fruits and vegetables causes a change in color from brilliant green to olive brown in processed foods and to yellow, brown, or colorless in senescent tissue (Terefe et al. 2014).

In green vegetables, the senescence process usually leads to a yellow coloration of the tissues, normally considered the major consequence of chlorophyll degradation. Pheophytin is an olive-colored pigment in vegetables. In some minimally processed green vegetables, the synthesis of pheophytin appears when the chlorophyll loses its bond with the magnesium atom and substitutes it with a hydrogen atom (Dea et al. 2011).

The biochemical pathway for the degradation of chlorophyll and loss of green color has not been well characterized. Up to 20 enzymes including chlorophyllase are thought to be involved in the series of reactions that transforms chlorophyll into a number of colorless catabolites. In general, the degradation pathway of chlorophyll depends on the type of products with intrinsic and extrinsic factors playing a role as well as the activities of chlorophyll-degrading enzymes (Terefe et al. 2014). For example, chlorophyll degradation in broccoli has been partly attributed to the

activities of chlorophyll degrading POD and chlorophyll oxidase as well as chlorophyllase. Increased POD activity has been correlated with chlorophyll degradation in broccoli (Funamoto et al. 2002; Funamoto et al. 2003).

Alliinase

As it is previously mentioned, allicin is a product of alliinase activity. Allicin is the most important beneficial flavor compound in garlic. Similarly, di(1-propenyl) thiosulfinate, a hydrolysis product of alliinase activity, gives onion its flavor. It is believed that this enzyme is also involved in the green color discoloration of processed garlic products as well as pink discoloration of onion. The relative effect of each enzyme depends on the product. There are two steps in both discoloration cases. In the first step, alliinase acts on alk(en)yl-*L*-cysteine sulfoxides to produce soluble organosulfur compounds called "color developers". Color developers then react nonenzymatically with amino acids and carbonyl compounds to form pigments (Terefe et al. 2014).

4.2.2.4 Endogenous Enzymes Related to Nutritional Quality

The initial nutritional value of a fresh-cut product can only be as good as its whole counterpart. Antioxidants such as ascorbic acid, lycopene, β-carotene, and phenolics as well as essential amino acids and fatty acids and some vitamins are of great interest regarding the nutritional quality of fruits and vegetables (Dea et al. 2011).

The amount of these nutritionally important compounds in minimally processed fruits and vegetables may change during storage period. Unfavorable environmental factors such as pH, temperature, UV lights, or oxygen as well as various endogenous and microbial enzymes may cause decrease in the amounts of essential nutrient of minimally processed fruits and vegetables, resulting in nutritional quality loss in minimally processed fruits and vegetables. For example, the compound ascorbic acid is easily oxidized during minimal processing. Ascorbic acid content in fresh-cut products may decrease or increase.

Certain enzymatic spoilage product(s) in minimally processed fruits and vegetables may also cause nutritional quality loss in minimally processed fruits and vegetables. For example, the activity of PPO, POD, and anthocyanase cause degradation of polyphenols including anthocyanins that are important phytochemicals. Enzymatic browning caused by mainly PPO as well as POD and anthocyanase results in the decrease of the available lysine content of proteins (Terefe et al. 2014).

Some nutritional loss is expected during the shelf life of fresh-cut products. However, it was indicated that the visual quality of several fresh-cut fruits (i.e., strawberry, persimmon, peach, papaya, mango, strawberry, pineapple, kiwifruit, cantaloupe, and watermelon) could be appreciably reduced before any significant nutrient decrease has occurred (Dea et al. 2011).

On the other hand, vitamin, mineral, and enzyme content of the raw ripened fruits and vegetables is adequate and desirable from a nutritional point of view. It is believed

that digestive enzymes in whole and minimally processed fruits and vegetables support the digestion of proteins, starch, and lipids in the human body. All raw, uncooked foods contain the exact types and amounts of enzymes necessary for the digestion (breakdown) of polysaccharides, proteins, and lipids. Amylases, cellulases, lipases, and proteases are the important digestive enzymes in raw fruits and vegetables. All the above, except cellulases, are produced in the human body. Digestive enzymes have only three main functions in the human body. Proteases are enzymes that digest proteins, amylases digest polysaccharides, and lipases digest lipids. Undigested food cannot adequately nourish cells. Digestive enzymes from raw food start food digestion in the stomach so that contents reaching the duodenum are easily broken down further, assimilated, and absorbed. As a result, the nutrients from food will be better digested, transported, and utilized, and waste will be more easily eliminated. When food is more completely digested, the body gets the nutrients rather than the pathogenic organisms (Howell 1985; Rojek 2003, 2004).

Relatively little information is available on the enzymes that affect the nutritional quality of horticultural products. Major enzymes responsible for changes in nutritional quality are discussed in the following paragraphs.

Polyphenol Oxidases (PPO)

Nutritional quality of fruits and vegetables may be impaired in several ways by the activity of polyphenol oxidases (PPO). PPO action usually results in the formation of highly reactive quinones. These enzymatically generated quinones can react with amino acids, peptides, and proteins or mediate oxidation of amino acids cysteine, methionine, and tryptophan. A reduction of only a small amount of essential amino acids may result in a marked reduction of nutritional value (Lamikanra 2002; Ludikhuyze et al. 2003). PPO-caused browning decreases the available lysine content of proteins (Terefe et al. 2014). The *o*-benzoquinone, the product of enzymatic browning reactions, may combine with the ε-amino groups of lysine residue in the plant proteins. Lysine in this combination is nutritionally unavailable. It is thought that blocking of free ε-amino group of lysine may cause decrease in biological value (ratio of retained and resorbed nitrogen) and in digestibility of proteins (Horigome and Kandatsu 1968). In addition to its negative effect on phenolic antioxidants, PPO is also believed to be involved in the oxidative degradation of ascorbic acid. The nutritional value can be impaired by quinone-mediated coupled oxidation of ascorbic acid (Ludikhuyze et al. 2003; Terefe et al. 2014).

Anthocyanase (Anthocyanin-β-Glucosidase)

As it is previously indicated, the activity of PPO, POD, and anthocyanase cause degradation of polyphenols including anthocyanins that are important phytochemicals. Enzymatic browning caused by mainly PPO as well as POD and anthocyanase results in the decrease of the available lysine content of proteins (Terefe et al. 2014).

Lipoxygenase (LOX)

As discussed previously, the hydroperoxides and the free radicals are formed by LOX reactions. The LOX-catalyzed oxidation of unsaturated fatty acids, which involves the formation of free radical intermediates, may have detrimental effects on nutritional quality of minimally processed fruits and vegetables.

The free radicals formed during LOX-catalyzed oxidation of polyunsaturated fatty acids can damage vitamins. The free radicals cause oxidation of carotenoids (vitamin A precursors as well as potent antioxidants), tocopherols (vitamin E), ascorbic acid, and folate. The free radicals can also damage cysteine, tyrosine, tryptophan, and histidine residues of proteins. Interaction with essential amino acids lowers the protein quality and functionality. Besides, the activity of LOX causes decrease in the amounts of essential fatty acids (linoleic acid and linolenic acid) or arachidonic acid, a conditionally essential fatty acid (Ludikhuyze et al. 2003; Terefe et al. 2014).

Ascorbic Acid Oxidase

Ascorbic acid oxidase causes degradation of ascorbic acid in vegetables and fruits. Ascorbic acid oxidase catalyzes the transformation of ascorbic acid into dehydroascorbic acid. Dehydroascorbic acid has the same physiological efficiency as ascorbic acid. Further degradation of dehydroascorbic acid into diketogluconic acid, oxalic acid, and other nutritionally inactive compounds may occur. However, this was reported to occur only at high temperature (Terefe et al. 2014).

Myrosinase

As it is previously discussed, the secondary degradation products (isothiocyanates, thiocyanates, nitriles, thiones) of the glucosinolate hydrolysis reaction of myrosinase can contribute either positively or negatively to the characteristic properties of many plant species, especially cruciferae. Myrosinase also enhances the nutritional quality of *Brassica* genus vegetables as it converts glucosinolates into the biologically active forms such as thiocyanates and isothiocyanates. Besides, myrosinase has positive effect on human health. It has been found recently that hydrolysis products of indole glucosinolates can induce anticarcinogenic properties, i.e., a reduced risk on tumor formation and proliferation (Ludikhuyze et al. 2003; Terefe et al. 2014).

Thiaminase

Thiaminase causes the degradation of thiamine. This enzyme catalyzes the replacement of the thiazole moiety of thiamine with a variety of nucleophiles. Thiamine is an essential cofactor in amino acid metabolism. The activity of this enzyme has been observed in marine organisms, silk worm, bacteria, and plants. Ingestion of a

significant quantity of thiaminase may lead to a serious thiamine deficiency even when there is sufficient amount of thiamine in the diet. However, the occurrence of the enzyme is limited to certain plants that are not normally consumed by humans except a few like *Marsilea drummondii* that is consumed cooked by Australian Aborigines and fishes that are commonly eaten after cooking and the enzyme is inactivated (Terefe et al. 2014).

4.3 Microbial Enzymes

Minimally processed fruits and vegetables retain much of their indigenous microflora after minimal processing. Mechanical damages, such as cutting, may increase susceptibility to decay and growth of microorganisms. Some operations such as washing can reduce the microbial load; however, they may also help to distribute spoilage microorganisms and moisten surfaces enough to permit growth of microorganisms during holding periods (Frazier and Westhoff 1978).

Microorganisms in minimally processed fruits and vegetables may affect food quality and human health. Pathogenic microorganisms and saprophytic ones may form part of this flora, possessing a potential safety problem and food quality problem. Saprophytic microorganisms may cause various spoilages in minimally processed fruits and vegetables. Microorganisms may degrade the sensory quality by affecting the appearance, cause off-odor/off-flavor, and, to a lesser extent, cause texture loss and nutritive value loss. Pathogenic or toxigenic microorganisms may cause infectious diseases or intoxications in man, and therefore they may produce some important health problems. When product is consumed raw, as is the case with fresh cuts, harmful microorganisms and/or their harmful products such as toxins may be present and ingested (Francis et al. 1999; Martinez et al. 2000; Rajkowski and Baldwin 2003; Dea et al. 2011).

The main enzymes responsible for quality degradation may also be of microbial origin. The types and numbers of microorganisms and their enzymes are important for the quality of minimally processed fruits and vegetables and human health.

4.3.1 Suitability of Minimally Processed Fruits and Vegetables Products for Microorganisms

Minimally processed fruits and vegetables deteriorate faster than its intact counterpart. Processing operations such as cutting, shredding, and slicing not only provide opportunities for contamination but also cause damage to fruit and vegetable tissues and cellular structure, leading to leakage of nutrients and cellular fluids (Heard 2002; Rajkowski and Baldwin 2003). In most cases, cut surfaces of any processed V and F are ideal for the growth of microorganisms, including human pathogens. Cutting destroys the internal cell compartment and creates wounds on the plant organs.

The wounded tissue releases plant juice or cell contents that serve as nutrients for microorganisms. A larger food source for microorganisms is plant biopolymers. Some of the microorganisms produce pectinolytic enzymes degrading texture and as such provide more nutrients for microbiological activity (Chen 2002; Ragaert et al. 2010).

Minimally processed fruits and vegetables are very susceptible to microbial spoilage due to their high water activity, the presence of nutrients at the cut surface, and the absence of preservative processes known to delay undesirable biological and biochemical changes, such as bleaching, freezing, or sterilization (Dea et al. 2011).

On the basis of nutrient content, fruits and vegetables can be a good source of nutrients for bacteria, yeast, and mold growth (Ayala-Zavala and González-Aguilar 2010). However, in general, less contamination is reported on fruits than on vegetables. This may be due partly to the lower pH of most fruits in comparison to vegetables (Leverentz et al. 2002). For most fruits (e.g., strawberries, raspberries, and nectarines), due to their low pH, the natural microflora is restricted to acid-tolerant microorganisms, such as fungi and lactic acid bacteria. The most significant spoilage microorganisms of fruits are fungi, mainly molds. On the contrary, since pH of vegetables is near neutrality, bacteria cause deterioration. Bacterial growth can be detected on the more pH-neutral vegetables (Ragaert et al. 2010). Because tomatoes have a lower pH, spoilage is similar to that of fruits, although bacterial spoilage also occurs (Banwart 1989). Fresh vegetables are all subject to bacterial soft rots. The rotting process could be as short as 3–5 days under favorable conditions. Through wounds, the soft rot bacteria enter plants and multiply quickly in the intercellular spaces. They produce a set of enzymes such as pectinases, cellulases, and proteases, dissolve the middle lamella, and separate the cells. This causes maceration and softening of the affected tissues (Chen 2002). The delicate balance of flavors in fruits could be more severely affected by the growth of lactic acid bacteria than vegetables, and this might contribute to the relatively rapid flavor loss in minimally processed fruits (Lamikanra et al. 2000). Typically, under conditions that do not favor bacterial growth (e.g., high acidity), fungi can proliferate with visible deleterious effects (Warriner and Zivanovic 2005).

4.3.2 Contamination Sources and Microbial Flora

Microbial contamination of agricultural products occurs at every stage of the production chain, from cultivation to processing. Raw fruits and vegetables have a naturally occurring microflora. Natural microflora of fruit and vegetable may be originated from the growing environment. During growth, the fruit or vegetable can become contaminated from different sources such as soil, water, air, animals, birds, and insects (Chen 2002; Rajkowski and Baldwin 2003; Dea et al. 2011). The microbial flora and load can further be enhanced by the different processing methods, such as handling, transporting, washing, peeling, cutting, shredding, slicing, grating, packaging, shipping, storage, and marketing. Poor sanitation of the processing facilities, dirty equipment, work surfaces, wash water and reuse of wash water or ice,

workers and inadequate personal hygiene among employees, packing materials, process equipment, and transportation vehicles are also potential sources of contamination (Oliveira et al. 2015a). During distribution and storage, temperature fluctuations and the high humidity present in packages provide a favorable environment and incubation time for proliferation of spoilage microorganisms and microorganisms of public health significance (Rajkowski and Baldwin 2003; Dea et al. 2011).

4.3.3 Counts of Microorganisms

Microbial spoilage appears to be one of the major causes of quality loss of fresh-cut products by formation of off-flavor, fermented aroma, and tissue decay. The shelf life of many food products may be accurately predicted by quantifying the population of microorganisms present on the food product (Zhuang et al. 2002). As it is known, initial microbial load dramatically influence the rate of the deteriorative effects of microorganisms and their enzyme activities on the products. The presence of high microbial populations leads to shortened shelf life. The lower initial microbial counts on the products generally do not produce big problems in product quality and its shelf life. Microbiological spoilage symptoms appear as microbial numbers increase, and as a result, quality is reduced and shelf life shortened. However, the degree of spoilage does not always correlate with high microbial populations (Heard 2002).

In general, total microorganism plate counts on minimally processed fruits and vegetables can range from 2 to 9 log colony forming units per gram (CFU g^{-1}), depending on the produce variety and time of year and geographic location It is indicated that fresh-cut vegetables harbor large and diverse populations of microorganisms, and counts of 10^5–10^7 CFU g^{-1} are frequently present (Francis et al. 1999; Leverentz et al. 2002). In minimally processed fruit and vegetable products, minimum detection levels of microorganism based on visual observation of spoilage may vary depending on the microorganism and type of product (Dea et al. 2011). The detection of off-odors or obvious visual defects on fresh-cut vegetables is often accompanied by a bacterial count exceeding 8 log CFU g^{-1} or a yeast count exceeding 5 log CFU g^{-1} (Ragaert et al. 2007). Production of organic acids on shredded mixed bell peppers and grated celeriac was detected when the psychrotrophic count exceeded 8 log CFU g^{-1}, dominated by lactic acid bacteria with the counts of 7–8 log CFU g^{-1} (Ragaert et al. 2010).

4.3.4 Common Microorganisms Found in Minimally Processed Fruits and Vegetables

The normal microflora found on the surface of products consists of large and diverse populations of microorganisms, including bacteria, yeasts, and molds (Leverentz et al. 2002; Garcia and Barrett 2004). Bacteria are predominant microorganisms on fruits and vegetables. Of these, 80–90% of bacteria are Gram-negative rods,

predominantly *Pseudomonas*, *Enterobacter*, or *Erwinia* species (Leverentz et al. 2002). The commonly encountered microflora of fruits and vegetables are *Pseudomonas* spp. (especially *P. fluorescens*) and *Erwinia* spp. (*E. herbicola*, *E. carotovora* and *E. agglomerans*), some species of lactic acid bacteria belonging to the genera of *Leuconostoc* (especially *Leuconostoc mesenteroides*) and *Lactobacillus*, and several species of yeast and molds (Heard 2002; Ayala-Zavala et al. 2009; Ayala-Zavala and González-Aguilar 2010; Ragaert et al. 2010; Dea et al. 2011). Molds and yeasts are often present on raw fruits and vegetables but in relatively lower numbers than those of bacteria. Yeasts and lactic acid bacteria are common microflora on fruits (Ahvenainen 1996; Martinez et al. 2000; Chen 2002).

In the case of fresh-cut fruits, intrinsic properties favor growth of yeasts, molds, and, in some cases, lactic acid bacteria mainly due to the lower pH value compared to vegetables (Ragaert et al. 2010). Among the deteriorative microflora, fungi are the most important microorganisms causing wastage of fresh-cut fruit, where the relatively acid conditions tend to suppress bacterial growth (Frazier and Westhoff 1978). On the contrary, bacterial infections are more common in vegetables due to their high pH. The major microbial concerns related to minimally processed fruit and vegetable products are mesophilic and psychrotrophic microorganisms affecting product shelf life and human health (Ayala-Zavala et al. 2009; Dea et al. 2011). Epidemiological surveys indicated that fresh-cut fruits and vegetables could also harbor pathogenic bacteria such as *Listeria monocytogenes*, *Salmonella* spp., and *Escherichia coli* O157:H7 (Oliveira et al. 2015a, b).

The most commonly isolated mold genera from vegetables are *Aureobasidium*, *Fusarium*, *Mucor*, *Phoma*, *Rhizopus*, *Botrytis*, *Cladosporium*, *Penicillium*, *Alternaria*, and *Aspergillus* (Banwart 1989; Jay et al. 2005). A wide range of mold species can be present on fruits. Molds reported on berries are *Botrytis cinerea*, *Rhizopus stolonifer*, *Mucor piriformis*, *Rhizoctonia solani*, and *Phytophtora cactorum*. Overripe or damaged berries can be invaded by *Penicillium* and *Cladosporium* species. However, on intact strawberry and raspberry fruit, bacterial survival is possible and even growth on the calyx of strawberries (Ragaert et al. 2010). Yeasts which are commonly isolated from fresh-cut vegetables include *Candida* spp., *Cryptococcus* spp. (particularly *Cryptococcus laurentii*), *Rhodotorula* spp., *Trichosporon* spp., *Pichia* spp., and *Torulaspora* spp. Yeasts which are commonly isolated from fruits include *Pichia* spp., *Rhodotorula* spp., *Candida* spp. (such as *Candida pulcherrima*, *C. lambica*, and *C. sake*), and *Debaryomyces polymorphus*. The more fermentative species, *Saccharomyces exiguus*, *S. cerevisiae*, and *S. dairensis*, occur mainly in salads dressed with mayonnaise (Heard 2002; Ragaert et al. 2010).

4.3.5 Microbial Spoilage and Safety of Minimally Processed Fruits and Vegetables

Microbial growth and activities can seriously limit the shelf life and safety of minimally processed fruits and vegetables. Microorganisms can cause quality loss of minimally processed fruit and vegetable products by formation of off-odors and off-flavor,

brown discoloration, fermented aroma, and loss of texture, deteriorated/rotten and moldy appearance, and loss or decrease of nutritive value.

Formation of off-odors and off-flavors is mainly caused by the activity of lipolytic and proteolytic enzymes and fermentation reactions. Loss of texture and soft rot is mainly caused by enzymatic degradation of the plant cell wall by pectinolytic enzymes. Brown discoloration is mainly caused by polyphenol oxidase (POD) activity of the microflora. Fermentative spoilage is caused by fermentation of carbohydrates to produce mainly acids, gases, and alcohols (Heard 2002). For example, lactic acid bacteria (LAB) are normal flora of fruits and vegetables and associated with spoilage organisms, causing unpleasant odors (Fleet 1992). Fruit products undergo fermentative spoilage by LAB or yeasts, resulting in the production of organic acids such as lactic acid and acetic acid, ethanol and CO_2, and volatile esters. However, pseudomonads may spoil less acidic fruits such as cantaloupe. Lipase activity and utilization of amino acids can alter the flavor of fruits, resulting in a loss of quality (Heard 2002; Arvanitoyannis and Bouletis 2012). Yeasts and molds are present in smaller numbers than bacteria but, when present in high numbers, can contribute to spoilage of fermented products and the development of soft rot (Fleet 1992).

Environmental factors, the types of fruits or vegetables, and the types of organisms present determine what type of spoilage occurs and how quickly the quality of the product deteriorates. Composition of fruits and vegetables can also determine the type of spoilage. Yeasts and lactic acid bacteria often use simple sugars. Therefore, the growth of yeasts and lactic acid bacteria is favored in sugar-rich fruits and vegetables such as carrots, bell peppers, and most of the fresh-cut fruits. Fermentative spoilage usually as off-odors occurs due to the microbial proliferation and the production of organic acids such as lactic acid, acetic acid, malic acid, succinic acid, and pyruvic acid as well as alcohol and CO_2 (Chen 2002; Heard 2002; Ragaert et al. 2010). On the cut surface of fresh-cut products, yeasts and lactic acid bacteria grow faster and often precede molds in the spoilage process (Chen 2002).

4.3.6 Role and Function of Microbial Enzymes in Microbial Spoilages

Microbial spoilages in minimally processed fruits and vegetables generally occur from microbial proliferation and enzyme activities. Enzyme activities are the major agent in the case of microbial spoilages. Each microorganism has a characteristic enzyme diversity determined by its genetics. Some microbial metabolites, which are derived from secondary metabolism, may also have negative effects on sensory quality of products. Common examples of secondary metabolism include volatiles, antibiotics, toxins, and siderophores (Warriner and Zivanovic 2005).

The enzymatic activity of microorganisms may have adverse effects on visual appearance and sensory properties of vegetables or fruits. However, it must be noted that many of the spoilage enzymes of microorganisms are also endogenous to plants.

A wide range of enzymes such as pectinases, cellulases, amylases, lipases, and proteases are known to be involved in the degradation of plant cell polymers. These enzymes are found in the plants endogenously, and they may be microbial in origin. For example, pectic enzymes and cellulase degrade pectic substances resulting in softening in fruits and vegetables. Most of the isolated microorganisms from minimally processed fruit and vegetable products are pectinolytic. These microorganisms can cause mainly soft rots of fruits and vegetables by their pectic enzymes. Polymeric substances of plants such as starch and pectin are firstly degraded into transportable units in order to be metabolized by the microbial cell. Many microorganisms isolated from products are known to produce extracellular enzymes. Microorganisms release extracellular enzymes to attack the plant cell walls, and some other cell organelles. With this background activity, it can be difficult to assess the significance of microbial enzymes in degradation reactions. However, microbial enzymes do play a key role in surmounting plant defenses and/or gaining access to nutrients (Chen 2002; Warriner and Zivanovic 2005).

Wounds on the plant host are required for penetration of the soft rot microorganisms. The plant pathogenic microorganisms feed and multiply on the plant juice from the wound surface. The production of large amounts of pectinolytic enzymes leads to further maceration of the tissues. Microorganisms continue to multiply and advance in the intercellular spaces, while the surrounding plant cells plasmolyze, collapse, and die. The invaded tissues soon become soft with the appearance of a slimy mass consisting of innumerable microorganisms swimming about in the liquefied substances (Chen 2002).

Major microbial enzymes and their importance for fruits and vegetables quality are summarized in Table 4.2.

4.3.6.1 Pectinases

Microorganisms do not contain pectins in their cell walls regardless of the species. However, in the absence of other carbon sources, microorganisms, both saprophytes and parasites, may hydrolyze pectins and utilize them as nutrients. Furthermore, enzymatic degradation of plant cell walls is the main pathway of attack by plant pathogens (Warriner and Zivanovic 2005).

Production of pectinolytic enzymes by microorganisms is known to be both constitutive and inductive. For plant pathogenic bacteria, the extracellular pectinases are regulated by the availability of the pectin polymer and the release of galacturonan units (Chen 2002).

Pectin methylesterase (PME) is a common enzyme in plants and microorganisms. However, plant PMEs remove methyl groups in blocks, leaving “pouches” of deesterified galacturonic acid residues, while fungal PMEs cause random deesterification, resulting in random distribution of unmethylated galacturonic acids along the pectin chain. Plant (endogenous) endo-polygalacturonases (endo-PGs) are responsible for softening during fruit maturation, while fungal growth on fruits and vegetables results in localized softening and spoilage due to rapid mercerization and

Table 4.2 Major microbial enzymes and their importance for fruits and vegetables quality

Enzyme/enzyme group	Microorganism/microorganism group	Fruits/vegetables	Effect type/spoilage type
Pectic enzymes (pectinases): Pectin methylesterase Polygalacturonase (exo type) Pectin lyase (endo type) Pectate lyases (endo and exo types) (usually) (Some pectinases are intracellular, others are extracellular)	*Erwinia* spp. *E. carotovora* subsp. *carotovora* *E. carotovora* subsp. *atroseptica* *E. chrysanthemi*	Vegetables and fruits Usually vegetables such as potatoes, climate crop, tropical and subtropical crops	Cell wall-degrading enzymes Soft rot
Pectic enzymes (pectinases): Polygalacturonase (endo type) Pectate lyases Pectate lyases and polygalacturonase (Extracellular)	*Pseudomonas* spp. Non-fluorescent spp. *P. cepacia* *P. caryophylli* *P. gladioli* Fluorescent spp. *P. marginalis* *P. fluorescens* B	Vegetables and fruits	Cell wall-degrading enzymes Soft rot
Pectic enzymes (pectinases): Endopectate lyase (usually) Polygalacturonase (exo type) Pectate lyase and polygalacturonase (exo type) Pectate lyase (Intracellular)	*Bacillus* spp. (such as *Bacillus subtilis*) *Xanthomonas* spp. *Clostridium* spp. (such as *Clostridium thermosaccharolyticum*) Some human pathogens: *Yersinia* spp. *Klebsiella pneumoniae*	Vegetables and fruits	Cell wall-degrading enzymes Soft rot They are used for growth and survival of microorganisms
Pectic enzymes (pectinases): Polygalacturonases Pectin esterases	Lactic acid bacteria (LAB)	Vegetables and fruits	LAB are not necessarily destructive to plant tissue

Pectic enzymes (pectinases): Polygalacturonases (mostly) Pectin methylesterase (mostly) Pectin lyases (mostly) (Extracellular or intracellular)	Most molds *Aspergillus* spp. *Cercospora* spp. *Fusarium* spp. *Penicillium* spp. *Rhizopus* spp. *Rhizoctonia* spp. *Trichoderma* spp. *Botrytis* spp. *such as B. cinerea* *Colletorichum* spp. *Byssochlamys* spp. *such as B. fulva*	Mainly fruits also vegetables	Cell wall-degrading enzymes Soft rot
Pectic enzymes (pectinases): Endopolygalacturonases (Mainly)	Some yeasts *Candida* spp. *Cryptococcus* spp. *Kluyveromyces* spp. *Pichia* spp. *Saccharomyces* spp. such as *S. fragilis* *Zygosaccharomyces* spp. *Fabospora* spp.	Vegetables and fruits	Not actively attacking plant tissue
Cellulases (Extracellular)	*Erwinia* spp. *E. chrysanthemi*	Vegetables and fruits	Cell wall-degrading enzymes Soft rot
Cellulases and xylanases	*Pseudomonas* spp. (a few species)	Vegetables and fruits	Cell wall-degrading enzymes Soft rot
Cellulases and hemicellulases: Endoglucanases (mainly)	Cellulolytic bacteria *Bacillus* spp. and *Pseudomonas* spp.	Vegetables and fruits	Cell wall-degrading enzymes Soft rot

(continued)

Table 4.2 (continued)

Enzyme/enzyme group	Microorganism/microorganism group	Fruits/vegetables	Effect type/spoilage type
Endocelluloses, exocelluloses and hemicellulases	*Clostridium thermocellum*		
Endoglucanases, exoglucanases and β-glucosidase (cellobiase)	Some fungi such as *A. niger* and *B. cinerea*		
Proteases: Metalloprotease-A Metalloprotease-B Metalloprotease-C Certain types (Extracellular)	*Erwinia* spp. *E. chrysanthemi* *E. carotovora* subsp. *carotovora*	Vegetables and fruits	Cell wall-degrading enzymes Soft rot Off-odors and off-flavors
Proteases: Acid proteases Aspartic proteinase Carboxypeptidase Proteolytic enzymes Proteolytic enzymes	Some fungi *Botrytis cinerea* *A. niger* *Pseudomonas* spp.	Vegetables and fruits	Cell wall-degrading enzymes Soft rot Off-odors and off-flavors
Lipolytic enzymes and lipoxygenase Lipolytic enzymes Phospholipase	*Pseudomonas* spp. Lactic acid bacteria Some fungi *Erwinia* spp. *E. chrysanthemi*	Vegetables and fruits	Cell wall-degrading enzymes Soft rot Off-odors and off-flavors
Cutinases	Many fungi A few bacteria	Vegetables and fruits	They are used for growth and survival of microorganisms

Lactic acid fermentation enzymes Some other catabolic enzymes	Lactic acid bacteria (homofermentative and heterofermentative)	Vegetables and fruits	Fermented flavor Off-odors and off-flavors
Ethyl alcohol fermentation enzymes Some other catabolic enzymes	Yeasts	Mainly fruits also vegetables	Fermented flavor Off-odors and off-flavors
Starch-hydrolyzing enzymes: α-amylase β-amylase Glucoamylase Pullulanase	 Most of the bacterial and fungal species Some bacteria, but relatively rare in fungi Often in fungi, but rare in bacteria In bacteria	Vegetables and fruits	Participate in softening Providing necessary nutrients for microorganisms
Riboflavin hydrolase	*Devosia riboflavina* (formerly called as *Pseudomanas riboflavina*)	Vegetables and fruits	Loss of nutritive value

liquefaction of the product. Exo-polygalacturonase (exo-PG) occurs in various fruits and vegetables. However, it has only been detected in a few fungi and bacteria such as *Clostridium thermosaccharolyticum*, *Erwinia chrysanthemi*, and *Ralstonia solanacearum*. Pectin and pectate lyases are common in microorganisms. Among all of cell wall-degrading enzymes, pectate lyases have a predominant role in plant tissue maceration. Pectin lyases are predominantly of fungal (e.g., *Aspergillus* spp.) and pectate lyases of bacterial (e.g., *Bacillus* and *Erwinia* spp.) origin. Since both polygalacturonases and lyases result in softening of fruits and vegetables or decreased viscosity of pectin-rich products, determination of the 4,5 unsaturated end products of β-elimination indicates microbiological sources of the enzyme. Microbial protopectinases, isolated from yeast and fungal species, mainly *Aspergillus*, *Geotrichum*, and *Trichosporon*, exhibit two forms of action. Protopectinase A type acts as an endoenzyme, splitting glycosidic bonds within the polygalacturonic region, while B-type protopectinase hydrolyzes side chains and cleaves the link between protopectin molecule and other constituents in the wall (Warriner and Zivanovic 2005).

Bacteria Produce Pectic Enzymes

Several species in the bacterial genera of *Erwinia*, *Pseudomonas*, *Bacillus*, and *Clostridium* are primary rotting pathogens of vegetables. The most frequently isolated pectinolytic bacteria regarding fresh-cut vegetables are species of *Erwinia* and *Pseudomonas* (Ragaert et al. 2010). *Erwinia carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica*, and *E. chrysanthemi* are three extensively studied soft rot erwinias (Fig. 4.2). The main characteristic distinguishing soft rot erwinias from other *Erwinia* species is the ability to produce large quantities of pectic lyases. The enzyme macerates parenchymatous tissue of a wide range of plant species (Chen 2002).

During the spoilage of fruits and vegetables, pseudomonads produce pectic enzymes to degrade the cell walls of the host tissue. This results in maceration of the tissue (Heard 2002). During cold storage of minimally processed leafy vegetables, pectinolytic strains of psychrotrophic *Pseudomonas* are responsible for bacterial soft rot (Ahvenainen 1996). Psychrotrophic pseudomonads are also capable of producing pectic enzymes that would have been expected to degrade the fruit tissue. *Pseudomonas cepacia*, *P. caryophylli*, and *P. gladioli* are three important pectic nonfluorescent *Pseudomonas* species. It is also well known that *P. marginalis*, a fluorescent species, causes soft rots of various vegetables. Endo-PG was reported to be the principal enzyme produced during infection of *P. cepacia*. *P. cepacia* also produces a pectate lyase. This bacterium is currently under the name of *Burkholderia cepacia* (Chen 2002). Strains of *P. fluorescens* B were predominantly pectinolytic, producing pectate lyase and polygalacturonase (Heard 2002). The role of *Xanthomonas* in the soft rots of fruits and vegetables was also discussed. It was reported that lactic acid bacteria can also produce polygalacturonases and pectin esterases. Lactic acid bacteria are not necessarily destructive to plant tissue. It is generally believed that lactic acid bacteria do not directly attack plant cell wall polymers (Chen 2002).

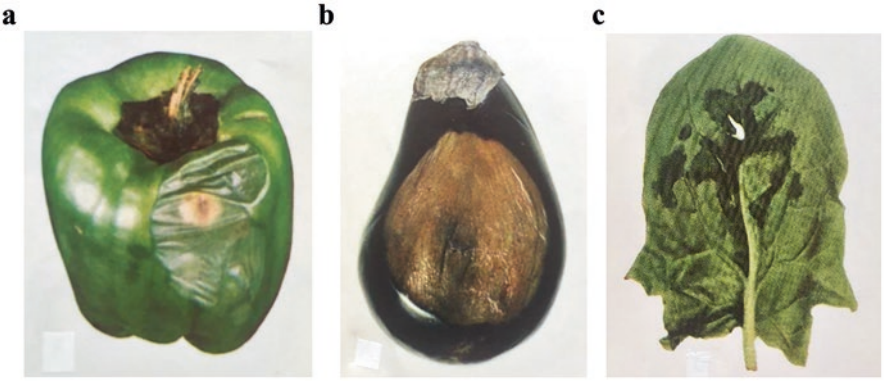

Fig. 4.2 Bacterial soft rot of various vegetables by pectic enzymes. (**a**) Bacterial soft rot on pod of green sweet pepper (McColloch et al. 1968). (**b**) Bacterial soft rot of eggplant (McColloch et al. 1968). (**c**) Bacterial soft rot on leaf of spinach (Ramsey et al. 1967)

On the other hand, the pectic enzymes from foodborne human pathogenic bacteria are becoming the focus of some researchers. The pectic enzymes from these bacteria are not extracellular. Knowledge about pectic enzymes from these saprophytic but human pathogenic bacteria becomes more relevant with the growth of the fresh-cut produce industry. These bacteria can utilize the readily available pectic substrates of plant produce for growth and survival purposes. In strains of *Y. enterocolitica* and *Y. pseudotuberculosis*, pectate lyase is a periplasmic and cytoplasmic enzyme (Chen 2002).

Molds Produce Pectic Enzymes

Many molds (filamentous fungi) such as *Aspergillus*, *Cercospora*, *Fusarium*, *Penicillium*, *Rhizoctonia*, *Trichoderma*, *Rhizopus*, and *Byssochlamys* are known to produce large amounts of extracellular pectic enzymes (Fig. 4.3). *Aspergillus oryzae*, *Penicillium frequentans*, *Botrytis cinerea*, *Byssochlamys fulva*, and *Aspergillus niger* are the common pectinolytic mold species examples (Banwart 1989; Chen 2002).

In contrast to the widespread occurrence of endopectate lyase in bacteria, fungi mostly produce polygalacturonases and pectin esterases. Most fungal polygalacturonases are endoenzymes. However, some fungi also produce exoenzymes. The synthesized pectinases are generally secreted from intact cells into the surrounding tissue. However, some enzymes might remain inside the cell, obviously for their catabolic function. *Botrytis cinerea* is an important pre- and postharvest pathogen of many fruits. *B. cinerea* produces a set of endopolygalacturonase isozymes. Pectin lyase and pectin methylesterase activities of *B. cinerea* were also noted. *Aspergillus niger* produces several polygalacturonase isoenzymes as well as pectin methylesterase. Similar to the bacterial counterpart, pectic enzymes in fungi are produced both inductively and constitutively (Chen 2002).

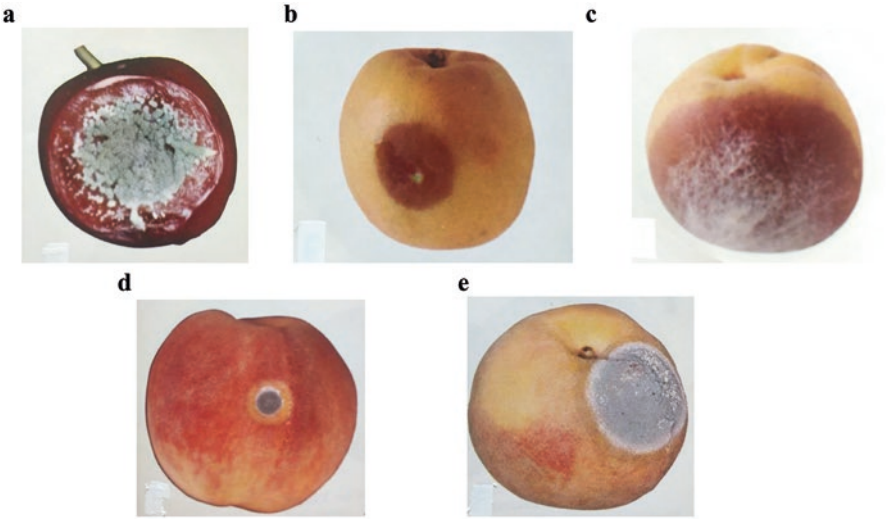

Fig. 4.3 Mold rot of various fruits by pectic enzymes. (**a**) Blue mold soft rot of cherry. (**b**) *Rhizopus* rot of apricot (early stage). (**c**) *Rhizopus* rot of apricot (late stage). (**d**) Blue mold rot of peach (early stage). (**e**) Blue mold rot of peach (advanced stage) (Harvey et al. 1972)

Yeasts Produce Pectic Enzymes

Pectic enzymes have been reported in several yeasts, belonging to the genera *Candida*, *Cryptococcus*, *Fabospora*, *Kluyveromyces*, *Pichia*, *Saccharomyces*, and *Zygosaccharomyces*. Pectic enzymes from yeasts are mainly endo-PGs, which could be important to consider during storage of fresh-cut fruits. The end products of endo-PGs reactions are always oligosaccharides with a varying number of galacturonic residues. Moreover, all of these enzymes preferentially attack pectate over pectin, and their activities decrease as the degree of methylation increases (Chen 2002; Ragaert et al. 2010).

4.3.6.2 Cellulases and Hemicellulases

Cellulose is a major structural component in plant cell walls. It is a linear polysaccharide chain of glucose linked by β-1,4 glycosidic bonds. Cellulose is a large polysaccharide build up to several thousand glucose monomers. Cellulose is insoluble in water. In general, enzymatic degradation of cellulose is a slow process. The major limiting factor in the hydrolysis of such materials is probably the sequestration of single molecules of substrate by the enzymes involved. In plant cell walls, the cellulose fibers are embedded in an amorphous complex of branched and linear mannans, xylans, arabans, and galactans, called hemicellulose, which provide support for the structural network (Chen 2002; Warriner and Zivanovic 2005).

A group of enzymes that degrade cellulose are referred to as cellulases, and enzymes that hydrolyze hemicellulose are called hemicellulases. The three major types of hydrolytic cellulases participate in the degradation of cellulose to glucose. These are numerous endoglucanases and exoglucanases. However, none of them can individually degrade crystalline cellulose fibers. Exoglucanase (exo-1,4-β–D--glucan 4-cellobiohydrolase, EC 3.2.1.91) releases either cellobiose or glucose from the nonreducing end of soluble cellulose. Endoglucanases (endo-1,4-β–D--glucanohydrolase, EC 3.2.1.4) randomly attack the cellulose chain and split the β-1,4-glucosidic bond. β-Glucosidase or cellobiase (β-1,4 oligoglucan glucohydrolase, EC 3.2.1.21) hydrolyzes cellobiose and other water-soluble cellodextrins to glucose (Chen 2002; Warriner and Zivanovic 2005).

Endogenous cellulases occur in numerous higher plants, and their activities increase during fruit ripening. Endogenous cellulases are not considered to be a significant factor in tissue softening and degradation, especially compared to pectinases. However, plant pathogens secrete high levels of cellulases along with pectic and hemicellulolytic enzymes. This combination of enzymatic activity plays a role in the softening and disintegration of cell wall material. Cellulolytic enzymes may further participate indirectly in spoilage by releasing soluble sugars from cellulose chains. These soluble sugars can serve as food for the plant pathogen. Nonpathogenic microorganisms may also participate in cellulolysis for food resources (Chen 2002; Warriner and Zivanovic 2005).

Various aerobic and anaerobic microorganisms can produce cellulolytic enzymes. Bacteria such as *Bacillus* and *Pseudomonas* secrete mainly endoglucanases that randomly cleave cellulose. Cellulases have been identified in *Erwinia chrysanthemi*. On the other hand, only a few pseudomonads have been identified to produce cellulases. Some fungi such as *A. niger* and *B. cinerea* can produce cellulase that degrade plant polymers. While fungi produce all of the three types of cellulases, all cellulolytic bacteria secrete a variety of endoglucanases (Chen 2002). Bacteria are not considered to be as efficient cellulose decomposers as fungi. However, there are some exceptions such as *Clostridium thermocellum*. This bacterium has several very efficient endocelluloses, exocelluloses, and hemicellulases. However, it can grow only on cellobiose and cannot utilize xylose, arabinose, mannose, and other sugars derived from hemicellulose. Microbial cellulases are inducible enzymes. They will be produced when cellulose is present in the medium (Warriner and Zivanovic 2005).

Hemicellulases are a diverse group of hydrolysis enzymes, including xylanases, β-mannanases, α-L-arabinofuranosidases, and α-L-arabinanases, among others. Similar to degradation of cellulose, hydrolysis of hemicellulose is carried out by microorganisms that can be found either freely in nature, mainly plant pathogens, or as a part of the digestive tract of ruminant animals. Although cellulose- and hemicellulose-degrading enzymes are mainly produced by microorganisms that grow and cause spoilage of fruits and vegetables, their optimum pH is generally between 4.5 and 6.5 (Warriner and Zivanovic 2005).

4.3.6.3 Proteases

In addition to its enzymatic function, proteins are constituents of cell membranes and structural components of plant cell walls. Proteases catalyze the hydrolysis of peptide bonds in proteins or peptides (Chen 2002). Proteases convert the proteins into diffusible polypeptides, oligopeptides, and amino acids, which can enter the microbial cells. The main reactions of microorganisms on amino acids are decarboxylation, deamination, and transamination. By the catabolic reactions of amino acids, many diverse products, such as various type of amines and organic acids, alcohols, ammonia, hydrogen sulfide, mercaptans, and carbon dioxide may be produced (Banwart 1989).

Proteases produced by bacteria and fungi are predominantly extracellular. Degradation of host proteins by proteinase secreted by microorganisms can profoundly affect the organization and function of the host cells. Proteases can be classified into four groups based on the essential catalytic residue at their active site. They include serine proteases (EC 3.4.21), cysteine proteases (also called thiol proteases) (EC 3.4.22), aspartate proteases (EC 3.4.23), and the metalloproteases (EC 3.4.24) (Chen 2002).

Activity of proteolytic enzymes and amino acid catabolism enzymes can cause formation of off-odors and off-flavors, as well as cell wall degradation (Heard 2002). It has been documented that *Erwinia* spp. produces and secretes several proteases that have been associated with virulence in plants. For example, three closely related metalloproteases (-A, -B, and -C) were noted for *E. chrysanthemi*. On the other hand, it was reported that an apple strain of *B. cinerea* produced extracellular acid proteases, aspartic proteinase, and carboxypeptidase. Isolated aspartic proteinase hydrolyzed proteins in the preparations of apple cell walls, and the excretion of aspartic proteinase preceded that of carboxypeptidase. Some fungi such as *A. niger* can produce protease with the other plant cell wall degradation enzymes (Chen 2002).

4.3.6.4 Lipolytic Enzymes

All lipids contain saturated or unsaturated fatty acids in their molecule structure. Lipids are found in many cells as energy storage compounds. Wax lipids are common on aerial epidermal cells. Phospholipids and glycolipids, along with proteins, are the main constituents of all plant cell membranes (Chen 2002).

Lipids are susceptible to hydrolysis, oxidation, and other chemical processes resulting in the production of various compounds. Desirable and undesirable flavor changes in foods may be associated with these compounds (Frazier and Westhoff 1978). Lipolytic enzymes or lipases hydrolyze lipids and liberate fatty acids. Fatty acids are presumably utilized by oxidation (Chen 2002). Oxidation of the unsaturated fatty acids yields aldehydes, ketones, and acids and results in off-odors and off-flavors (Frazier and Westhoff 1978; Heard 2002). Activity of lipolytic enzymes and oxidative enzymes can cause formation of off-odors and off-flavors, as well as cell wall degradation.

Many of the aerobic, actively growing proteolytic bacteria are also lipolytic. Lipolytic activity was usually noted for pseudomonad isolates. *Pseudomonas fluorescens*, for example, is strongly lipolytic. Some *Erwinia* spp. contain a phospholipase activity (Frazier and Westhoff 1978; Chen 2002). The ability of lactic acid bacteria to alter food flavor is, however, well known. A possible pathway of fruit flavor deterioration by lactic acid bacteria is by way of an increased lipase production (Chen 2002). Some mold genera such as *Geotrichum*, *Penicillium*, *Aspergillus*, *Cladosporium*, and *Monilia* can also produce lipases (Frazier and Westhoff 1978; Chen 2002).

4.3.6.5 Cutinases

The structural component of plant cuticle, called cutin, is an insoluble aliphatic biopolymer composed of hydroxy and hydroxyepoxy fatty acids. The natural plant surface of leaves, flowers, fruits, and young stems are covered by cuticle layer. This is a barrier protecting plants from invasion of pathogenic microorganisms. However, many fungi and a few bacteria are able to produce cutinases. Many fungal pathogens can penetrate the intact barriers of the plants. With the production of cutinase, some fungi could grow on cutin as the sole source of carbon (Chen 2002).

4.3.6.6 Enzymes Related to Ethyl Alcohol Fermentation of Yeasts

Yeasts are highly efficient in metabolizing simple sugars in fruits. Pectic enzymes of yeasts could be involved in substrate colonization on fruits, causing the breakdown of plant tissues with a concomitant release of sugars from plant cells. When the cell content is released, yeasts can multiply quickly in fruit juice by the fermentation of simple sugars. Therefore, yeasts play an important role in fruit spoilage under favorable conditions (Chen 2002).

A common characteristic of yeasts is the production of ethyl alcohol and CO_2 by ethyl alcohol fermentation. Fruit products can undergo fermentative spoilage by ethyl alcohol fermentation of yeasts (Arvanitoyannis and Bouletis 2012; Oliveira et al. 2015a). Fermentation reactions of yeasts can cause formation of off-odors and off-flavors in various fruits and fruits products (Chen 2002; Ragaert et al. 2010). The species of various yeast genera such as *Saccharomyces*, *Candida*, *Hanseniaspora*, *Hansenula*, *Pichia*, *Kloeckera*, *Debaryomyces*, and *Torulopsis* can cause fermentative spoilage on fruits. *Saccharomyces*, *Candida*, *Hanseniaspora*, *Pichia*, *Kloeckera*, and *Debaryomyces* can cause fermentative spoilage on tomatoes. Alcohol dehydrogenase is the principal enzyme of ethyl alcohol fermentation of yeasts (Banwart 1989).

Many molds are capable of utilizing alcohols as sources of energy, which are produced by ethyl alcohol fermentations of yeasts in fruits tissues. When these and other simple compounds have been depleted, these molds proceed to destroy structural polysaccharides and other remaining parts of fruits (Jay et al. 2005).

4.3.6.7 Enzymes Related to Lactic Acid Fermentations

Lactic acid bacteria (LAB) can use simple sugars especially hexoses and pentoses. The activity of cell wall degrading enzymes of certain microorganisms can cause concomitant release of sugars from plant cells by degradation of structural polysaccharides in plant cell wall. When the cell content is released, LAB can multiply quickly in fruit juice by the fermentation of simple sugars. Therefore, LAB can play an important role in vegetable or fruit spoilage under favorable conditions.

A common characteristic of LAB is the production of lactic acid by lactic acid fermentations. Lactic acid is produced by LAB by two types of lactic acid fermentations, "homofermentative lactic acid fermentation" and "heterofermentative lactic acid fermentation". Lactic acid only is produced by homofermentative lactic acid fermentation, while lactic acid and ethanol and/or acetate and CO_2 are produced by heterofermentative lactic acid fermentation.

Fruit products can undergo fermentative spoilage by lactic acid fermentations of LAB. (Arvanitoyannis and Bouletis 2012; Oliveira et al. 2015a). Fermentation products and volatile compounds (such as ammonia, volatile acids, and the like) produced by LAB can cause formation of off-odors and off-flavors in various fruits and fruits products (Banwart 1989; Chen 2002; Jay et al. 2005; Ragaert et al. 2010).

The common genera of lactic acid bacteria are *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Enterococcus*, *Carnobacterium*, *Oenococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella* (Chen 2002; Jay et al. 2005). Aldolase, hexose isomerase, and lactate dehydrogenase are the principal enzymes of homofermentative LAB, while phosphoketolase, alcohol dehydrogenase, and lactate dehydrogenase are the principal enzymes of heterofermentative LAB. On the other hand, some LAB such as N group of lactococci and *Leuconostoc* spp. can also make citrate metabolism. By this metabolic pathway, flavor compounds such as acetoin and diacetyl are produced from citrate (Jay et al. 2005).

4.3.6.8 Enzymes Related to Starch Hydrolysis

Starch is the main reserve polysaccharide in plants and the main carbon source for animals, plants, and microorganisms. Starch contains two kinds of glucose polymer: α-amylose and amylopectin. The former consists of long, unbranched chains of D-glucose in a unit connected by α-1,4 glycosidic bonds. The glycosidic linkage of an amylopectin chain is α-1,4, but the branch points are α-1,6 glycosidic bonds (Chen 2002; Warriner and Zivanovic 2005).

Starch hydrolysis enzymes can be classified as endo- or exoenzymes depending on their activity on starch molecules. Endoenzymes, such as α-amylase (EC 3.2.1.1; α-1,4-D-glucan glucanohydrolase) or pullulanase (EC 3.2.1.41; α-dextrin-6-glucono hydrolase), hydrolyze glycosidic bonds randomly within the starch molecule, while exoenzymes, such as β-amylase (EC 3.2.1.2; α-1,4-glucan maltohydrolase) or glucoamylase (EC 3.2.1.3; α-1,4-D-glucan glucohydrolase; amyloglucosidase), release molecule of maltose or glucose one by one from the nonreducing end of the polysaccharide. Generally, endoenzymes cause rapid loss in viscosity, while exoenzymes do not affect viscosity but increase sweetness (Warriner and Zivanovic 2005).

Most of the bacterial and fungal species produce α-amylase. However, *Bacillus cereus*, *B. subtilis*, *B. stearothermophilus*, *Lactobacillus cellobiosus*, *Streptococcus* spp., *Clostridium butiricum*, *Cl. thermosaccharolyticum*, *Aspergillus niger*, *A. oryzae*, *Fusarium oxysporum*, *Candida japonica*, and *Pichia polymorpha* produce this enzyme in significant amounts. β-amylase is commonly found in plants and some bacteria, but it is relatively rare in fungi. *Bacillus* spp. and *Cl. thermosulfurogenes* are good bacterial producers of β-amylase. Glucoamylase is often detected in fungi, especially in *Aspergillus* spp., and yeast such as *Pichia*, *Candida*, and *Saccharomyces*. Glucoamylase is rare in bacteria but has been detected in *B. stearothermophilus* and *Cl. thermosaccharolyticum.* Similarly, α-glucosidase (EC 3.2.1.20; α-D-glucoside glucohydrolase; maltase) releases one glucose molecule from the nonreducing end, with a higher affinity toward oligosaccharides than toward high-molecular-weight molecules. Contrary to glucoamylase, α-glucosidase is widely distributed not only in fungi and yeasts but also in bacteria. Pullulanase has been detected in bacteria and plants. *Bacillus acidopullulyticus* has a characteristically high pullulanase activity (Warriner and Zivanovic 2005).

α-Amylase is often referred to as "liquefying enzyme" due to the rapid loss in viscosity. As a result, α-amylase may participate in softening of fruits and vegetables in a way. When there are no other, preferable, carbon sources available in the surrounding media, microorganisms begin to produce extracellular starch-degrading enzymes to provide necessary nutrients. Then, the simple sugars can be readily metabolized according to the microorganism's enzyme producing nature. The metabolites which are produced by microbial enzyme activities can cause off-flavors in fruits and vegetables.

4.3.6.9 Enzymes Related to Loss Nutritional Quality

Riboflavin Hydrolase

Riboflavin hydrolase causes degradation of riboflavin. However, this enzyme is only found in some microorganisms (Whitaker 1996). *Devosia riboflavina*, formerly called as *Pseudomonas riboflavina*, is a riboflavin hydrolase-producing bacterium (Nakagawa et al. 1996).

4.3.7 Microbial Metabolites Affecting Fruit and Vegetable Quality

4.3.7.1 Microbial Flavor Metabolites Enhancing Product Quality

Many microbial metabolites can be considered detrimental to the quality of fruits and vegetables. However, some microbial metabolites may have positive effects on the quality of fruits and vegetables. For example methylotrophs (e.g., *Methylobacterium* spp.)

have specifically been identified as enhancing the flavor volatiles in fruit. Some *Methylobacterium* spp. strains can produce plant regulators such as cytokinins, pyrroloquinoline quinone, urease, and polyhydroxybutyrate that stimulate plant development. A diverse range of fungi can produce the odorous secondary metabolites such as esters, terpens, alkanes, and fatty acids. Most of them provide fruity odors that are perceived as beneficial for fruit fermentation. For example, *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* both produce low levels of 2-phenylethanol and terpenes that enhance fruity odors during fermentation (Warriner and Zivanovic 2005).

4.3.7.2 Microbial Metabolites Causing Vegetable and Fruit Spoilage

Some microbial metabolites may be responsible for the certain type of spoilages of fruits and vegetables. For example, vegetables stored under aerobic conditions are spoiled by the action of *Erwinia* and *Pseudomonas* spp., Gram-negative obligate aerobes. Spoilage by *Pseudomonas* spp. is often caused before significant visual damage has occurred due the accumulation of volatile substances (off-odors) such as ammonia and hydrogen cyanide. The common spoilage bacteria associated with potato rot are the aerobes *Erwinia carotovora* and *E. chrysanthemi*. Under anaerobic conditions, *Clostridium scatologenes* can grow and produce significant amounts of skatole, indole, and *p*-cresol. The contamination of potatoes typically occurs in the field, augmented by tissue damage and plant stress (Warriner and Zivanovic 2005).

Pseudomonads may contribute to the yellowing of vegetable products during storage, through the production of the ripening hormone ethylene. Ethylene production was observed by *Pseudomonas syringae* strains, and various strains of *P. fluorescens* A and G from broccoli origin (Heard 2002).

Molds such as *Penicillium italicum* and *P. digitatum* cause pigmented rot spoilage of fruits. These molds can also cause spoilage of fruits via the production of the volatiles such as 4-vinylguaiacol. Methyl esters of acids such as butanoic acid also lead to sulfurous and rancid odors due to a combination of mold and fruit autolytic pathways. Apples are frequently spoiled by *Penicillium expansum*, which causes discoloration and a strong aroma due to the formation of geosmin (Warriner and Zivanovic 2005).

Acid-tolerant lactic acid bacteria can take part in fruit spoilage by the production of volatile flavor compounds in products such as citrus juice. Another example is haloperoxidase. Haloperoxidase is widely distributed in plants. However, the acidophile spore-forming bacterium *Alicyclobacillus acidoterrestris* also contain this enzyme. The key difference of the bacterial enzyme is that it does not require cofactors or halide ions for activity. There is debate on the significance of the halophenols formed by this bacterium due to the presence of the reaction pathway in fruits. Nevertheless, it has been demonstrated that fruit juice inoculated with *Ali. acidoterrestris* accumulates 2,6-dibromophenol and 2,6-dichlorophenol, confirming its potential role in spoilage (Warriner and Zivanovic 2005).

4.4 Increase Shelf Life of Minimally Processed Products

Fruits and vegetables are generally consumed fresh, minimally processed, pasteurized, or cooked by boiling in water or microwaving. Fresh and minimally processed products are characterized by a short shelf life due to activities of deteriorative enzymes together with microbial growth (with associated enzymatic activity) and/or other nonenzymatic (usually oxidative) reactions.

The classical strategy to control enzyme activities is the rapid and complete inactivation of the enzymes by using effective methods like heat inactivation and chemical inhibition. Thermal treatment is the most common method of microbial and enzyme inactivation used by the food industry. Thermal treatment is generally effective in microbial and enzyme inactivation. However, it may cause undesirable changes in sensorial quality and nutritional quality of minimally processed products (Terefe et al. 2014). Refrigeration, controlled atmosphere packaging, and modified atmosphere packaging are the most common strategies to suppress and minimize deleterious effects of minimal processing and extend the shelf life of fresh products. Among the known pretreatments for extending storage life of minimally processed products include using edible coating, dipping in organic acid solutions (e.g., lactic acid, citric acid, acetic acid, tartaric acid) or in calcium salt solutions, using reducing agents to reduce or eliminate cut surface discoloration, sanitation and sanitizers, osmotic-dehydration, using of biocontrol methods (protective cultures such as lactic acid bacteria), control of relative humidity to help minimize the rate of water loss, using of ionizing irradiation and ultraviolet light (UV), using of high-pressure processing, using of certain cultivars and maturity levels, induction of other metabolic pathways that are naturally antagonistic to wound responses, tissue vacuum impregnation with glucose and hot water dipping, using cold plasma, and using of essential oils and their constituents (Zhuang et al. 2002; Paviath and Orts 2009; Ayala-Zavala and González-Aguilar 2010; Ragaert et al. 2010; Valencia-Chamorro et al. 2011; Oliveira et al. 2015a; Ramazzina et al. 2015; Siroli et al. 2015). If processing of fruits and vegetables is minimal and damages to cell integrity are local (at surface, stem, etc.), alternative methods like removal of oxygen, surface coating with edible films, application of chemical finning agents, and low-temperature heating procedures to increase cellular integrity might be applied to suppress and minimize activities of enzymes (Yemenicioğlu 2015). These types of treatments should be kept to a minimum, since they add cost and complexity to an already complex system. Temperature control and reduction of mechanical injuries of the intact produce before processing are key factors in maintaining their quality and suitability for processing.

4.5 Conclusions

Consumers are increasingly demanding convenient and ready-to-eat fruits and vegetables with fresh-like quality. However, endogenous enzymes and/or microbial enzymes may cause various quality defects in minimally processed fruits and

vegetables. Numerous preservation technologies such as refrigeration, controlled atmosphere packaging and modified atmosphere packaging, high-pressure processing, and edible coating are being performed to inactivate or control the enzymes with deteriorative action. These preservation technologies use minimal heat and preservatives with the aim of obtaining safe and fresh-like products of superior nutritional quality and acceptable shelf life. However, each technology has some advantages and disadvantages, with the latter predominating. In recent years, increased awareness of consumers on the relationship between diet and health has created a greater impetus and effort for the investigation of alternative food processing technologies. The maintenance of the quality of fresh products is still a major challenge for the food industry (Sonti 2003). In this regard, novel technologies such as pulsed electric field, ultrasonication, UV irradiation, and alternative thermal processing technologies such as microwave, radio frequency, and ohmic heating are being investigated (Terefe et al. 2014).

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Chapter 5
Biological and Biochemical Changes in Minimally Processed Refrigerated Fruits and Vegetables

Patrick Varoquaux and Robert C. Wiley

5.1 Introduction

In recent years, there has been a rapid expansion in the sale of prepacked/precut fresh fruits and vegetables in North America and in Europe. Because the tissue integrity of these products has been altered, during processing, they are more perishable than the original raw materials (Rolle and Chism 1987; Shewfelt 1986). Like whole fruits and vegetables, minimally processed refrigerated (MPR) produce deteriorates after harvesting due to physiological aging and microbial spoilage. Injury stresses (Figs. 5.1 and 5.2) caused by processing also result in cellular decompartmentalization or delocalization of enzymes and substrates which leads to various biochemical deteriorations such as browning, off-flavors, and texture breakdown (Varoquaux 1987). Moreover, peeling and cutting facilitate primary infection of the plant tissues by epiphytic and phytopathogenic microorganisms.

Ready-to-use fruits and vegetables were developed about 50 years ago in the United States (Garrott and Mercker 1954). Recent investigations in the United States, Japan, and Europe have sought to improve the like-fresh characteristics of these products and to extend their shelf-life, thus allowing distribution within an adequate area (Huxsoll and Bolin 1989). Achievement of this aim is possible through optimization of all unit operations during processing, preservation, and marketing.

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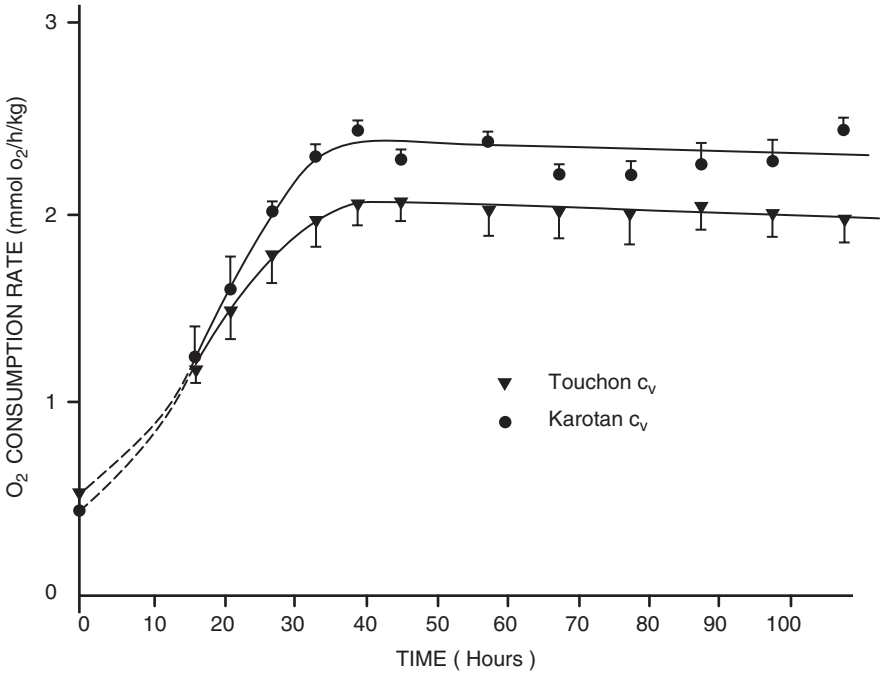

Fig. 5.1 Change in respiratory intensity of fresh grated carrots after standard processing (two cultivars). Grated carrots were stored in air at 10 °C (From Carlin 1989)

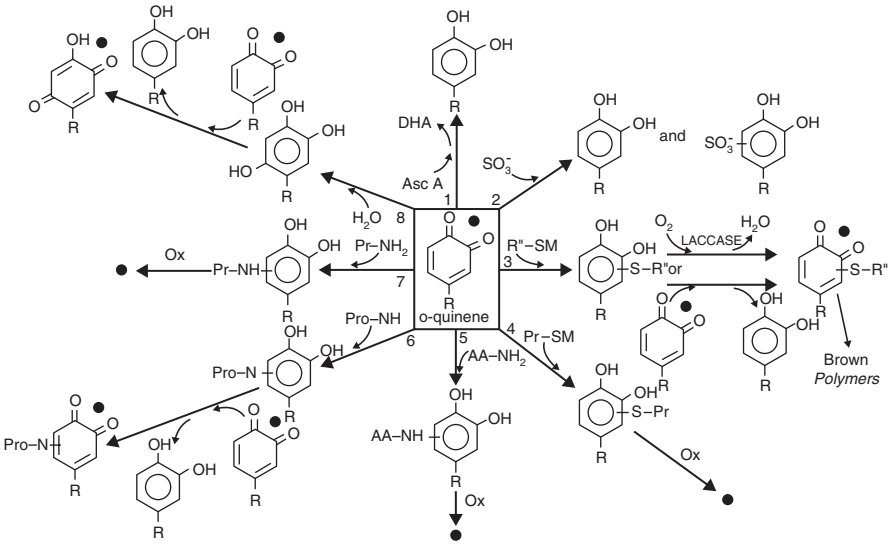

Fig. 5.2 Enzymatic browning; the role of inhibitors on the formation of brown polymers from o-quinone (From Rouet-Mayer et al. 1993)

The first part of this chapter is devoted to a review of the physiological, biochemical, and microbial degradation mechanisms of MPR fruits and vegetables. The second part deals with the effects of processing and distribution techniques on the mechanisms of quality deterioration.

Some minimal treatments use chemical compounds applied by spraying or dipping. Chemical preservative treatments may result in a change in taste or smell. Unfortunately, some very efficient chemicals such as sorbic and benzoic acids or sulfiting agents have been found to be potentially harmful to some segments of the population. These treatments, applied to MPR fruits and vegetables, are being more carefully scrutinized by government regulators in most countries. Moreover, all additives, whether natural or useful nutrients, are increasingly rejected by individual consumers of ready-to-use fresh fruits and vegetables. Safe additives, such as critic and ascorbic acids or their combinations, are not effective enough in controlling browning of shredded lettuce (Bolin et al. 1977), and their beneficial effects are short term for pear (Rosen and Kader 1989) and apple slices (Varoquaux and Varoquaux 1990). Chemical preservatives are covered in Chap. 6.

There are many postharvest physiology reviews of intact plant tissues, including Wills et al. (1989) and Kays (1991). This chapter attempts to cover additional information that relates primarily to biological and biochemical changes that may occur in MPR fruits and vegetables. These are physiological, biochemical, and microbiological in nature.

5.2 Mechanisms of Quality Deterioration

The effects of processing, packaging, and storage on the maintenance of the quality of minimally processed fruits and vegetables are analyzed in the following sections.

5.2.1 Physiological Disorders (Primarily Injury Stress)

Desiccation, chilling injury, and CO_2 injury, which are widely known disorders in stored intact fruits and vegetables, are well covered in Wills et al. (1989) and Kays (1991). Wounding stress results in metabolic activation. The main physiological manifestations of this phenomenon include increased respiration rate (Fig. 5.1) and, in some cases, ethylene production (Rosen and Kader 1989). The response depends on the magnitude of the stress.

The O_2 consumption rate of shredded endive is only 1.2 times that of intact endive (Chambroy 1989). This ratio increases to 1.4 for broccoli (Ballantyne 1987) and to 2 for shredded lettuce (Ballantyne 1986). For more damaged plant tissue, respiration averages three to seven times that of the intact tissue, for example, four to seven for grated carrots (Carlin 1989; MacLachlan and Stark 1985). This increase in the metabolism of minimally processed fruits and vegetables results in rapid consumption of

oxygen in the packaging. Bolin and Huxsoll (1991) found about four times the oxygen concentration in an intact head of lettuce compared with shredded lettuce after about 16 days of modified atmosphere packaging (MAP) storage at 2°C.

Many examples of wound-induced ethylene production in fruit and vegetable tissues have been extensively reviewed. Because ethylene contributes to the neosynthesis of enzymes involved in fruit maturation (Yang and Hoffman 1984), it may play a part in physiological disorders of sliced fruits.

The stimulation of ethylene production by stress typically occurs after a time lag of 10–30 min and subsides later after reaching a peak within several hours (Yang and Pratt 1978). When tomato is cut into small disks, ethylene production increases to about 20-fold that of the whole fruit (Watada et al. 1990).

Immediately after slicing, and for 2 h at 20 °C, the ethylene production rate of kiwifruit decreases. Then, 2–4 h later, it increases sharply, peaks at seven times that of intact fruit, and decreases slightly or remains constant after about 10 h (Varoquaux et al. 1990). This confirms the results of Watada et al. (1990), who found ethylene production rates 16-fold higher in sliced kiwifruit than in intact fruits. These authors suggested that the continual increase in rate was probably due to stimulation of ethylene production by endogenous ethylene as well as slicing. The ethylene production rate was found to be proportional to the injured surface area and hence to the intensity of the stress. Ethylene production by sound, unstressed kiwifruit tissues is negligible, compared to uninjured tissue, whatever the maturity of the fruit (Vial 1991).

Rosen and Kader (1989) found an increase in ethylene production in sliced strawberry but not in sliced pear. Injury stress may also enhance the susceptibility of plant tissue to ethylene (Lafuente et al. 1989).

5.2.2 Biochemical Reactions

Enzymes and substrates are normally located in different cellular compartments, and their transfer is actively regulated. Processing results in destruction of surface cells and injury stress of underlying tissues. Enzymatic reactions cause sensory deteriorations such as off- flavor, discoloration, and loss of firmness.

5.2.3 Off-Flavor

Enzymatic peroxidation of unsaturated fatty acids is the most dramatic example of the biochemical modifications of natural aromas of vegetables that have been minimally processed. This peroxidation is catalyzed by lipoxidase and leads to the formation of numerous aldehydes and ketones (Hildebrand 1989).

It has been shown that the concentration of n-hexanal, a by-product of hydroperoxide degradation, is well correlated with postharvest development of off-flavor in peas (Bengtsson et al. 1967). Gowen (1928) reports that vine-shelled peas develop

a strong off-flavor within 4–6 h at room temperature. Bruising of peas has been shown to be an important factor in the development of delayed off-flavor. Hand-shelled peas do not deteriorate in flavor as rapidly as vine-shelled peas. This oxidative reaction also occurs, to a lesser extent, in French beans and potatoes, both of which are currently minimally processed. The hydroperoxides are unstable, may be cytotoxic, and particularly affect proteins and membranes (Watada et al. 1990). Damage to the membrane can result in disruption of the diffusion barrier and thus generation of physiological disorders.

5.2.4 Discoloration

The main color deterioration that occurs in bruised plant tissues is enzymatic browning (Mayer 1987). The enzymatic reactions involved in the brown discoloration are still under investigation (Fig. 5.2). The enzymatic activities markedly depend on pH; a 0.5 reduction in the natural pH of apple results in a 50% decrease in chloroplast polyphenoloxidase (PPO) activity (Harel et al. 1964).

Ortho-benzoquinones are very reactive and unstable in aqueous solutions. They are converted into phenolics by a reducing agent such as ascorbic acid and also undergo polymerization into melanins (Bu'Loch 1960; Whitaker 1972).

Other reactions can alter the natural color of fresh fruits and vegetables but color changes are not specifically caused by minimal processing. Conversion of chlorophylls into pheophytins, for example, may be caused by acidification of cellular cytoplasm, a reaction that is responsible for the degreening of broccoli (Ballantyne et al. 1988b).

Destruction of chlorophyll by ethylene has been reported to be due to increased chlorophyllase activity (Amir-Shapira et al. 1987). The chlorophyll change may also result from the loss of membrane integrity that occurs with senescence hastened by ethylene (Rolle and Chism 1987). Other degradative enzymes have been reported, such as chlorophyll oxidase, chlorophyllase, lipolytic acid hydrolase, and peroxidase-hydrogen peroxide systems. The results reported by Watada et al. (1990) indicate that the chlorophyll degradation pathway probably differs among plant species, and it is unknown if ethylene activates other pathways. It seems that chlorophyll degradation constitutes a good marker of the physiological condition of green plant tissues (Yamauchi and Watada 1991). Coupled oxidation of carotenoids with lipoxidase-catalyzed hydroperoxides may result in discoloration of grated carrots.

5.2.5 Loss of Firmness

Slicing plant tissue generally results in loss of firmness, as observed with apple slices by Ponting et al. (1972). There have been many reviews on loss of firmness in intact plant tissues (Doesburg 1965; Kertesz 1951)

Kiwifruit slices lose 50% of their initial firmness in <2 days at 2 °C; Varoquaux et al. (1990) suggested that textural breakdown of kiwifruit slices during storage is due to enzymatic hydrolysis of cell wall components. Pectinolytic and proteolytic enzymes liberated from cells damaged by slicing could diffuse into inner tissues. The migration rate of macromolecules through kiwifruit tissue, determined with labeled enzymes, is unexpectedly high, because the radioactive front progresses at about 1 mm/h (Cuq and Vial 1989). The mechanisms of hydrolysis of cell wall component after slicing differ from those involved in the normal maturation of kiwifruit in which solubilization of protopectins is predominant. Watada et al. (1990) emphasized the role of ethylene in the loss of firmness of sliced kiwifruit packed together with banana sections. The average firmness of 1 cm thick slices decreased by about 25% after 24 h and by 40% after 48 h at 20 °C. Exposure of slices to 2 or 20 ppm ethylene accelerated the loss of firmness. But, as stated by Varoquaux et al. (1990), the loss of firmness of sliced kiwifruit begins immediately after cutting at the same softening rate as that after 6 or 12 h. Therefore, the texture breakdown is not primarily provoked by the neosynthesis of enzymes initiated by ethylene. Nevertheless, Watada et al. (1990) suggested that "wound ethylene" can increase the permeability of membranes and perhaps reduce phospholipid biosynthesis, which can upset the dynamic processes of cellular structure and membrane integrity.

5.2.6 Microbial Spoilage

Microflora responsible for spoilage of MPR fruits and vegetables include a large number of fungi and bacterial species. These are reviewed in Chap. 19. Among gram-negative bacteria, *Pseudomonadaceae* and *Enterobacteriaceae* prevail. Gram-positive microorganisms, mainly represented by lactic acid bacteria and numerous yeast species, have so far been detected in mixed salads and grated carrots (Denis and Picoche 1986). Only phytopathogenic or epiphytic bacteria able to induce sensory deteriorations affected by processing and packaging conditions are considered in this chapter.

Pectinolytic bacteria such as *Erwinia carotovora* (Brocklehust et al. 1987), *Pseudomonas marginalis* (Nguyen-The and Prunier 1989), and *Pseudomonas viridiflava* (Carlin et al. 1989) were identified in minimally processed and fresh vegetables (Manvell and Ackland 1986) and in vacuum-packed sliced carrots (Buick and Damoglou 1987). These microorganisms were identified in both raw and processed vegetables (Lund 1988; Mundt and Hammer 1968).All spoilage mechanisms are interdependent and contribute to disorders in MPR fruits and vegetables. The plant response to these stresses is cellular derealization, which results in biochemical reactions alone or may be superimposed on other spoilage manifestations (viz., alcoholic or lactic acid fermentations).

5.3 Effects of Processing and Marketing Techniques on Quality

5.3.1 Processing

The successive operations in the processing of MPR vegetables are summarized in Fig. 5.3 (Anon 1989). (Also see Fig. 1.1). Each step may play a role in the spoilage mechanisms. Bruises in minimally processed fruit and vegetables lead to further deterioration, with loss of quality and shelf-life. Damage that occurs to cells next to

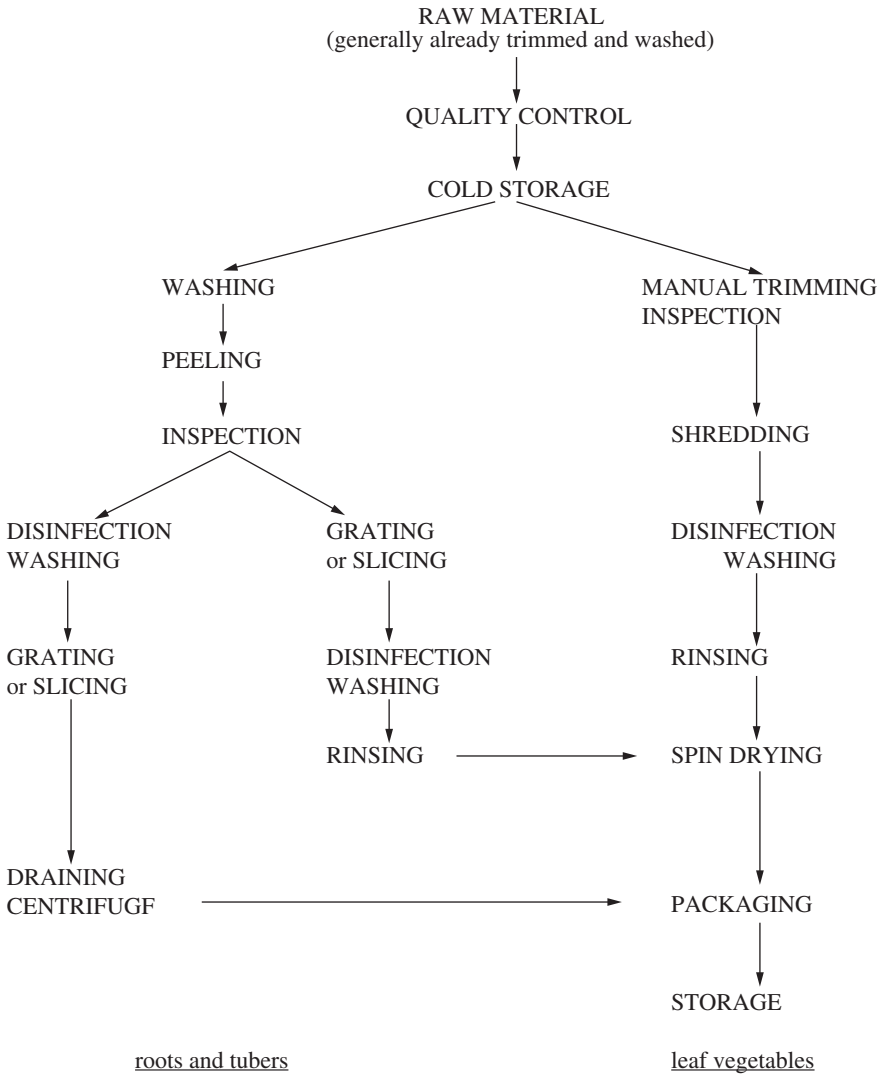

Fig. 5.3 Minimally processed vegetables. Flow diagram of processing lines

the cut surfaces will also be very detrimental (Bolin and Huxsoll 1991; Huxsoll and Bolin 1989). The most damaging unit operations are those that alter tissue integrity.

MPR fruit and vegetable production plants must follow the scientific rules which are given below:

(a) Entering raw materials must always move in forward direction.
(b) Trimming room, the washing room, and the packing rooms must be separated in order to prevent cross-contamination.
(c) Temperature in trimming room, washing room, and packing rooms must be controlled, but never exceed 10 °C.
(d) Before processing and after packaging, the products must be kept at 0–2 °C temperatures.
(e) Waste evacuation conveyors and product moving conveyors must be in the opposite directions to prevent cross-contamination.
(f) Airflow and ventilation and pressures must be from inside of the processing room to outside with a positive pressure in the rooms (Varoquaux and Mazollier 2002).

Bolin et al. (1977) showed that compared to chopping, slicing improved the shelf-life of shredded iceberg lettuces and that the cutting blades should be as sharp as possible (Fig. 5.4). Later work has shown that tearing by hand was more beneficial to lettuce than shredding by machine (Bolin and Huxsoll 1991). In general, shelf-life for most commodities is enhanced by reducing machine-to-product and product-to-product impacts.

Bolin et al. (1977) also claimed that total microbiological pollution of shredded lettuces was closely correlated to quality deterioration (Fig. 5.5). To reduce microbial pollution, it is necessary to remove all the heavily contaminated external parts of the raw material, especially those in contact with soil. For example, roots and tubers should be carefully peeled and green salads severely trimmed. After cutting,

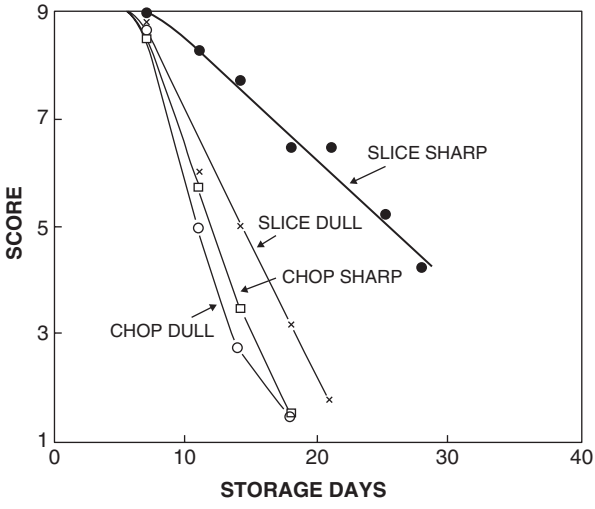

Fig. 5.4 Effect of cutting method on storage life of shredded lettuce (From Bolin et al. 1977)

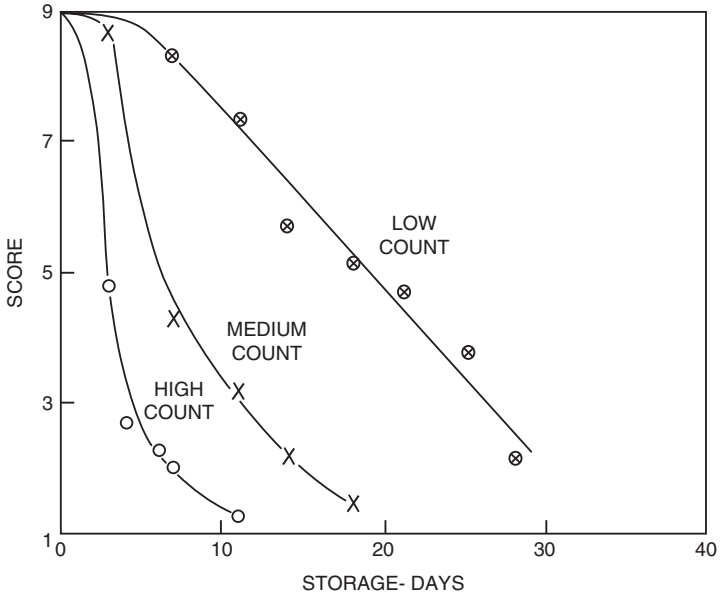

Fig. 5.5 Effect of initial microbial count on storage stability of shredded lettuce (From Bolin et al. 1977)

the plant fragments should be thoroughly washed, although this operation may be very detrimental to the taste and flavor of grated roots or tubers and, to a lesser extent, shredded foodstuffs, because of the possibility of leaching flavor compounds. Disinfection can be performed using chlorinated water (Adams et al. 1989). Chlorine in the disinfection bath reduces the count of mesophilic aerobic bacteria according to an apparent first-order reaction (Fig. 5.6).

An optimal concentration of 120 ppm active chlorine on shredded salads was suggested by Mazollier (1988). It is noteworthy that chlorine only delays microbial spoilage and does not show any beneficial effects on biochemical or physiological disorders (Bolin et al. 1977). Washing with clean water removes the free cellular contents that are released by cutting. Cellular fluids contain active PPO and phenolic compounds responsible for rapid brown discoloration (Bolin et al. 1977).

Draining should be efficient because water droplets on the product surface result in microbial proliferation. Herner and Krahn (1973) indicated the importance of keeping cut lettuce dry, even advocating not rinsing at all before storage.

Conversely, excessively efficient draining, using a centrifuge, bruises plant tissue and is responsible for rapid biochemical deteriorations, although the shelf-life of lettuce was extended by centrifugation (Bolin and Huxsoll 1991). According to Ryall and Lipton (1972), a detectable texture breakdown is noted if moisture loss exceeds 5%.

After draining, the minimally processed product must be packed for retail sale. Proper packaging should protect the fresh product from physical damage and surface abrasion caused by handling. It should also prevent microbial cross-contamination during distribution.

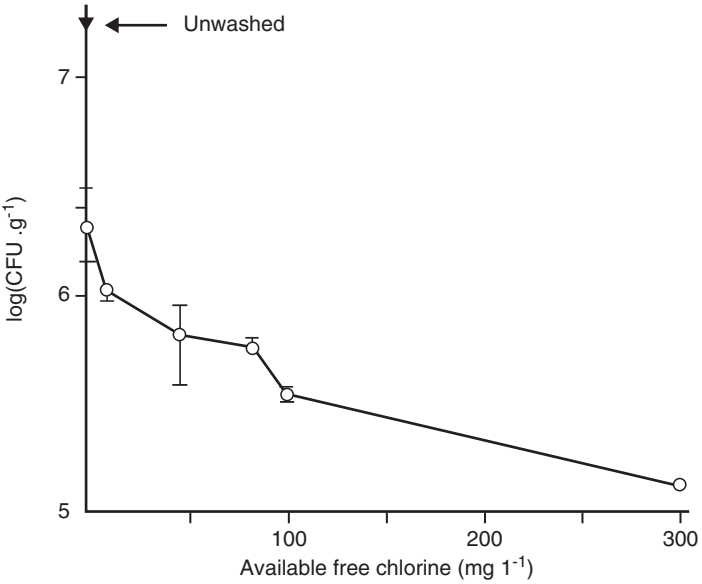

Fig. 5.6 Effect of chlorine concentration on the count of mesophilic aerobic bacteria on washed lettuce leaves. pH increased from 7.3 for unchlorinated water to 9.4 for 300 mg/l of free chlorine indicates ranges in replicate experiments (From Adams et al. 1989)

Polymeric membranes generally exhibit a high resistance to the diffusion of water vapor (see Chaps. 6 and 7). Maintenance of high relative humidity is essential to the development of defense mechanisms. Below 75% relative humidity (RH), cells surrounding an injury are damaged by desiccation and are incapable of lignin synthesis (Ben-Yehoshua 1987).

High RH maintains the turgor of fruit and vegetable tissues, but it may cause condensation on the commodity, creating conditions favorable for the growth of phytopathogenic and epiphytic flora (Zagory and Kader 1988). Excessive RH may also result in the exudation of cellular sap which causes proliferation of saprophytes (Tomkins 1962).

Among other possible functions of packaging reviewed by Smith et al. (1989), pouches or overwrapping films should create an optimal modified atmosphere to keep the product under optimal physiological conditions.

The effects of MAP on MPR fruits and vegetables are reviewed in this chapter and in Chap. 2

5.3.2 Temperature

The chill chain used with MPR fruits and vegetables should begin as soon as possible after harvesting. Early precooling of raw material dramatically extends the shelf-life of minimally processed products.

A substantial proportion of French salads grown for minimal processing are vacuum-cooled less than 4 h after harvesting. High-humidity air precooling is also workable for leafy vegetables. As stated by Bolin et al. (1977), temperature has one of the most pronounced effects on the storage life of shredded lettuce (and of any MPR fruits or vegetables).

French regulations imposed 8 °C as a maximum temperature for MPR fruits and vegetables in 1987. This limit was lowered to 4 °C in 1988 (Scandeila 1988), but minimally processed commodities are often stored or distributed at higher temperatures (Anon 1988; Scandeila 1989; Scandeila et al. 1990). The temperature chosen for investigations should range from 8 °C to 10 °C. The English Guidelines for Handling Chilled Food recommend a storage temperature range of 0–8 °C for salad vegetables, noting that some vegetables may suffer damage if kept at the lower end of this temperature range

The quality of raw ingredients and the suitable control throughout the food chain are the most significant factors that will normally predetermine the shelf-life of MPR fruits and vegetables before they even enter the distribution system (Lioutas 1988).

5.4 Effect of Temperature on Physiological Activity

Lowering the temperature reduces respiration and delays senescence. There is a linear relation between the logarithm of the O_2 consumption rate and temperature. The respiration rate of shredded endive as a function of temperature is shown in Fig. 5.7; also included is the equation for endive. The effect of a 10 °C increase in temperature on the respiration rate, Q_{10}, averages at 2 for most fruits and vegetables but may range from 1 to 5.

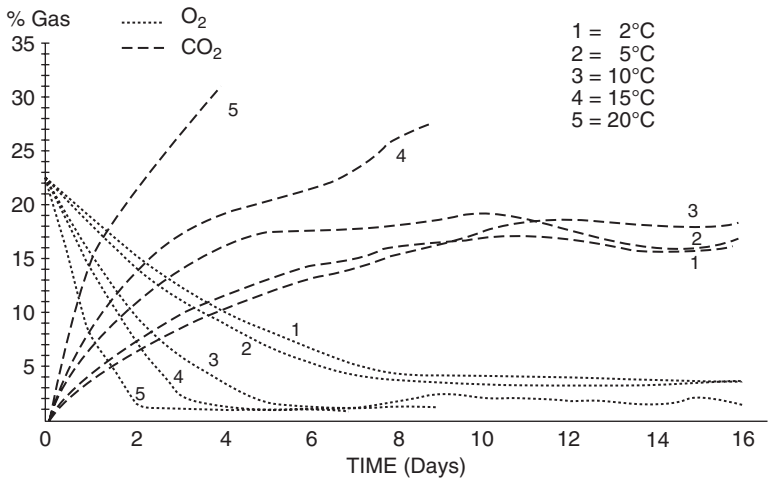

Fig. 5.7 Effect of temperature on O_2 consumption rate of shredded endives in air (From Chambroy et al. 1990)

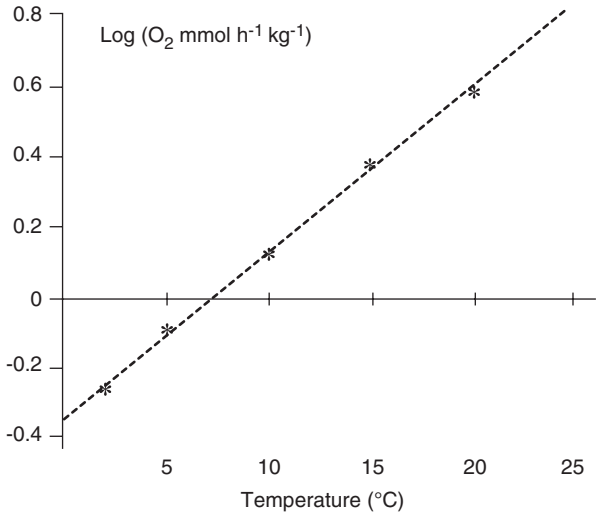

Fig. 5.8 Effect of temperature on atmosphere change within propylene packs of shredded endives versus duration of storage (From Chambroy 1989)

Maintenance of a stable, low temperature is the key to success for packed MPR fruits and vegetables. When the storage temperature is increased to 10 °C, the steady state is reached sooner (Ryall and Lipton 1972) and the gas composition within the pouches during at temperatures higher than 10 °C, CO_2 concentration increases sharply due to enhanced metabolism and microbial proliferation. The chill chain temperature must therefore be taken into account for modified atmosphere packaging developments (Fig. 5.8).

5.4.1 Effect of Temperature on Biochemical Reactions

Because biochemical reactions are catalyzed by enzymes, biochemical change in MPR fruits and vegetables is, in part, the consequence of the effect of temperature on enzyme activities (Arrhenius' law).

The kinetics of loss of firmness of kiwifruit slices, as a function of temperature, are shown in Fig. 5.9. All other enzymatic reactions are temperature dependent. For example, difference in flavor is not readily apparent in peas held at 4 °C for up to 4 h. At 25 °C, flavor differences are noticeable within 2 h, and at 37 °C, off-flavor is inhibitory after only 1 h (Weckel et al. 1964).

Decreasing temperature also alleviates degradative change in color of injured plant tissues, thereby reducing tyrosinase and o-diphenoloxidase activities. As shredded lettuces darken during storage, discoloration is accompanied by a loss in visual green pigmentation (Bolin et al. 1977; Bolin and Huxsoll 1991), likely due to coupled oxidations. Loss in green color, measured as reflectance (a) versus storage duration at several temperatures, is shown in Fig. 5.10. In bruised fruits and vegetables, the effect of temperature on enzymatic activities responsible for biochemical

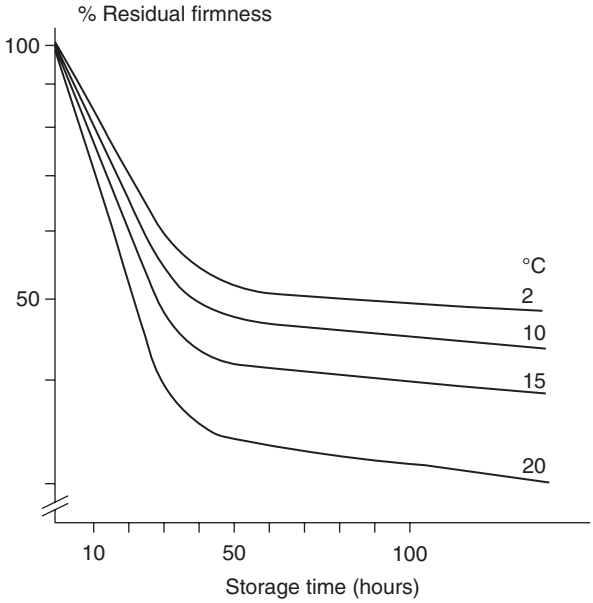

Fig. 5.9 Logarithm of loss of firmness in kiwifruit slices as a function of storage time in sealed "clarylene" pouches (From Varoquaux et al. 1990)

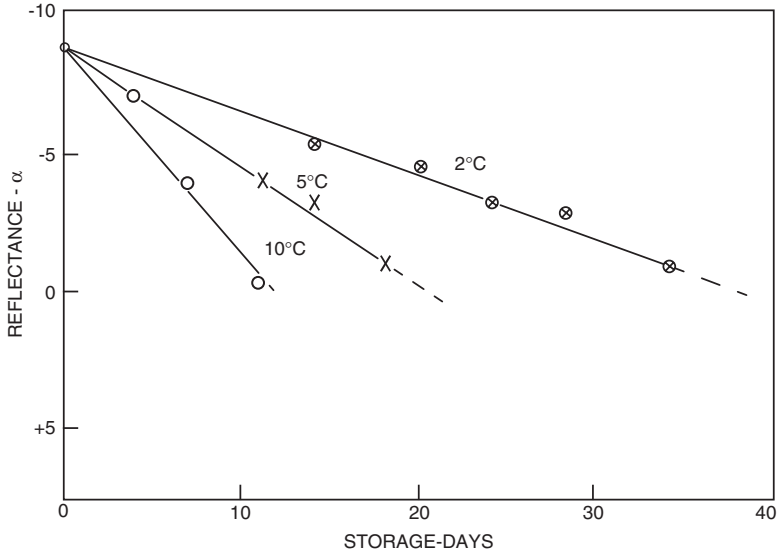

Fig. 5.10 Effect of storage temperature on green color loss in shredded lettuce (From Bolin et al. 1977)

damage is indissociable from its effect on normal postharvest changes. Thus when plant tissues are stored at temperatures inducing chill injuries (Marcellin 1982), the inner structures of cells disintegrate, and biochemical change occurs more intensively than in controls kept at higher temperatures Hence, the optimal temperature minimizes tissue senescence and thus delays cell delocalization.

5.4.2 *Effect of Temperature on Microorganism Growth*

Lowering temperature also reduces microbial proliferation on MPR fruits and vegetables. In the properly controlled chill chain, cold-tolerant microorganisms would grow slowly and eventually cause spoilage with consequent reduction in the shelf-life of the commodity (Manvell and Ackland 1986).

Lactic acid bacteria grow above 2 °C in shredded endive packed in polypropylene (40 µm), and at 6 and 10 °C, they develop faster than total flora, as shown in Fig. 5.11.

In salad trays overwrapped with a cling film held at 7 °C, lactic acid bacteria formed a low proportion of the total population, whereas at 30 °C lactic acid bacteria formed a dominant population. These differences between population types led

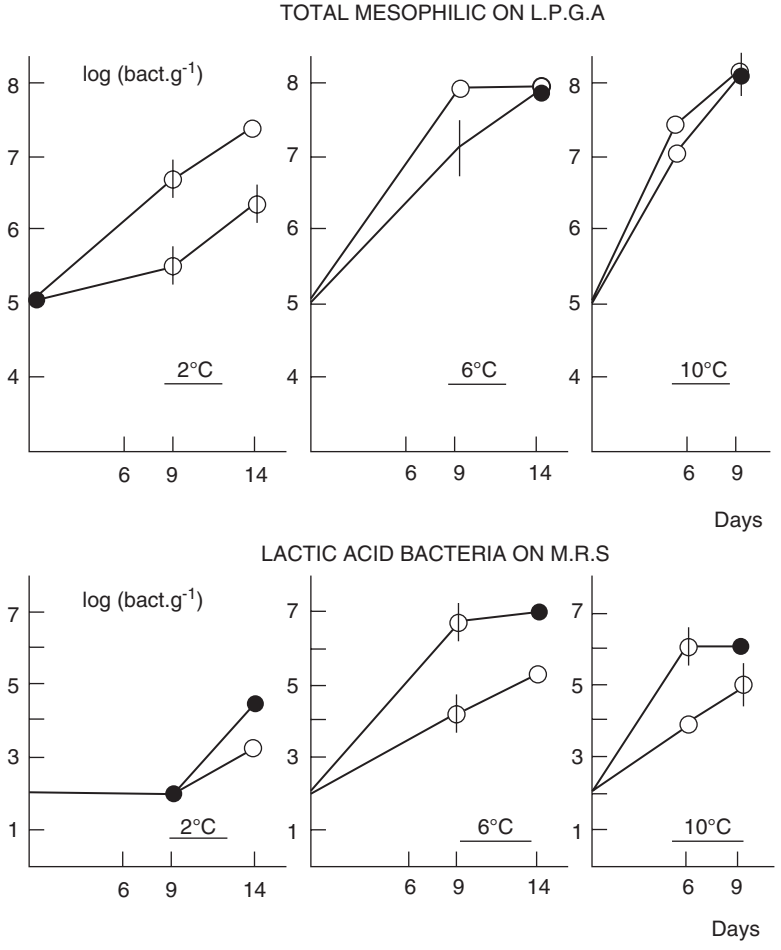

Fig. 5.11 Changes in total flora and lactic acid bacteria in shredded endive packed in polypropylene pouches under air (O—O) or air +20% CO_2 (®—®) as a function of storage duration at 2, 6, and 10 °C (From NGuyen-The and Carlin 1988)

to the development of tests indicating shelf-life expiration or temperature abuse (Manvell and Ackland 1986) (see temperature-time indicator [TO], Chap. 7).

Enrichment of the storage atmosphere with CO_2 results in a slower development of mesophilic flora compared to the air control and in more rapid growth of lactic acid bacteria at 6 and 10 °C (Nguyen-The and Carlin 1988).

5.5 Modified Atmosphere Packaging

Retail sale demands that ready-to-use commodities be packaged, and as a consequence the atmosphere composition within the pack changes due to the respiration of living tissues. This change can be detrimental or beneficial to the overall quality of the commodity; it can also produce contradictory effects on the different spoilage mechanisms. Controlled atmosphere (CA) and MAP have become the subject of a tremendous number of research projects over the last decade. This research has provided a good, basic understanding of CA/MAP which is useful in developing MAP applications for minimally processed fruits and vegetables (Brecht 1980; Isenberg 1979; Kader 1986; Marcellin 1977; Smock 1979; Wolfe 1980).

Several studies have examined the potential for the use of sealed polymeric films to generate a favorable modified atmosphere within the package environment (Cameron et al. 1989; Daun and Gilbert 1974; Geeson et al. 1985; Zagory and Kader 1988).

Modified atmosphere (MA) can reduce the incidence of physiological disorder, microbiological spoilage, and biochemical deterioration, each of which alone or in conjunction results in changes in color, texture, flavor, and, as a consequence, in the commercial value of the commodity (see also Chap. 6).

5.5.1 Effects of MA on the Physiology of MPR Fruits and Vegetables

Respiration in plants is the oxidative metabolism of sugars and organic acids to end products CO_2 and H_2O with concurrent production of energy. MAP may lower the metabolism and decrease both O_2 consumption and $C0_2$ production (Laties 1978). The effects of low O_2 and high CO_2 on respiration are additive. The optimal concentrations of both gases in combination are difficult to predict without actual measurements in a variety of atmospheres. However, the potential respiration rate of most roots or bulbs is stimulated when stored under elevated CO_2 concentrations. This phenomenon has been shown for celeriac (Weichmann 1977a), carrots (Weichmann 1977b), and onions (Adamicki 1977). If 0_2 is reduced or CO_2 elevated beyond the tolerance levels of the commodity, respiration is then associated with anaerobic metabolism.

It is established that high CO_2 concentrations inhibit several enzymes of the Krebs cycle including succinate dehydrogenase (Ranson et al. 1957). This would inhibit the aerobic pathway and result in accumulation of succinic acid, which is toxic to plant tissue (Bendall et al. 1960).

The difference between external and internal O_2 concentrations is determined by the resistance of the plant tissue to gas diffusion which depends on the species and stage of maturity. Water condensation on the commodity reduces diffusion, whereas temperature has little effect (Cameron and Reid 1982). The anaerobic metabolism pathway is responsible for the production of CO_2, ethanol, aldehydes, and other chemical compounds that produce off-flavors, off-odors, and discoloration. In theory, the O_2 level within the cell which induces anaerobic metabolism is as low as 0.2%, and that outside the product l–3% (Burton 1974).

The ratio of CO_2 production to O_2 consumption, known as the respiratory quotient (RQ), is, theoretically, 1 in true aerobic metabolism. In actual measurements, it ranges from 0.7 to 1.3 (Forcier et al. 1987). CO_2 levels as low as 5% may induce physiological disorders in common mushroom (Lopez-Briones 1991), and asparagus exhibits surface pitting when stored in >10% $C0_2$ (Lipton 1977). The average CO_2 toxicity threshold ranges from 10% to 30% depending on plant and storage factors. Crisp head lettuce in storage with elevated CO_2 is strongly affected by O_2 concentration (Stewart and Uota 1972); however, this is not the case for romaine lettuce (Lipton 1987). Cultivation conditions such as irrigation, climate, and fertilization can modify plant tissue susceptibility to CO_2 injury. Krahn (1977) found that the outer leaves of crisp head lettuce are not injured by 2% CO_2, but the inner leaves and midribs show damage. Also, shredded head lettuce seems to tolerate higher levels of CO_2.

The effect of CO_2 on cell ultra-structures (Frenkel and Patterson 1974) and membranes (Sears and Eisenberg 1961) could account for its toxicity. It can be postulated that CO_2 dissolution, which enhances acidity in the cell medium, may participate in the physiological disorder. Optimum concentrations of O_2 and CO_2 should minimize the respiration rate without danger of anaerobic metabolism. Commodities vary widely in their tolerance of different atmospheres (Lougheed 1987). A classification of fresh fruits and vegetables according to their tolerance to reduced O_2 and elevated CO_2 has been presented by Kader et al. (1989). Yet little is known about the atmosphere requirements of minimally processed commodities.

Although many polymeric films are available for packaging purposes, relatively few have been used to wrap or pack fresh fruits and vegetables. Until recently none exhibited suitable permeabilities for commodities with high 0_2 requirements. Proper permeabilities to both O_2 and CO_2 should range for the most susceptible plant tissues from 6000 to 150,000 ml $m^{-2}.atm^{-1}.day^{-1}$ and up.

The extent to which the MA differs from the external atmosphere is determined primarily by the permeability of the polymeric film, the ratio of its area of gas diffusion to the mass of the plant tissue, the respiration rate of the enclosed product, and the package headspace. The gas diffusion rate through the polymeric film is proportional to the difference in partial pressures between the internal and external media.

Table 5.1 Effect of film permeability on fresh minimally processed grated carrots stored for 2 days at 10 °C

Films	D950	A	B	C	D
Thickness (μm)	40	30	30	30	30
Permeability[a] to O_2	6060	6000	9000	11,000	22,000
Permeability[a] to CO_2	18,000	6000	9000	11,000	22,000
Gaseous composition at steady state (n = 5)					
CO_2 (%)	19.6	21.6	19.7	27.0	16.5
O_2 (%)	1.5	1.6	2.2	1.6	5.1
Respiration rate in air (mol/kg h)	2.2	2.3	2.1	2.6	1.8
Confidence interval at 5% level (n = 5)	±0.04	±0.10	±0.11	±0.19	±0.11
(RRp/RRa) × 100	3	11	33	27	61
Respiratory quotient inside the pack	17	6.2	1.5	2.2	1.2

From Carlin et al. (1990b)
RRp respiration rate pouch, *RRa* respiration rate air
Supplier D950: Grace-Cryovac, Epernon, France
Supplier A, B, C, and D: Courtaulds Packaging, Avignon, France. "Permeability to gas in $ml.m^{-2}.day^{-1}.atm^{-1}$ – Atari, at 25 °C

This gas flow tends to compensate respiratory exchanges. Mathematically, the two phenomena should generate within the package a steady-state MA. As shown in Table 5.1, the respiration rates of grated carrots after 2 days at 10 °C in pouches in the least permeable films, D 950 and A, are the most reduced, respectively, to 3 and 11% of that of the control that is placed in air. The respiratory quotient with these films reached a value above 6, indicating a shift to anaerobic metabolism. In B, C, and D films, the RQ was similar to the RQ of control in air that is about 1.5.

Gas exchanges might also be affected by the metabolism of microorganisms present on grated carrots. Because the respiration of grated carrots does not markedly increase during 2-day storage at 10° C and the number of mesophilic aerobic bacteria increases from 10^5 to 10^9 per gram, the contribution of microorganisms to gas exchange is small in pouches stored for only 2 days (Carlin 1989)

The relationship between MA composition and respiration rate is unclear. For example, the CO_2-O_2 concentrations were similar in A and D 950 films (Table 5.1), whereas the respiration rate (RR) was 10 times higher in film A. The O_2 and CO_2 concentrations within packs cannot fully account for the actual turnover of these gases. Conversely, there is a good relation between the respiratory quotient of the commodity and the gas turnover within the package.

This is in accordance with the results of Tomkins (1967), who assumed that MA conditions can alter the RQ which in turn affects the atmosphere created by the respiration of the commodity within the package (Carlin et al. 1990a).

Passively modified atmospheres develop very slowly in pouches of vegetables whose O_2 consumption rate is low, such as lettuce or endive. Biochemical reactions may cause deterioration long before an efficient equilibrated MA can be established and shelf-life is only slightly extended.

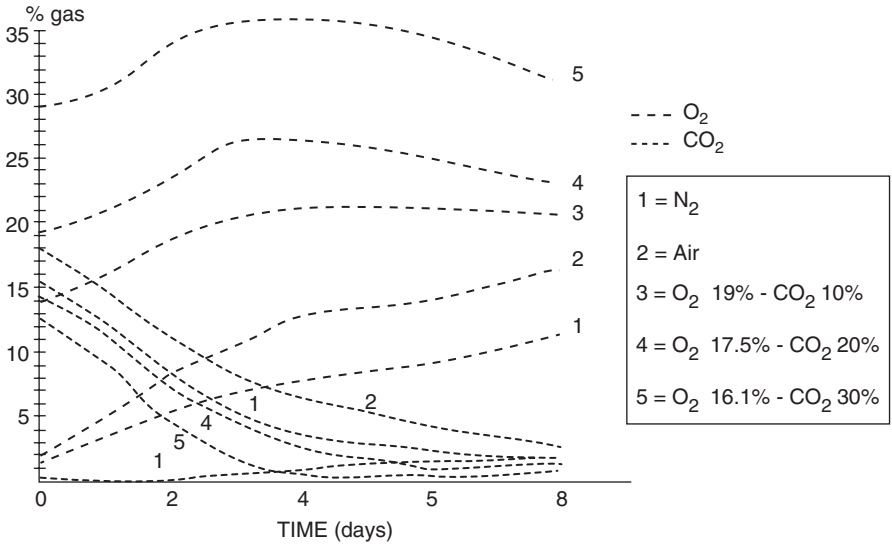

Fig. 5.12 Effect of initial atmosphere composition on gas concentration changes within polypropylene packs of shredded endives versus duration of storage at 10 °C (From Chambroy 1989)

The atmosphere may be modified initially just before sealing. This can be done by pulling a slight vacuum and injecting a controlled gas mixture or by flushing with the same gas mixture. Flushing is less efficient than compensated vacuum, but it is compatible with high-speed filling machines, and for fragile plant organs, it is less detrimental to tissue integrity.

Figure 5.12 shows the gas composition changes in shredded endives packed in polypropylene (40 μm) at 10 °C as a function of time and percentage of CO_2 in the mixture injected at sealing. In this example, O_2 consumption rates are not markedly affected by additional CO_2, but for elevated CO_2 concentrations, diffusion rates exceed CO_2 production through respiration, and therefore the CO_2 concentration decreases. In endives, anaerobic metabolism seems difficult to trigger because injection of pure N_2 in the bags results in a decrease in CO_2 production rate compared to other samples (Chambroy 1989).

Several workers have attempted to model the interaction between foodstuff respiration and package atmosphere in an effort to provide an analytical basis for MAP design (Kader et al. 1989). As stated by Zagory and Kader (1988), prediction of the equilibrium gas composition and the time taken to reach equilibrium should take into account at least:

1. The effect of changing O_2 and CO_2 concentrations on respiration rate
2. The effect of switching, even partially, to anaerobic metabolism
3. The permeability of the film to O_2 and CO_2 (and the effect of moisture on the gas diffusion coefficients)
4. The effect of temperature on film permeability to both O_2 and CO_2 and on the respiration rate

5. The surface area and headspace of the package
6. The resistance of the commodity to gas diffusion through its tissue
7. The optimal atmosphere for the commodity of interest, including biochemical reaction and microorganism growth

No model to date has integrated all of these variables.

Mathematical equations that fit gas composition changes in packaged plant organs have been developed recently (Cameron et al. 1989; Yang and Chinnan 1988). All models are based on two ordinary first-order differential equations representing first the gas exchange through polymeric films and second the plant tissue respiration; the gas exchange through the film or through perforations placed between two volumes obeys Fick's law (Emond et al. 1991). The respiration rate of living tissues is affected by the atmospheric composition. But Henig and Gilbert (1975) found with packaged tomatoes that the O_2 consumption rate with complete absorption of CO_2 was constant in the range of 11–21% O_2; below this value, O_2 consumption rate decreased linearly with O_2 concentration. Henig and Gilbert (1975) also claimed that when CO_2 accumulation occurred concomitantly with O_2 reduction, there was a significant but surprisingly low reduction in the O_2 consumption rate. They therefore suggested that two straight lines could be used to approximate the relation between O_2 consumption rate and O_2 concentration (Also see Chap. 2.)

This model was dismissed by Cameron et al. (1989) as approximate and not based directly on O_2 measurements. Nevertheless, their own model is valid for scientific purposes but is of little use for optimizing parameters for MAP.

By using the Henig and Gilbert (1975) approach, it becomes possible to solve the two differential equations:

$$X\left(\mathrm{O}_2\right) = \frac{KSx_0}{KS+\alpha m} + \frac{\alpha m x_0}{KS+\alpha m} e^{\frac{-(KS+\alpha m)t}{V}} \tag{5.1}$$

when $t \to \infty$ $XO_2 \to O_2(EMA)$

$$\frac{1}{\mathrm{O}_2\left(EMA\right)} = \frac{\alpha m}{KSx_0} + \frac{1}{x_0} \tag{5.2}$$

where

X = concentration O_2 (%) at time t
x_0 = initial concentration of O_2
K = O_2 diffusivity through the film
S = surface area of the film
V = headspace
m = weight of plant tissue
t = storage time
α = proportionality between respiration intensity and O_2 concentration

The approximate Eq. (5.1) is not useful in practice, but as time passes, the O_2 concentration tends toward the steady-state concentration (equilibrated modified atmosphere, EMA).

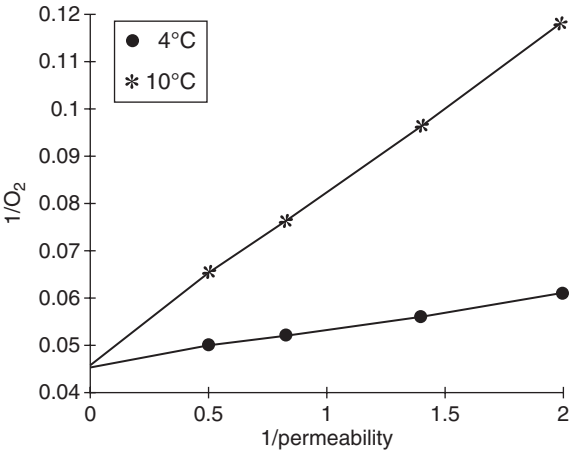

Fig. 5.13 Double-reciprocal plot of O_2 concentration (%) within pouches at steady state (EMA) and film permeabilities (ml O_2/m^{-2} . atm^{-1} . day^{-1}). Packaged whole mushroom stored at 4 and 10 °C (From Lopez-Briones 1991)

Equation (5.2) shows that the reciprocal of O_2 at steady state is directly proportional to the reciprocal of the film permeability. This model was validated with experimental data concerning apricots stored under MA (Chambroy et al. 1990) and common mushroom (Lopez-Briones 1991). Figure 5.13 shows that experimental data on MAP mushrooms fit the model reasonably well over a very large range of film permeability. However, there are great differences between mathematical and experimental data for CO_2 content within the pouches as already stated by Hayakawa et al. (1975).

It has been recently demonstrated that the approximate model is not valid for MA containing less than 3% O_2 or over 15% CO_2. The proportionality between respiration rate and O_2 concentration cannot be extrapolated to anoxic conditions.

It may be assumed that apricot and mushroom metabolisms rapidly switch to partial anaerobic metabolism under MA storage. The metabolism switch has little effect on O_2 concentration but markedly affects CO_2 production. These models permit good predictive estimations of generated MA as a function of simple physical parameters such as surface area, thickness of the film, and weight of tissue. This approach may prove useful once the optimal film permeability has been determined by CA and experimental testing.

As described above in the review of physiological disorders, slicing plant tissue may increase ethylene production, especially in pre-climacteric fruit. High CO_2 levels inhibit the action of ethylene so that plant tissues do not respond to the presence of this compound (Burg and Burg 1969) and reduce ethylene synthesis (Buescher 1979).

Conversion of 1-aminocyclopropane-carboxylic acid to ethylene catalyzed by ethylene-forming enzyme is an oxidative reaction and is therefore reduced at low oxygen partial pressure (Kader 1980) and inhibited under anaerobic conditions (Yang 1985). Curiously CO_2 inhibits ethylene production during tomato ripening but has little or no effect on wound-induced ethylene production by tomato (Buescher 1979). A similar result was found by Rosen and Kader (1989) with sliced pear.

The effects of ethylene neoformation on physiological and biochemical changes in MPR fruits have yet to be investigated

5.5.2 *Effects of MA on Biochemical Reactions in MPR Fruits and Vegetables*

MAs may inhibit enzymatic systems responsible for deterioration in quality during storage but may also reduce tissue senescence and microbial spoilage, both of which result in cellular delocalization.

5.5.3 *Effect of MA on Enzyme Activities*

Lowering O_2 in a storage atmosphere reduces the reaction rate of enzyme-catalyzed oxidations because O_2 is a substrate (Murr and Morris 1974). Polyphenoloxidase and tyrosinase, the enzymes responsible for brown discoloration of plant tissue, have a low affinity for O_2 compared with cytochrome oxidase. Hence, the packaging of plant tissues with high browning potentiality under vacuum or nitrogen in high-barrier film prevents any discoloration even after 10 days at 10 °C, but the process may trigger anaerobic metabolism and growth of lactic acid bacteria (Varoquaux and Varoquaux 1990).

Mazollier et al. (1990) claim that nitrogen flushing shredded lettuce before sealing reduces browning of the sliced surfaces but enhances the risk of lactic fermentation. This confirms the results of Ballantyne et al. (1988a), who optimized color stability of shredded lettuce with MA stabilized at 1–3% 0_2 and 5–6% CO_2. Color change in broccoli florets is minimal in a 2–3% O_2, 2–3% CO_2 equilibrium MA, but lower concentrations result in off-odors that markedly shorten shelf-life (Ballantyne et al. 1988b). Because of the experimental design of the reported research, the effect of low 0_2 cannot be separated from the effect of increased CO_2.

CO_2 may inhibit polyphenoloxidase activity (Murr and Morris 1974), but the direct inhibition of this enzyme was not fully demonstrated. Another important effect of CO_2 is increased acidity in plant tissues. Because intracellular pH values are normally regulated within narrow limits, only elevated CO_2 concentrations (as high as 5%) will lower intracellular pH. Bown (1985) proposed that the accumulation of respiratory CO_2 is responsible for the reduction in pH, as dissolved CO_2 diffuses slowly compared to gaseous CO_2. Bertola et al. (1990) determined that the specific resistance to CO_2 diffusion of tomato peel was about 200 times as great as that of the stem scar. Dissociation of carbonic acid into bicarbonate and hydrogen ions could affect the activity of enzymes. Tolerance of plant tissues to CO_2 can be determined by their buffering capacity. Using nuclear magnetic resonance, Siriphanich and Kader (1986) estimated cytoplasmic and vacuolar pH in lettuce tissue as affected by elevated CO_2 concentrations. Lettuce exposed to air at 20 °C and then stored for 6 days at 0 °C with 16% CO_2 in air showed pH decreases of about 0.4 and 0.1 units in the cytoplasm and vacuole, respectively.

Since MRP fruits and vegetables are stored at low positive temperatures and peripheral tissues are bruised, dissolution of CO_2 should be much greater than in

intact organs kept at ambient temperature. This acidification could explain either the marked reduction in activity of enzymes under MA or the phytotoxicity of CO_2. High CO concentrations reduce texture loss of strawberries even after transfer of the berries to air (Kader 1986).

Maintenance of firmness of strawberry in MAP is the result of the improvement of the physiological conditions compared to normal air storage. CAs retard senescence and delay softening of fruit (Knee 1980). MAP does not alter the softening rate of kiwi slices, demonstrating that atmosphere composition has no effect on pectinolytic or proteolytic enzymes (Lecendre 1988).

5.5.3.1 Effect of MA on the Microbiological Spoilage of MPR Fruits and Vegetables

Published reviews have mentioned the effects of MAP and CA on the postharvest diseases microorganisms can cause (Eckert and Sommer 1967; El-Goorani and Sommer 1981; Harvey 1978; Lougheed et al. 1978; Smith 1963).

Microbial deterioration of minimally processed fruits and vegetables is covered in Chap. 19. Here we review the interdependence of the physiological, biochemical, and microbial spoilage mechanisms as affected by processing and packaging techniques. Interaction among these mechanisms will be analyzed for two models: grated carrots and shredded endives. These commodities account for about 85% of ready-to-use fruits and vegetables sold in France (overall production: 30,000 tons in 1989) (Varoquaux et al. (1990).

Spoilage of Grated Carrots

Loss of firmness and off-flavor occurring in spoiled grated carrots are associated with the following characteristics: MA with excessively high CO_2 levels (over 30%) and low O_2 levels (below 15%), high number of lactic acid bacteria and yeasts, and production of ethanol, acetic, and lactic acids. Therefore, the deterioration of fresh grated carrots is typically a lactic acid fermentation, which can spontaneously occur in preparations of fermented sliced carrots (Andersson 1984; Niketic-Aleksic et al. 1973).

All isolated lactic acid bacteria were identified as *Leuconostoc mesenteroides,* commonly present on plants (Mundt and Hammer 1968) and on minimally processed vegetables (Denis and Picoche 1986). However, initial contaminations by *L. Mesenteroides* do not markedly differ from one pack to another, so the count of this bacterium at packaging is not sufficient to determine the storability of the commodity (Carlin et al. 1989).

The next step in the attempt to improve grated carrot shelf-life is to study spoilage mechanisms under CAs. Carlin et al. (1990b) demonstrated that the growth of both lactic acid bacteria and yeasts on grated carrots was faster when CO_2 content increased from 10% to 40%, regardless of O_2 concentration (Fig. 5.14). Lactic acid bacteria were not found to be the primary cause of spoilage since the growth of

L. Mesenteroides on a sterile medium is unaffected by low O_2 content (Lucey and Condon 1986) or by high CO_2 concentrations (Fig. 5.15). Storing grated carrots in a CO_2-enriched CA produces high K^+ leakage. Other electrolytes and nutrients, especially sugars, are exuded.

Leakage of alpha-amino compounds was measured by Romo-Parada et al. (1989) to determine the increase in membrane permeability of cauliflower during storage

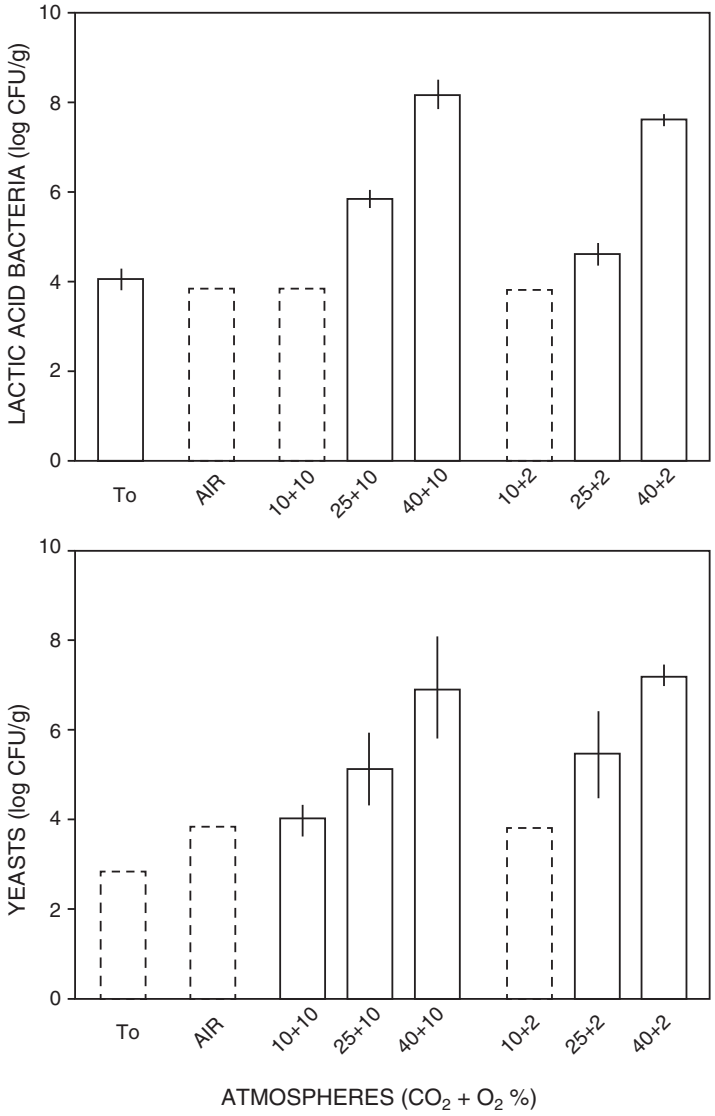

Fig. 5.14 Counts of lactic acid bacteria and yeasts on grated carrots under controlled atmospheres after 10 days of storage at 10 °C. Counts in *dashed lines* were below the detection level, comparng to initial count. *Bars* represent standard deviation (From Carlin et al. (1990b)

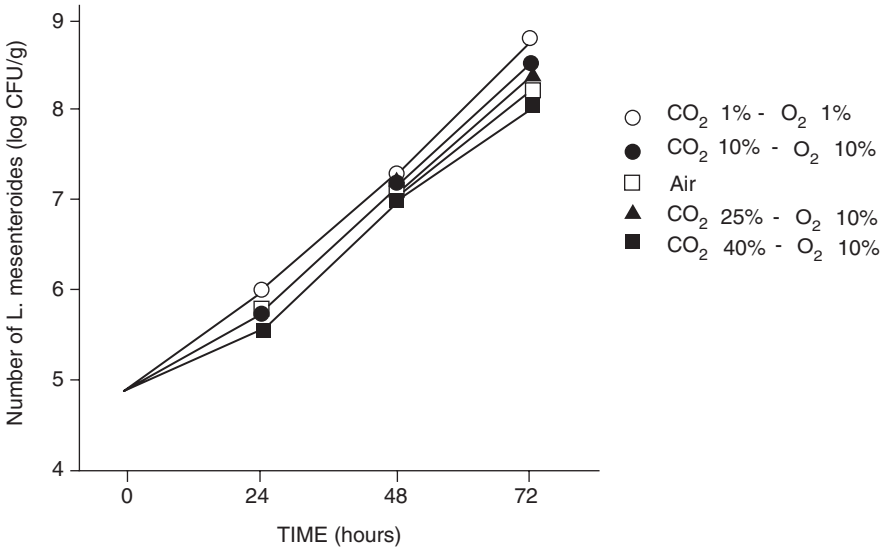

Fig. 5.15 Growth at 10 °C of *Leuconostoc mesenteroides* on a sterile carrot medium under controlled atmospheres (From Carlin et al. (1990c)

under various CAs. They found that O_2 in the CA did not affect leakage, but CO_2 over 10% significantly enhanced it. This exudate provides a substrate for microbial growth (Tomkins 1962). Moreover, Atkinson and Baker (1987) have shown that the activation of K^+/H^+ exchange in beans by *Pseudomonas syringae cv syringae* induces host plasmalemma transport of sucrose and allows the proliferation of the pathogen in intercellular spaces.

Potassium leakage is lower in CA containing both 10% CO_2 and 10% O_2 than in air. In the same way, the catabolism of sucrose, whose concentration is the main factor in the taste quality of carrot (Rumpf and Hansen 1973), is lower in 10, 25, and 40% CO_2, 2% O_2,and in 25% CO_2 and 10% O_2 than in air or in other CAs (Fig. 5.16).

Thus, MAs containing 15–20% CO_2 and 5% O_2 would retard the senescence and microbial spoilage of grated carrots by reducing their physiological activity (Carlin et al. 1990c). These results confirm that the film currently used in France for packaging grated carrots, namely, polypropylene, 40 μm in thickness, is not permeable enough to both O_2 and CO_2 to ensure good preservation of the commodity.

Ethanol production in the headspace of grated carrot markedly decreases when the film permeability to gases increases. Ethanol is a good marker of spoilage though it may be either a by-product of anaerobic fermentation or an end-metabolite of several microorganisms or both. As expected, grated carrots packaged with the least permeable film (permeability to $O_2 < 6000$ ml·m^{-2} ·atm.$^{-1}$ ·day^{-1})

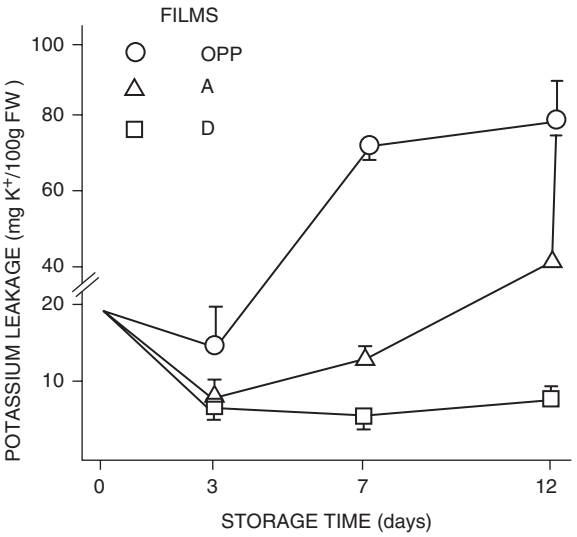

Fig. 5.16 Potassium leakage from carrots in pouches as a function of storage time at 10 °C for different film permeabilities (From Carlin 1989)

Table 5.2 Effect of film permeability on the quality of minimally processed grated carrots stored at 10 °C

Films			
	Polypropylene	P-Plus A	P-Plus-D
Permeability to O_2 ml/m^2.day.atm	1,000	6,000	22,000
% of spoiled packs			
After 7 days	100	0	0
After 14 days	100	75	8

From Carlin et al. (1990c)

switched to anaerobic metabolism, with K^+ leakage as a consequence (Fig. 5.16). The use of highly permeable films (20,000 ml·m^{-2} ·atm.$^{-1}$ ·day^{-1} and over) results in a better physiological condition of the commodity and, therefore, prevents any microbial spoilage.

Conversely, these highly permeable films favor a high respiration rate (about 1 mmol O_2 kg^{-1} h^{-1}) and induce a faster consumption of carbohydrates which causes a noticeable loss in palatability of the carrots. It must be noted that the MA generated in packs depends on the storage temperature. At low temperature (about 2 °C), physiological activity and microbial growth are reduced sufficiently to delay the development of spoilage, even with the least permeable film. But at storage temperatures at 10 °C, the use of highly permeable films such as P-Plus C is justified to reduce spoilage (Table 5.2).

Table 5.3 Effect of CA on the development of soft rot on inoculated endive leaves

CA			Inoculum		
				Filtrate	Filtrate
% CO_2	% O_2	H_2O (control)	*Ps. m* (heavy)	*Ps. m*	A. *niger*
40	10	0	0	0	+ + +
20	10	0	0	(+)	+ + +
0	22	0	+ +	+ +	+ +

From Nguyen-The and Carlin (1989)
Ps. m, Pseudomonas marginalis; *A. niger, Aspergillus niger*; 0, no spoilage; (+), no browning, slight soft rot; + +, browning soft rot; + + +, general browning and soft rot

Spoilage of Shredded Endives

Phytopathogens such as *Erwinia carotovora* have been found on pre-packed fresh vegetables but are not markedly pathogenic in minimally processed commodities (Lewis and Garrod 1983). Pectinolytic *Pseudomonas fluorescens* and *Pseudomonas viridiflava are* well known as soft rot bacteria on stored vegetables (Lund 1983). They may also induce spoilage of shredded endives (Nguyen-The and Prunier 1989). Strains of pectinolytic *Pseudomonas marginalis* isolated from minimally processed Shredded endives show a strong spoilage capacity on the commodity though these bacteria are present in both spoiled and apparently sound packs (Nguyen-The and Carlin 1988). CA enriched in CO_2 up to 50% reduces the in vitro growth of pseudomonads and *Erwinia* spp. (Fig. 5.17). Surprisingly, the same CA does not modify epiphytic proliferation of *Pseudomonas marginalis* on salad leaves, but CA or MA containing over 15% CO_2 reduces or eliminates soft rot on endive leaves that were previously inoculated with a heavily concentrated *Pseudomonas marginalis* suspension (Table 5.3). The same phenomenon is observed when leaves are inoculated with a sterile growth medium of *P. marginalis.* The presence, in the ultrafiltrate, of active pectinolytic enzymes, can account for the considerable soft rot also shown in Table 5.3. Increasing CO_2 to 20% reduces the necrosis, and at 40% it prevents any damage.

It is remarkable that MA, with high CO_2 concentrations up to 40%, have no effect on soft rot induced by *Aspergillus niger* growth medium (Nguyen-The and Carlin 1988). Since *P. marginalis* produces pectate lyases (Lund 1983) with an optimum pH of 6–8, and *Aspergillus niger* a polygalacturonase active at pH 4–5, it is postulated that the beneficial effects of $C0_2$ on soft rot induced by *P. marginalis* are due to the acidification of cell medium provoked by dissolved $C0_2$ (Table 5.3).

Deterioration of shredded endives by both pectinolytic and lactic acid bacteria can be prevented by storing and marketing the commodity under a MA containing 20–30% of $C0_2$ and 1–3% of O_2 depending on the maturity stage and cultivation conditions of the raw endive. A MA suitable for shredded endives can be created by the use of a polypropylene film (35-40 μm thick), provided that the storage and distribution temperature never exceeds 10 °C with a sell-by date of 1 week, which may be too short for North American markets.

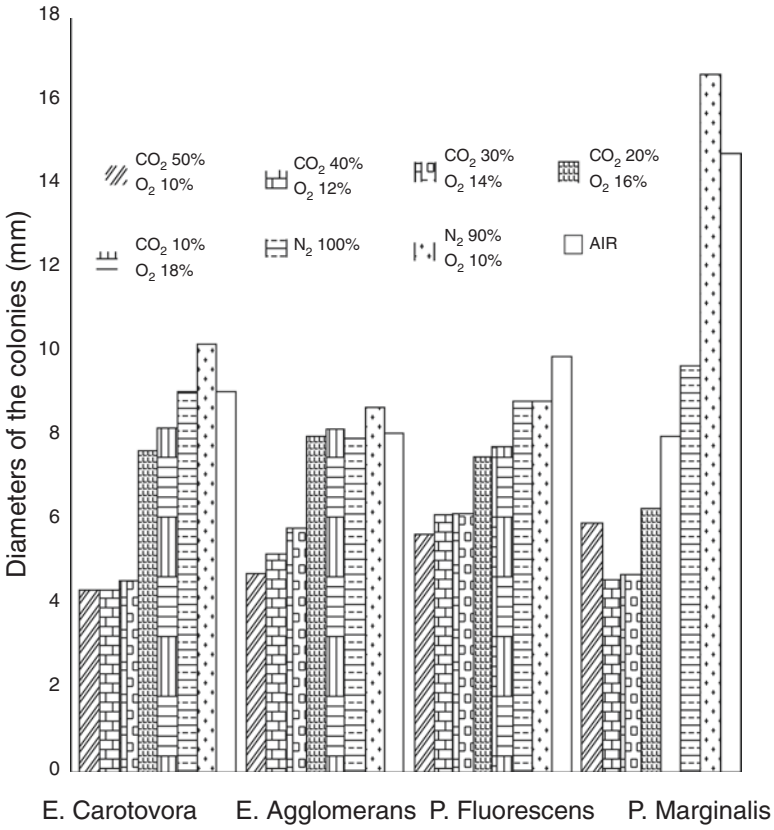

Fig. 5.17 Effect of CO_2 on the in vitro growth of bacteria isolated from minimally processed endive. The percentage of $C0_2$ varies from 10% to 50% (Colony diameter in mm on LPGA after a 9-day incubation at 10 °C) (From NGuyen-The and Prunier 1989)

5.6 Conclusions and Future Directions

The mechanisms of MPR fruit and vegetable deterioration are similar to those of intact plant organs, observed differences being quantitative and not qualitative.

Development of these new commodities for the retail market is strongly dependent on their microbiological safety and on freshness.

Freshness may be improved by two nonexclusive means: (1) adaptation of processing technology to the raw material and (2) matching of the raw material to the processing and preservation methods. Both approaches rely on multidisciplinary research that simultaneously uses the basic principles of plant physiology, biochemistry, microbiology, and engineering. Examples include attempt to minimize browning of endives by selection of cultivars and determination of the optimal harvest time.

The suitability of carrot cultivars for "ready-to-use" processing has also been investigated (Hilbert 1990), and the susceptibility of carrot tissue to both increasing

CO_2 and decreasing O_2 was found to be the primary spoilage factor. Current investigations are focused on the mechanisms of cell derealization in ready-to-use commodities.

5.6.1 Optimization of Processing of MPR Fruits and Vegetables

Optimization of ready-to-use processing method requires:

- Limitation of initial bruising of plant tissues
- Minimization of wound injury during peeling and cutting and other size reduction operations
- Determination of optimal draining conditions to remove moisture
- Identification of an optimal MA which slows senescence, enzyme activity, and microbial growth but that does not trigger anaerobic metabolism (this must be studied for each commodity)
- Ensuring refrigerated temperatures by using temperature-time indicators (TTI) placed on packaging.

Recent investigations have pointed out the importance of MA composition on the spoilage mechanisms of plant tissues. For some commodities, the permeability of commercially available film is not high enough for both O_2 and CO_2 to match their respiratory requirements. New films have recently been developed in the United States and England and tested in France. Curiously, intact plant organs with high respiration rates such as asparagus, spinach, mushroom, and to a lesser extent cauliflower and broccoli are the first to benefit from these new films.

5.6.2 Matching of Raw Material and Processing Requirements

Minimal processing is too young an industry for the plant geneticists to have selected or created cultivars and hybrids adapted to its specific requirements. The first step is to define selection criteria in terms of objective chemical or physical determinations as established for fruits and vegetables for freezing or canning. This may prove a difficult task. For example, the browning potential of blended tissues is proportional to their phenolic compound content (Carlin et al. 1990a). Phenolics in plants such as peaches (Lee et al. 1990) and endives (Varoquaux et al. 1991) *generally decrease during maturation*, theoretically leading to reduced sensitivity of overmature fruits and vegetables to enzymatic browning. However, it is well established that overripe produce scores poorly in MPR processing. Biochemical parameters are not the limiting factors of the brown discoloration of MPR commodities.

Cellular derealization seems to be the key mechanism that induces enzymatic browning of sliced plant tissues (Watada et al. 1990). The most promising measurement would be rapid testing of susceptibility to cell derealization. This requires more basic research into the cell response to various stresses.

5.6.3 Matching of Raw Material and Processing Requirements

5.6.3.1 Injection of Various Gases at Packaging

Carbon monoxide is utilized in the United States for long-distance bulk transportation of some fruits and vegetables. The toxicity of this gas is not compatible with its use in retail packaging for distribution in Europe.

The effects of nitrogen protoxide on MPR fruits and vegetables are still unclear, but a beneficial effect on the browning of sliced apples and peeled potatoes has been reported. Further investigation of the effect of this gas on bacterial growth and enzyme activities is needed.

5.6.3.2 Ionization

Ionization with gamma radiation or accelerated electron beams allows disinfection of pre-packed minimally processed commodities. However, even at very low doses (0.5–1 kGy), irradiation induces dramatic softening and off-odors. The effect of irradiation on prepacked salads is still controversial.

5.6.3.3 CA Atmosphere Packaging

Gas composition in CA packaging can be actively regulated by means of O_2 and CO_2 generators or scavengers (Lioutas 1988; Myers 1989). This technique is well adapted to nonrespiring produce. The feasibility of CA packaging for fresh plant tissues seems very remote.

Research carried out in Europe on highly perishable products aims to achieve a shelf-life of 1 week or 10 days at most. As pointed out by Lioutas (1988), the same operation will require a shelf-life of 21 days to have a chance in the US market. Such a goal, if realistic, would be a tremendous challenge for fundamental and technical researchers.

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Chapter 6
Preservation Methods for Minimally Processed Refrigerated Fruits and Vegetables

Robert C. Wiley

6.1 Introduction

The preservation of foods, an important manufacturing step that is used to provide food safety, maintain quality, extend shelf life, and prevent spoilage, has long been called "food processing." In the context of this book, "process" is an operation or treatment and, especially in manufacture, a procedure for forward movement such as cutting, slicing, dicing, washing, etc. (Anon., Webster's 1987). To "preserve" is the act or process of preserving, by canning, pickling, or similarly preparing food for future use (Anon., Webster's 1987). Preservation methods then are the "actual" acts of preserving to reduce spoilage. Nicolas Appert in 1810 was probably the first person to explain preservation methods primarily by heating in his treatise "The Art of Preserving Animal and Vegetable Substances." He originally stated (before the completion of his work) that preserving foods could be reduced to two principal methods, "one in which desiccation is employed and the other in which more or less of a characteristic foreign substance is added to prevent fermentation and putrefaction." In his book the latter treatment refers to the use of sugar, vinegar, or salt. His primary method of preservation was "1st. to enclose in the bottle or jar the substances that one wishes to preserve; 2d, to cork these different vessels with the greatest care because success depends chiefly on the closing; 3d, to submit these substances thus enclosed to the action of boiling water in a water-bath for more or less time according to their nature in the manner that I shall indicate for each kind of food; 4th. to remove the bottles from the water-bath at the time prescribed." This method is obviously heat preservation; current synonyms are canning, thermal processing, or heat processing.

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The inception of minimally processed refrigerated (MPR) foods that in one way or another may undergo all of the preservation methods referred to in Appert's book, and the many more to follow in this text are examples of the great sophistication that has developed in the field of food science and technology over the last 200 years.

Some workers in the field tend to equate the word preservation before, during, or after packaging with (1) near endpoint destruction of all microorganisms and (2) endpoint destruction of all enzyme systems. Others use a broader interpretation of the word preservation to describe a procedure in which some but not all species of microorganisms are reduced in count and specific enzyme systems may be partially or fully inactivated in the package or prior to packaging. In this text, MPR preservation is considered in the latter context and is developed with the purpose of providing maximum food safety, like-fresh quality, and substantial increase in shelf life.

The question arises whether packaging per se is a preservation method. Packaging acts as a vehicle to protect or provide a suitable atmosphere for MPR fruits and vegetables but does not usually constitute the "actual" act of preserving (see Chaps. 4, 5, and 7). The actual preservation method, however, may precede packaging or may occur by treating the food material and packaging simultaneously. Other options include treatment immediately after packaging or continuously during the shelf life period. In the latter case, it is the modified atmosphere developed during packaging that gives a degree of preservation. Refrigeration also is a good example of this land of situation. Packaging is inherent in the protection of food to prevent spoilage, and this idea seems to have been put forth first by Appert (1810).

MPR fruits and vegetables must be held continuously at refrigeration temperatures and guarded from temperature abuse in distribution and retailing. Refrigeration probably should be considered an active act of preservation which is well known to reduce adverse quality and nutritional changes and greatly extend shelf life of many food product types including dehydrated, canned, and irradiated foods as well as minimally processed fruits and vegetables. Refrigeration itself is not a requirement, however, for dehydrated, canned, and some other types of food products.

Preservation of MPR fruits and vegetables is especially complex in that treatment is required for damaged or killed plant cells as well as those cells that are intact and not wounded or damaged (Fig. 6.3). In other words, some cells are respiring at normal rates, some damaged cells may be respiring at very high rates, and other cells are virtually dead or inactive (Rolle and Chism 1987). The volume-to-surface ratio of a whole minimally processed product that has been subjected to size reduction operations, for example, might be used to give an accurate prediction of the kind of preservation methods that would be most effective to extend shelf life. Human and plant pathogens as well as endogenous enzyme systems found in fruits and vegetables should be susceptible to the "hurdles" or "barriers" concept (Scott 1989).

Preservation methods to extend shelf life of MPR fruits and vegetables can utilize many of the classic procedures to preserve foods. These well-known methods which may be used to target MPR foods include heat preservation, utilizing mild heat treatments with quick cooling; chemical preservation, including acidulants, antioxidants, chlorine, antimicrobials, and the like; gas and controlled modified atmosphere

preservation; refrigeration preservation and preservation by irradiation; oxidation-reduction (O/R) potential preservation; and, in some cases, moisture reduction by lowering water activity (a_w), which in MPR fruits and vegetables would seriously reduce turgidity and crispness of the product. Combinations of the above preservation methods in specific or random order, taking advantage of the synergisms of the various preservation hurdles or barriers, may be used. These barriers have to consider intact or damaged enzyme systems in the living tissues, particularly polyphenol oxidases (PPOs), peroxidases (POs), and the various pectinases, polygalacturonases (PGs), and pectinesterases (PEs) and respiratory-related enzymes (Table 6.1).

Freezing preservation should not be used for most MPR foods because freezing tends to cause changes in texture and other like-fresh characteristics. However, from a safety standpoint, it is clear that certain MPR foods (entrees) are being frozen to avoid regulatory problems in the United States. Freezing is a preservation method that manufacturers should not use if they wish to market an authentic MPR food. Precut salads, sliced tomatoes, fresh soup mixes, and the like do not lend themselves well to freezing as an alternate preservation method because of the abovementioned quality constraints. This is not an attempt to downgrade freezing as a preservation method, but is simply intended to emphasize that MPR fruits and vegetables should be considered a specific food product type. A number of unit operations are performed before freezing on fruits and vegetables destined for freezing preservation that yield high-quality frozen food (Desrosier and Tressler 1977).

Table 6.1 Enzymes related to food quality

Enzyme	Catalyzed reaction	Quality defect
Flavor		
Lipolytic acyl hydrolase (lipase, esterase, etc.)	Hydrolysis of lipids	Hydrolytic rancidity (soapy flavor)
Lipoxygenase	Oxidation of polyunsaturated fatty acids	Oxidative rancidity ("green" flavor)
Peroxidase/catalase	?	"Off-flavor" (?)
Protease	Hydrolysis of proteins	Bitterness
Color		
Polyphenol oxidase	Oxidation of phenols	Dark color
Texture consistency		
Amylase	Hydrolysis of starch	Softness/loss in viscosity
Pectin methylesterase	Hydrolysis of pectin to pectic acid and methanol	Softness/loss in viscosity
Polygalacturonase	Hydrolysis of x-1,4 glycosidic linkages in pectic acid	Softness/loss in viscosity
Nutritional value		
Ascorbic acid oxidase	Oxidation of L-ascorbic acid	Loss in vitamin C content
Thiaminase	Hydrolysis of thiamine	Loss in vitamin

From Svensson 1977

6.2 Microbiological and Enzyme Considerations to Prevent Spoilage of MPR Fruits and Vegetables

MPR fruits and vegetables with wounded or intact plant tissues, or both, require special manipulation of preservation methods for the purpose of extending storage life and preventing spoilage. These problems can relate to both microbe and enzyme control. In the recent past, conventional wisdom suggested that a refrigeration temperature from −2.2 °C to 4.4 °C (Anon. 1989a) would generally control outgrowth of pathogenic and spoilage psychrotrophic types (see Chap. 19). Today, there is considerable evidence that the low-temperature or refrigerator temperature treatment or storage of MPR foods is not enough to control some psychrotrophic types. It has been suggested that all refrigerated foods be divided into two categories for labeling purposes, and this approach tends to be helpful in attempting to classify MPR fruits and vegetables by preservation method (Anon. 1989a, b). They are as follows (Anon. 1989a):

- "Group A Foods: Highly perishable, packaged [minimally]* processed foods that must be refrigerated for safety reasons." [Most of the vegetable products]
- "Group B Foods: Products intended to be refrigerated that do not pose a safety hazard if temperature abused." [Most of the fruit products and properly acidified vegetable products]

"Products that possess all of the following attributes should be considered Group A products that could potentially cause a public health hazard if improperly handled:

1. Product has a pH >4.6; and
2. Water activity >0.85; and
3. Does not receive a thermal process adequate to inactivate foodborne pathogens, which could, through persistence or growth in the product, cause a health hazard under moderate conditions of temperature abuse during storage and distribution; and
4. Has no barrier(s) imparted by either intrinsic factors (e.g., presence of nitrites, salt content, the presence of competitive flora, etc.) or intrinsic factors (e.g., a heat treatment to control pathogens) scientifically demonstrated to eliminate or prevent the growth of food borne pathogens."

The above suggestions by the Microbiology and Food Safety Committee of the National Food Processing Association (NFPA) (Anon. 1989a) have been drawn up for all refrigerated foods and are helpful in discussing the preservation of MPR fruits and vegetables. Several aspects of these labeling requirements that are not addressed are the twin problems of respiration and enzyme activity which must be considered in successful handling of MPR fruits and vegetable to prevent spoilage and extend shelf life. This is more fully discussed in the postharvest physiology area (see Chap. 2).

An interesting approach to inhibit the growth of microorganisms in foods is the development of the "hurdle concept" developed by Leistner and Rodel (1976).

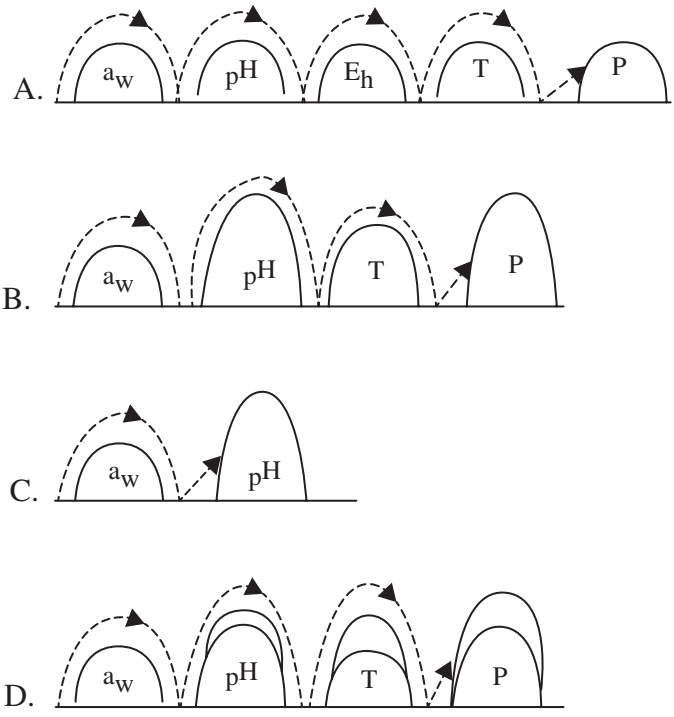

Fig. 6.1 The hurdle concept (From Scott 1989)

Scott (1989) has suggested that it is not productive from a quality standpoint to provide extreme treatment conditions to inhibit the growth of microorganisms in refrigerated foods. She suggests that suitable combinations of growth-limiting factors can be used for preservation, so that no growth can take place, and suggests that this may involve several subinhibitory treatments or preservation methods. Figure 6.1, taken from Scott (1989), diagrammatically presents the "hurdle concept."

In Fig. 6.1 it is assumed the products will be properly packaged to prevent the entry of microorganisms and they will be stored at the optimum refrigerated temperature for the specific fruit or vegetable. The examples are only for explanation; however, hurdles will have to be developed for the many individual products and formulas that will be minimally processed and refrigerated. Figure 6.1A shows five hurdles—a_w, pH, E_h, heat treatment, and a preservative—all at the same intensity (size of solid line arc). The preservative finally stops growth of the "weakened" microorganisms. Figure 6.1B shows several hurdles used on a product at different intensities with the microbes again unable to overcome the preservative hurdle. Figure 6.1C shows only two hurdles (a_w and pH) used to preserve a product. Figure 6.1D shows the synergistic effect in combining hurdles to provide greater difficulty for microbial reproduction. Because MPR fruits and vegetables are in large part "living" tissue, treatments to reduce or eliminate enzyme activity should be included in the microbe hurdle concept (Fig. 6.2).

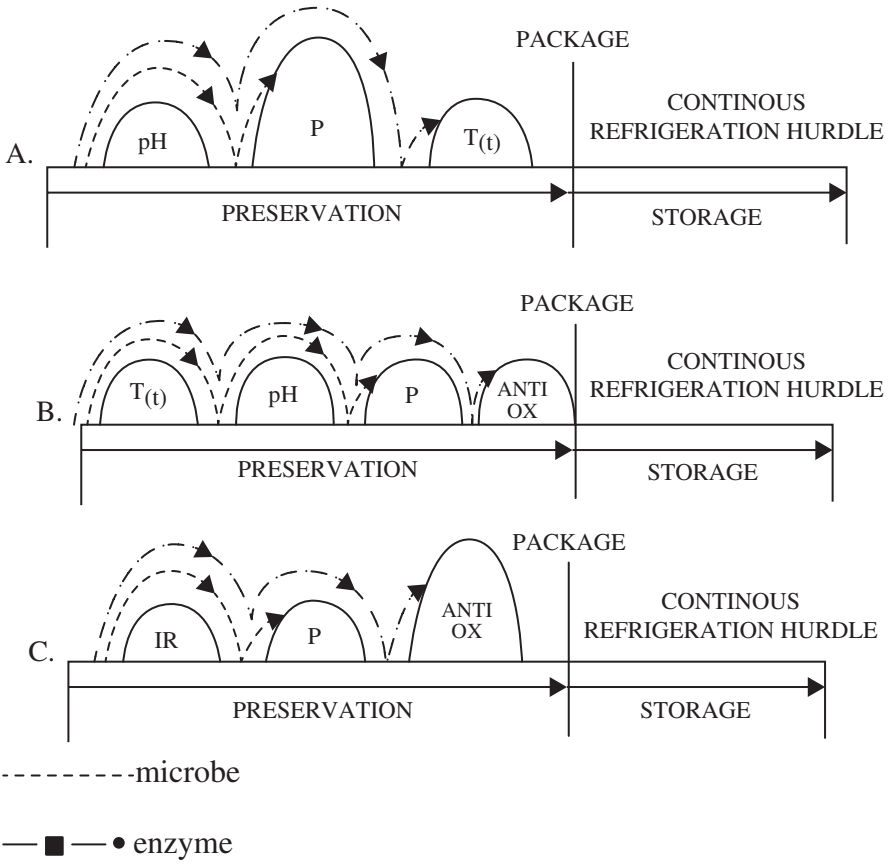

Fig. 6.2 Applying the hurdle concept to microbe and enzyme control in MPR fruits and vegetables. Enzymatic browning reaction

In Fig. 6.2A, a fruit product has a natural or added acidity to lower the pH to 4.6 or below, which will reduce microbial activity and increase the effectiveness of the added antioxidant. When an antioxidant such as ascorbic acid is added to inhibit PPO, it may not have much effect on the microbial systems present; however, as long as the ascorbic acid is present in the acid medium, little browning will take place. Finally a light heat treatment is provided to reduce the level of remaining enzymes and lower the number (count) of fungi, yeasts, and bacteria present. This product would be packaged and subject to refrigerated temperatures during storage, distribution, and marketing. This hurdle concept considers two levels of temperature for microbe and enzyme control, the initial heat treatment for a limited period and then low-temperature control at 4.4 °C (Anon. 1990) over an extended but undefined shelf life period.

In the example shown in Fig. 6.2B, a vegetable product may receive a short heat treatment; acidification to provide a more acid pH; treatment with a preservative to

reduce microbial activity, followed by an antioxidant treatment to reduce enzymatic browning; and finally refrigerated storage which would act as the final preservation barrier. If a packaged product is subjected to a modified atmosphere (MA) (Fig. 6.2C), a low-dosage radiation of a fruit and vegetable at the prescribed level, treatment with a preservative to reduce microbial activity, and a final treatment to reduce browning a refrigerated storage temperature could complete this preservation sequence. The information in Fig. 6.2A is illustrative of various preservation treatments. Refrigerated storage temperatures that improve the safety characteristics of MPR fruits and vegetables from a microbiological standpoint inhibit enzymatic activity and improve or sustain the quality of the product during the extended shelf life period. It appears that the hurdle or barrier concept should be factored in for both microbe destruction and enzyme inhibition. Preservation principles should be applied to both microbial and enzymatic (chemical) problems in MPR fruits and vegetables. This point is supported by Peri (1991), who suggested chemical hazards in fact are chemical or enzymatic reactions that cause oxidation, proteolysis, lipolysis, isomerization, polymerization, etc. in food products.

Fruit and vegetable technologies can draw on the experience with meats and poultry. The pathogenic microorganisms that present the greatest human safety problems in refrigerated foods containing meats and poultry are considered to be *Listeria monocytogenes*, *Salmonella* spp., and nonproteolytic and proteolytic *Clostridium botulinum* including the psychrotrophic species (Anon. 1990). Suggestions are made for a minimum 4-decimal (4-D) reduction of *L. monocytogenes* and the elimination of toxin production by *C. botulinum.* Most species of *Salmonella* would be destroyed by the 4-D reduction of *Listeria monocytogenes.* Similar recommendations are needed for MPR fruits and vegetables, particularly low-acid vegetables.

L. monocytogenes, which has been isolated in coleslaw (Schlech et al. 1983), lettuce (Radovich 1984), and cabbage juice (Conner et al. 1986), is a major organism of concern for MPR low-acid vegetables, because many of the hurdles discussed above applied to these foods to control *Listeria* have not been successful (Scott 1989). Unfortunately these have included low-temperature preservation treatments. On the other hand, high-temperature studies showed *Listeria* could not be isolated from milk heated to 76.4–77.8 °C for 15.4 s (Doyle et al. 1987), whereas Bunning et al. (1986) found a D-value of 1.6 s at 71.7 °C for the freely suspended bacteria in milk. D-values are the time it takes to reduce a microbial population by *90%* or the time it takes to make a similar reduction of specific enzyme activity at a defined temperature. The *z*-values are sometimes called the slope of the thermal resistance curve (Pflug and Esselen 1963). Also read Farber (1989). Heat treatment of this magnitude (to get 70–75 °C center temperatures) for many fruits and vegetables could be deleterious in preserving their like-fresh quality. When considering the possible presence of *L. monocytogenes,* it should be noted that MPR fruits have the best opportunity to provide safe products because of their natural acidities and pH values.

Plant pathogens provide problems for MPR fruits and vegetables, and control of these spoilage organisms must be considered in order to provide high-quality shelf

life (Chap. 7). Decayed and discolored tissues have to be eliminated from products destined to be minimally processed. The preservation treatments or hurdles applied to fruits and vegetables will be destructive to plant pathogens that have not been removed by peeling, trimming/cutting, etc.

Enzymes are of particular concern in MPR fruit and vegetable preservation. Table 6.1 summarizes the quality defects that may be caused by enzyme activity. The most important enzyme in MPR fruits and vegetables is PPO, which causes browning, usually a very undesirable reaction in terms of appearance. The second in importance from a quality defect standpoint are probably the endogenous and exogenous pectin methylesterases (PEs) and polygalacturonase (PG) which are related to softness, sloughing, and wholeness of fruit and vegetable tissue. The POs, catalases, and lipoxygenases, which are primarily associated with flavor changes, but also can be related to color changes in plant tissue, are also very important. In the freezing preservation of fruits and vegetables, the inhibition of PO, which is one of the most heat-resistant enzymes, is the standards of determining the adequacy of blanch (Desrosier and Tressler 1977).

6.3 Heat Preservation

Heat preservation, one of the oldest forms of preservation known to man, has potential to provide hurdles or barriers to reduce microorganisms and inhibit enzyme activity. The major problem in MPR fruits and vegetables is that heat is associated with destruction of flavor, texture, color, and nutritional quality. Heat resistance of *Listeria monocytogenes* appears to be greater after a light heat shock of about 48 °C which increased the D-value 2.2-fold at 55 °C in nonselective agar (Linton et al. 1990). Also, consideration probably should be given to whether heat-stressed spores and vegetative cells could recover better under MPR conditions than the non-heat-stressed organism. Heat may also reduce microorganisms that would be competitive with existing pathogens. This means heat treatments, if used, must be carefully controlled and used sparingly, or not at all, to maintain like-fresh quality

6.3.1 Modes of Heat Transfer

6.3.1.1 Steam

Systems that use live steam to treat MPR fruits and vegetables are somewhat difficult to control in terms of the time and exact temperature needed to maintain proper texture and prevent overcooking, yet provide benefits.

6.3.1.2 Hot Water

This commonly used mode of heat treatment has been reported by workers too numerous to mention. Among its many applications, it has been used on fruit and vegetable products as a means to reduce pathogens in fruit and pieces of peeled fresh root vegetables, to reduce endogenous microflora on mushrooms, to reduce microbial levels, and to partially reduce PPO activity (Orr 1990). Losikoff (1990) has used a boiling water blanch of celery to provide thermal inactivation of *L. monocytogenes.*

6.3.1.3 Hot Air (Gases)

There is little current data available relating to the use of hot gases to reduce microbes and enzymes in MPR fruits and vegetables. Properly used, hot gases might reduce moisture levels in centrifuged products but would have the disadvantage of adding heat to the product.

6.3.1.4 Ionizing Radiation (Warm)

Also see section on Irradiation.

The two types of ionizing radiation (warm) used in the food industry are infrared heating and microwave heating. Practically no information is available regarding the use of this method for preserving MPR fruits and vegetables.

6.3.2 Types of Heat Preservation

One of the best reviews of the use of heat for refrigerated foods comes from the National Advisory Committee on the Microbiological Criteria for Foods (Anon. 1990) for uncured meat or poultry products. They suggest nine heat processes which are divided into three major categories relative to the microbial risks during pre- and post-preservation. These categories could easily be applied to MPR fruits and vegetables:

Category 1. Assembled and Cooked

Products are assembled and packaged. The product is given a final heat treatment to destroy non-spore-forming pathogens and normal spoilage flora. This heat treatment does not kill spore formers. Category 1 includes sous vide and cook-in-bag products with raw or slightly precooked components assembled and packaged, then cooled.

Category 2. Cooked and Assembled

Ingredients are cooked and then assembled into the final package with no further heat treatment applied after final packaging. The microbial flora can reflect flora originally in the ingredients and those added during packaging and those in the package. All spore formers could be present.

Category 3. Assembled with Cooked and Raw Ingredients

Components are cooked individually, combined with raw ingredients, then assembled and packaged. Raw vegetables are added prior to final packaging and may introduce pathogens into the product. There may be no further heat treatment prior to distribution.

(Category 4. [Added by author] Assembled with Raw Ingredients

Components such as raw fruits or vegetables are not heat treated but are treated with a preservative and an antioxidant, assembled, and packaged. The products may contain human and plant pathogens and full or partial enzyme activity. Although this category does not strictly relate to heat preservation, it is a category required for MPR fruits and vegetables.)

The following process types (Anon. 1990) are primarily heat preservation methods with packaging and refrigeration included. They are mainly for cooked, uncured meat and poultry products but also have application in the MPR fruit and vegetable field. Actual time and temperature recommendations are not yet available for the many products involved in this compilation. These are not standardized and depend on the food manufacturer or source of the product:

Type 1. Example: sous vide (Fig. 6.3; Table 6.2)

Raw ingredients Precook (optional) —» Formulate —» Vacuum package —» Pasteurize —» Chill —» Distribute

Type 2. Examples: rolls and roasts

Raw ingredients Formulate → Vacuum package → Cook —» Chill* —» Distribute

Type 3. Examples: roast or fried chicken, other roasts, and uncured sausages

Raw ingredients Cook → Chill* → Package → Distribute

Type 4. Examples: some uncured luncheon meats and diced meats

Raw ingredients Formulate → Cook → Chill* → Slice or dice → Package → Distribute

Type 5. Examples: meat and pasta, meat and sauces, dinners, sandwiches, and pizza

Raw ingredients Cook → Chill* → Assemble → Package → Distribute

Type 6. Examples: chef salad, chicken salad, and sandwiches or pizza with raw ingredients

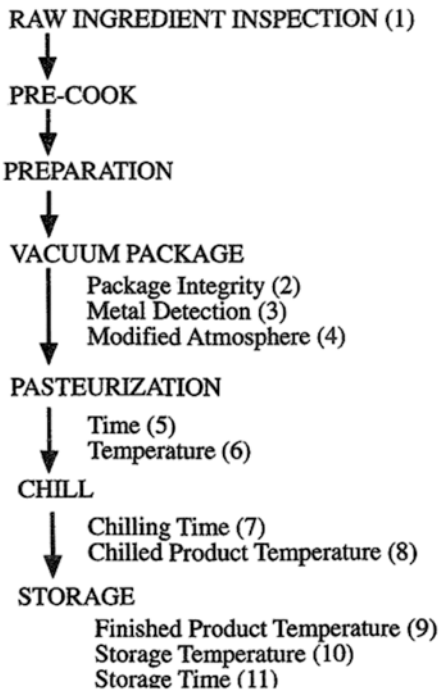

Fig. 6.3 Pasteurized in container with sous vide (From Anon., Chilled Food Association Anon. 1990)

Raw ingredients Chill* → Formulate —» Recook and chill (optional) → Package —» Distribute

Type 7. Examples: meat pies, quiches, patties, and pates

Raw ingredients Formulate → Cook (optional) → Fill into dough → Cook (optional) —» Chill* → Package → Distribute

Type 8. Examples: uncured jellied meats

Raw ingredients Cook → Chill* → Add raw ingredients → Final chill → Package → Distribute

Type 9. Examples: stews, sauces, and soups

Raw ingredients Formulate → Cook → Fill while hot → Seal → Hold (optional) → Chill* → Distribute

MPR fruits and vegetables that use mild heat treatments could be projected to fall into some of the heat-treated meats and poultry chilled food examples supplied here. Many of these examples can include like-fresh low-acid vegetables and the less sensitive to microbial problems, like-fresh high-acid fruit products.

* Denotes continuous chilling at the given step with the asterisk and through all subsequent steps to consumption.

Table 6.2 Critical control points for products pasteurized in container with vacuum (sous vide)

No.	Control point	Potential hazard	How monitored	Action to take for deviation
1	Raw material inspection	Microbial	Establish specifications	Notify vendor reject if not within specification
			Vendor certification and warranty	
			Inspect shipping vehicle	Audit quality
			Measure record temperature of product when received	
			Visual examination of product. Note physical condition of packaging material	Rework/reject as per PQC/TQC program
2	Package integrity	Microbial	Seal integrity check	Rework
			Vacuum check	Rework
			Date code check	Rework
3	Metal detection	Metal fragments in product	Metal detector	Reject positive detentions
4	Modified atmosphere	Microbial	Vacuum test	Reject
5, 6	Pasteurization time and temperature	Microbial	Establish correct pasteurization	Rework or reject
			Measure pasteurization temperatures	
			Monitor pasteurization times	
			Periodically check internal temperature at coldest point	
7	Chilling time	Microbial	Automatic or manual chiller	Rework or reject
8	Chilling temperature	Microbial	Chill water temperature	Hold for QC evaluation
			Chlorine level in chilling water	
9	Finished product temperature	Microbial	Temperature monitoring device	Reject
10	Storage temperature	Microbial	Chart recorder TTIs on packages	Hold for QC evaluation
11	Storage time	Microbial	Date code checks TTIs on packages	Reject

Adapted from Chilled Food Association (Anon. 1990)

For the sake of amplification of the problems in refrigerated (chilled) foods, Type 1 pasteurized in container with vacuum, for example, *sous* vide, will be presented as an example of heat preservation and critical control points in these types of foods which actually may take place in two distinctive time frames (Fig. 6.3; Table 6.2) (Ebert, 1990, Personal Communication). Precooking can reduce microbes in MPR

fruits and vegetables and also provide for inactivation of enzymes. As the second heat treatment, a specific temperature may be used for further reduction of microbes to inactivate enzymes and still allow the fruit and vegetable product to be like-fresh in quality. It is hard to visualize the use of sous vide for MPR fruits or vegetables using heat alone. One scenario may be vegetarian meals where the entire entree may be fruits and vegetables. Another could be the preparation of bulk (sized to consumer requirements) MPR fruits and vegetables that are prepared for the institutional market. The term individually quick-frozen (IQF) foods, which operates on the fluidized bed principle, has long been used in the frozen food industry (Desrosier and Tressler 1977). The same concept could be applied to MPR fruits and vegetables as mixed commodities or as individual commodities such as sliced, diced, or shredded carrots. Schlimme (1990) has suggested the term individually quick chilled (IQC) fruits and vegetables which could be linked with heat or other preservation methods.

In the sous vide process, there are two effective heat preservation steps to consider. The first is the precook, which is optional, to slightly soften the tissue and reduce pathogens. The product should still exhibit like-fresh characteristics. Any number of methods may be used for this precook including high-humidity cooking ovens, steriflow retort with racks to hold product, water baths using different temperatures of water, and microwave (especially for pasta). Meats require a center temperature up to *77* °C depending on the species. The product should be chilled to 7 °C or less as quickly as possible. Then size reduction operations may take place, and the product is filled in a package of standard thickness or shape and vacuumized. After vacuumizing the product is pasteurized at temperatures which will kill vegetative cells and further reduce enzyme activity. This includes a 4-D process for *Listeria monocytogenes* or a 7-D process for *Salmonella* (Table 6.3). The initial temperature (IT) for these processes is considered for safety's sake to be 0 °C.

There have been numerous studies related to the use of heat to preserve refrigerated fruits and vegetables. Some studies such as the one of Losikoff (1990) have considered diced raw celery and determined the effects of a short boiling water blanch on the thermal inactivation of *Listeria.* The information in Fig. 6.4 illustrate the problem for control of injurious enzymes in MPR fruit and vegetable tissue by heat alone. The D-values (in seconds) are very high at lower temperatures, making it difficult to inactivate enzymes and retain like-fresh quality.

Peroxidase, which affects flavor and color of many vegetables and fruits, best illustrates this point. It is clear that additional hurdles for enzymes (as well as for microbes) must be included for the preservation regimen. One such hurdle may be

Table 6.3 D-values at various pasteurization temperatures for *Salmonella* and *Listeria*

°C	°F	*Salmonella*(7-D time in min)	*Listeria*(4-D time in min)
54	130	121	87.8
60	140	12	11.4
66	150	1.2	1.48
71	160	0.12	0.19

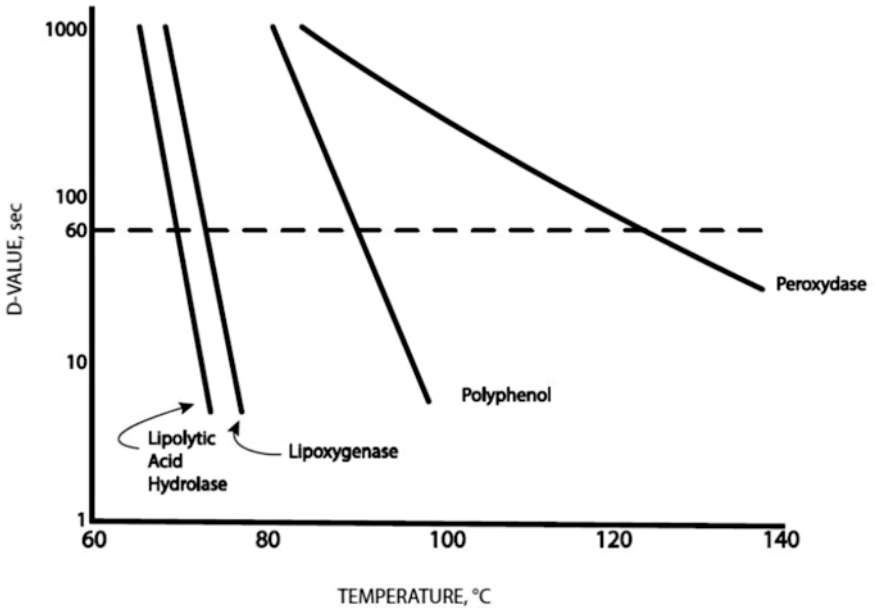

Fig. 6.4 Thermal inactivation of the thermostable fraction of the potato lipolytic acyl hydrolase, lipoxygenase, polyphenol oxidase, and peroxidase as a function of temperature (From Svensson 1977)

adjustment of the pH of the food away from the isoelectric point of key enzymes causing less heat stability. For example, Svensson (1977) has reported that pea lipoxygenase, which has its isoelectric at pH 5.8 and heated to 65 °C has a D-value of 400 min, has a D-value at pH 4.0 and 65 °C of only 0.1 min, a tremendous decrease in heat stability. On the other hand, there have been reports that decreasing a_w (a method to control microbes) has considerably increased the thermostability of enzymes (Svensson 1977). Much research is required on the interaction of pH, heat, and a_w in thermostability of enzymes in MPR fruits and vegetables.

6.4 Chemical Preservation/Preservatives

Chemical compounds both natural and synthetic have been used to control spoilage and maintain quality in low-acid vegetables, acidified low-acid vegetables, and high-acid fruit products. Preservatives that act as antimicrobials best fit the definition of preservation as expressed in this text; however, those preservatives that serve as antioxidants are also very important in MPR fruits and vegetables to prevent browning, reduce discoloration of pigments, and protect against loss of flavor, changes in texture, and loss of nutritional quality.

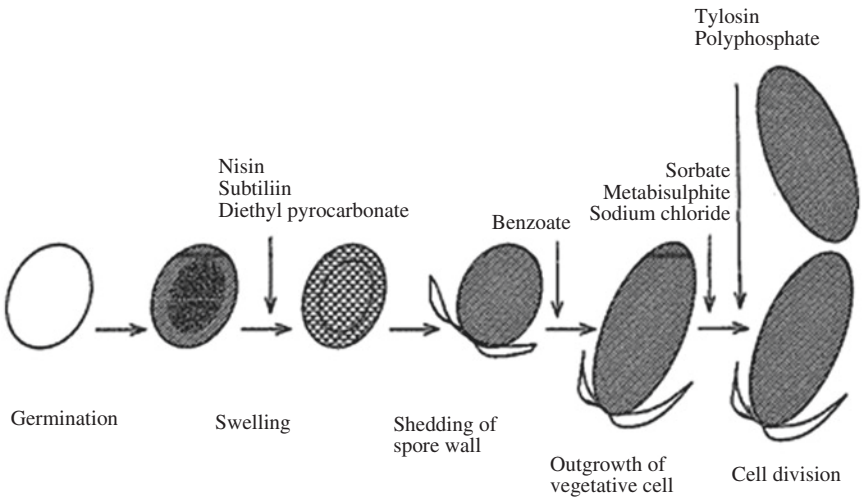

Fig. 6.5 Diagrammatic representation of growth of an endospore into vegetative cells showing stages arrested by minimum inhibiting concentrations of some food preservatives (From Jay 1986b)

The preservative action of antimicrobials depends on the type, genus, species, and strain of the microorganism tested. Antimicrobials are also effective at various stages of growth of the endospore into a vegetative cell as shown in Fig. 6.5. Efficiency of an antimicrobial also depends to a great extent on environmental factors, such as pH, water activity (a_w), temperature, atmosphere, initial microbial load, and low-acid or high-acid food substrate, each factor acting singly or in combination. If they are varied to their extremes, many of these environmental factors, such as a_w, temperature, gaseous atmosphere, etc., can be considered individually as methods of preservation. Some of these treatments were the basis of the hurdle or barrier concept of using less than extreme preservation treatments in a logical sequence to provide like-fresh quality in food products. The isobolograms in Fig. 6.6 by Davidson and Parish (1989) suggest that antimicrobial compounds used together can produce three types of results: synergism, additive effect, and antagonism. Similar results might be expected by combining heat, pH change, etc. with antimicrobials. It is obvious that combination of preservation methods including the use of antimicrobial combinations must be tested in the specific food product systems before application. In other words, the hurdles provided must at minimum be additive in action (preferably synergistic) and not antagonistic. A great deal of research must be conducted on MPR fruits and vegetables using specific food or food mixtures to determine preservation results of hurdle or treatment variables.

Those preservatives that serve as antioxidants to extend shelf life and preserve fruits and vegetables are of great interest. As indicated earlier, their efficiency depends on a number of environmental factors such as pH, water activity (a_w), temperature,

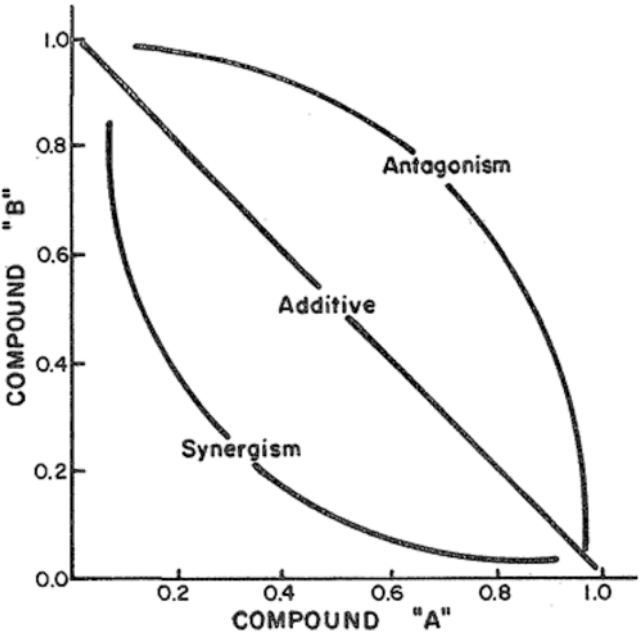

Fig. 6.6 Isobolograms displaying the three types of results possible with combinations of *antimicrobials* (From Davidson and Parish 1989)

light, type and activity of the enzyme system, gaseous atmosphere, food substrate, and heavy metal content (Buck 1985). The effectiveness of antioxidants is controlled by environmental conditions for the preservative in the food system, its concentration, and longevity during the storage or shelf life of the product. Those working with MPR fruits and vegetables must consider the use of both antimicrobials and antioxidants to provide a safe and high-quality like-fresh product over an extended shelf life period. This suggests that the preservation hurdles used for MPR fruits and vegetables must test both type and load of microbial flora and key enzymes that could cause quality problems. Some "generally recognized as safe" (GRAS) chemical food preservatives are found in Table 6.4 (Jay 1986b). (Also see Lewis 1989.)

6.4.1 Antimicrobials

6.4.1.1 Organic Acids and Related Compounds

Organic acids can be naturally present in fruits and vegetables (Table 6.5), accumulate as a result of fermentation, or be added during processing. For a good review and detail relating to antimicrobials occurring naturally in foods, see Beuchat and Golden (1989). Acid foods or organic acids added to low-acid foods as a

Table 6.4 Summary of some GRAS chemical food preservatives

Preservatives	Maximum tolerance	Organisms affected	Foods
Propionic acid/ propionates	0.32%	Molds	Bread, cakes, some cheeses, rope inhibitor in bread dough
Sorbic acid/sorbates	0.2%	Molds	Hard cheeses, figs, syrups, salad dressings, jellies, cakes
Benzoic acid/ benzoates	0.1%	Yeasts and molds	Margarine, apple cider, pickle relishes, soft drinks, tomato catsup, salad dressings
Parabens[a]	0.1%[b]	Yeasts and molds	Bakery products, soft drinks, pickles, salad dressings
SO_2/sulfites	200–300 ppm	Insects, microorganisms	Molasses, dried fruit winemaking, lemon juice (not to be used in meats or other foods recognized as sources of thiamine)
Ethylene/propylene oxides[c]	700 ppm	Yeasts, molds, vermin	Fumigant for spices, nuts
Sodium diacetate	0.32%	Molds	Bread
Dehydroacetic acids	65 ppm	Insects	Pesticide on strawberries squash
Sodium nitrite[c]	120 ppm	Clostridia	Meat-curing preparations
Caprylic acid	–	Molds	Cheese wraps
Ethyl formate	15.200 ppm[d]	Yeasts and molds	Dried fruits, nuts

From Jay 1986b
GRAS (generally recognized as safe) per Section 201 (32)(s) of the US Federal Food, Drug, and Cosmetic Act as amended
[a]Methyl, propyl, and heptyl esters of *p*-hydroxybenzoic acid
[b]Heptyl ester—12 ppm in beers, 20 ppm in noncarbonated and fruit-based beverages
[c]May be involved in mutagenesis and/or carcinogenesis
[d]As formic acid

Table 6.5 Some natural acids of fruits and vegetables

Fruits	Acids[a]
Apples	*Malic*, *quinic*, α-ketoglutaric, oxaloacetic, citric, pyruvic, fumaric, lactic, and succinic acids
Apricots	Malic *and* citric *acids*
Avocados	Tartaric *acid*
Bananas	*Malic*, *citric*, tartaric, and traces of acetic and formic acid
Blackberries	*Isocitric*, *malic*, lactoisocitric, shikimic, quinic, and traces of citric and oxalic acids
Blueberries	*Citric*, malic, glyceric, citramalic, glycolic, succinic, glucuronic, galacturonic, shikimic, quinic, glutamic, and aspartic acids
Boysenberries	*Citric*, malic, and isocitric acids
Cherries	*Malic*, *citric*, tartaric, succinic, quinic, shikimic, glyceric, and glycolic acids
Cranberries	*Citric*, malic, and benzoic acids
Currants	*Citric*, *tartaric*, malic, and succinic acids

(continued)

Table 6.5 (continued)

Fruits	Acids[a]
Elderberries	*Citric*, malic, shikimic, and quinic acids
Figs	*Citric*, malic, and acetic acids
Gooseberries	*Citric*, malic, shikimic, and quinic acids
Grapefruit	*Citric*, tartaric, malic, and oxalic acids
Grapes	*Malic* and *tartaric* (3:2), citric and oxalic acids
Lemons	*Citric*, malic, tartaric, and oxalic acids (no isocitric acid)
Limes	*Citric*, malic, tartaric, and oxalic acids
Orange peel	*Malic*, *citric*, and oxalic acids
Oranges	*Citric*, malic, and oxalic acids
Peaches	*Malic*, *citric*, tartaric, and oxalic acids
Pears	*Malic*, *citric*, tartaric, and oxalic acids
Pineapples	Citric *and* malic *acids*
Plums	*Malic*, *tartaric*, and oxalic acids
Quinces	*Malic* acid (no citric acid)
Strawberries	*Citric*, malic, shikimic, succinic, glyceric, glycolic, and aspartic acids
Youngberries	*Citric*, malic, and isocitric acids

Vegetables	Acids[a]
Beans	*Citric*, *malic*, and small amounts of succinic and fumaric acids
Broccoli	*Malic* and *citric* (3:2) and oxalic and succinic acids
Carrots	*Malic*, *citric*, *isocitric*, succinic, and fumaric acids
Mushrooms	*Lactarimic*, cetostearic, fumaric, and allantoic acids
Peas	*Malic* acid
Potatoes	*Malic*, *citric*, *oxalic*, phosphoric, and pyroglutamic acids
Rhubarb	Malic, citric*, and* oxalic *acids*
Tomatoes	*Citric, malic*, *oxalic*, succinic, glycolic, tartaric, phosphoric, hydrochloric, sulfuric, fumaric, pyrrolidine carboxylic, and galacturonic acids

From Gardner 1966

[a]Acids that occur in appreciable quantities are shown in italics. The relative amount of each varies widely with the variety, degree of ripeness, and seasonal effects. Complete identification of all the acids present in many of the products is obviously lacking in many instances

preservation measure are usually considered as acidification in foods destined to be thermally processed. The main objective of this procedure is to adjust the pH of the product below pH 4.6 which is generally accepted as the minimum pH for sporulation and growth of *C. botulinum.* Acidification is a safety measure also used for MPR fruits and vegetables. Some organic acids may act as fungicides or fungistats, whereas others tend to be more effective at inhibiting bacterial growth. Beuchat and Golden (1989) suggest that the mode of action for organic acids can be related to direct pH reduction of the substrate, some reduction of internal pH of the cell due to ionization of the undissociated acid molecule, or disruption of the transport mechanism through the cell membrane. It appears that the antimicrobial effectiveness of organic acids is primarily related to the dissociation constant (**pK_a**) of the acid.

Table 6.6 Proportion of total acid undissociated at different pH values

	pH values				
Organic acids[a]	3	4	5	6	7
Acetic acid	98.5	84.5	34.9	5.1	0.54
Benzoic acid	93.5	59.3	12.8	1.44	0.144
Citric acid	53.0	18.9	0.41	0.006	<0.001
Lactic acid	86.6	39.2	6.05	0.64	0.064
Methyl, ethyl, and					
propylparabens[b]	>99.99	99.99	99.96	99.66	96.72
Propionic acid	98.5	87.6	41.7	6.67	0.71
Sorbic acid	97.4	82.0	30.0	4.1	0.48

From Dziezak 1986)
[a]Values given as percentage
[b]Parabens, *p*-hydroxybenzoic acid

Table 6.6 shows proportion of total acid undissociated at different pH values for some common organic acids (Dziezak 1986). The pK_a values of most organic acids used in foods are somewhere between pH 3 and 5; thus acidification of low-acid foods greatly improves the antimicrobial characteristics of the food. However, this mechanism relies on complete diffusion of the acid throughout the sample and to the center of every food particle involved in acidification.

Citric Acid Citric acid ($C_6H_8O_7$) is the main organic acid of fruits such as citrus, cranberries, currents, figs, strawberries, etc. and vegetables including beans and tomatoes (Gardner 1966). Citric acid has been shown to inhibit the growth of bacteria (Fabian and Graham 1953), flat sour bacteria in canned tomato juice (Murdock 1950), and to be more inhibitory to *Salmonellae* in milk than lactic and hydrochloric acids (Subramaniun and Marth 1968). Unfortunately most of the above examples of efficiency of citric acid as an antimicrobial relate to thermally processed products. There have been a number of studies that have suggested the antimicrobial activity of citric acid is due to the chelation of metal ions which are essential for microbial growth {Beuchat and Golden 1989). Citric acid can be used to prevent browning by chelating copper in PPO. Inactivation of enzymes and potentiation of antioxidants in fruits and vegetables such as ascorbic acid, erythorbic acid, or sodium erythorbate can be achieved by the use of citric acid. Usage levels for citric acid are typically 0.1–0.3% with an antioxidant at 100–200 ppm (Dziezak 1986).

Benzoic Acid (C_6H_5COOH) and Parabens The earliest reference to benzoic acid was made in 1608 by Blaise de Vigenère in "Traite' du Feu et du Sel," which described its preparation by the sublimation of gum benzoin (Smith 1938). Benzoic add occurs naturally in cranberries, prunes, cinnamon, cloves, raspberries, plums, and other fruits and vegetables. Its sodium salts ($C_7H_5NaO_2$), along with the esters of *p*-hydroxybenzoic acid (parabens), are some of the most important food preservatives. The sodium salt is particularly helpful in products with pH values below 4.6. It can be used in fruit products, fruit drinks, and juices as an antimicrobial.

For example, Rushing and Senn (1962) found levels of 0.033–0.066% of sodium benzoate could extend the shelf life of chilled citrus salads to 5–6 weeks at 4.4 °C and 12–16 weeks at 1.11 °C. However, it appeared that off-flavor development in the salad occurred before bacterial count had increased significantly. A number of workers have suggested that the undissociated benzoic acid molecule, which appears to be responsible for antimicrobial activity, diffuses through the microbial cell membrane, where it ionizes, causing rather complete acidification of the cell (Beuchat and Golden 1989; Jay 1986a). This acidity in turn has been postulated to interfere with substrate transport and oxidative phosphorylation systems (Freeze et al. 1973). Figure 6.5 suggests that the stage of endospore germination after shedding the spore wall is most sensitive to benzoate treatment. The benzoate compounds are most active in the lower pH acid foods such as apple cider, soft drinks, tomato catsup, and the like and not as effective in low-acid vegetables such as corn, peas, beans, lettuce, etc. The pK_a of benzoate is 4.2. This means this acid would be much more effective around a pH of 4.0–5.0. At a pH of 6.0, which is normal for many vegetables, only 1.5% of the benzoate is undissociated (Jay 1986b).

The benzoates are more effective against molds and yeasts rather than bacteria. In acid foods, they can be effective against bacteria in the 50–500 ppm range. In the pH 5.0–6.0 range, at 100–500 ppm, they are effective in inhibiting yeast, whereas at 30–300 ppm they are inhibitory to molds (Jay 1986b). Care has to be taken with the addition of benzoates to acid foods in that they can deliver a "peppery" or a burning taste sensation at levels of about 0.1%.

The useful parabens in food systems methylparaben (methyl *p*-hydroxybenzoate), propylparaben (propyl *p*-hydroxybenzoate), and heptylparaben (*n*-heptyl *p*-hydroxybenzoate) are permitted in the United States, whereas the butyl- and ethylparabens are permitted in some other countries (Jay 1986b). Parabens are most effective against molds and yeast and are less effective against bacteria, especially gram-negative bacteria. The pK_a of these compounds is around pH 8.47. Their antimicrobial activity tends to increase with the length of the alkyl chain and extends up to pH 7.0 (Dziezak 1986), which should make these useful in vegetable salads and vegetable soup stocks. Both methyl- and propylparabens are affirmed as GRAS under 21 CFR 184.1490 and 21 CFR 184.1670 (Anon. 1992), respectively, and are limited to a combined usage level of 0.1%. The length of the alkyl chain is related to solubility in water which is usually necessary for use on fruits or vegetables. The parabens are available as a free-flowing white powder. The methyl ester imparts a slight odor and taste reminiscent of sodium benzoate, whereas the propyl ester is essentially odorless (Dziezak 1986).

Acetic Acid Acetic acid ($C_2H_4O_2$) and its salts Na acetate, Ca acetate, Na diacetate, and Ca diacetate have preservative antimicrobial properties. In addition acetic acid is known for its sequestering ability and its flavoring properties. Most vinegars contain acetic acid at the 4% level. Acetic acid and its salts are GRAS and are effective to a pH of 4.5. Acetic acid itself, which is a product of lactic acid bacteria fermentation or saccharomycete fermentation in such products as pickles, sauerkraut, olives, and cider, may also be added directly to the product. The limiting

factor of acetic acid/vinegar addition to foods is the loss of desirable flavors and "bite" from high levels of use.

The main targets of acetic acid are yeast and bacteria and it has less effect on molds. The antimicrobial effect of acetic acid appears to be due to the depression of pH below the optimum growth range of microorganisms and metabolic inhibition by the undissociated molecules. In vegetable, meat, and fish products, 1–2% of the undissociated acid is sufficient to inhibit or kill all nontolerant microbes (Dziezak 1986). Acetic acid is used as a flavoring agent in condiments such as catsup, mayonnaise, and salad dressing. The treatment levels for acetic acid set forth by the International Commission on Microbiological Specifications for Foods (ICMSF) (Christian 1980) indicate 0.1% inhibits the growth of most food poisoning and spore-forming bacteria, whereas 0.3% of the undissociated acid is required to inhibit the growth of mycotoxigenic molds.

In studies by Oscroft et al. (1989) acetic acid showed good antimicrobial properties both at 0.05% and 0.5% wt/vol combinations in a combined heat 95 °C acetic acid treatment of cocktails containing *Bacillus* spores. The results were the best when the precooked chilled ready-to-eat meals were cooled to 12 °C. Colder refrigerated temperatures should provide even more safety for these meals. Acetic acid/vinegar in dressings and condiments could be helpful in preserving MPR vegetables.

Lactic Acid This organic acid ($C_3H_6O_3$) is widely employed as a preservative in foods as a result of the action of lactic acid bacteria and related inhibiting substances other than organic acids (see Doores 1983; Jay 1986a; Daeschel 1989, for more information). Lactic acid can also be added directly to food and provide antimicrobial properties by depression of pH below growth range and metabolic inhibition of the undissociated acid molecules. It should be emphasized that it is the titratable acidity rather than hydrogen ion concentration (pH) per se that is important in a treatment using an organic acid such as lactic since there is incomplete ionization of the acid even at lower acid pH values.

Banks et al. (1989) used lactic acid alone at 0.05% and 0.5% levels to treat 10^6 spores/ml *Bacillus* spp. cocktail heated to 65 °C/60 min and stored at 30, 20, and 12 °C for 42 days. Results indicated the lactic acid treatments were not effective inhibitors in storages of 20 and 30 °C. The treatments stored at 12 °C showed outgrowth at pH 6.0 but no outgrowth at pH 5.7, 5.4, 5.1, 4.8, 4.5, and 4.2. The results were similar in experiments carried out with 10^4 spore/ml and 10^2 spore/ml. Similar experiments were conducted by Oscroft et al. (1989) using a thermally stressed (95 °C/15 min) cocktail of *Bacillus* spp. at 10^2, 10^4, and 10^6 spores/ ml in trypticase soy broth (TSB). The treated samples were incubated for 42 days at 12, 20, and 30 °C. The control treatment had lower counts than the Banks et al. (1989) experiments, and there was outgrowth at 12 °C storage in the pH 6.0 and 5.7 treatments but none at pH 5.4, 5.1, 4.8, 4.5, and 4.2. These are similar results to the heat treatments for a longer time at a lower temperature of 65 °C. The integrated sterilizing (IS) values for these heat treatments were not reported.

Propionic Acid (CN_3CH_2COOH) and Its Salts This acid and its sodium and calcium salts are primarily used to prevent mold growth in breads, bakery products, cheeses,

Table 6.7 Effect of pH on sorbic acid dissociation

pH	Undissociated (%)
7.00	0.6
6.00	6.0
5.80	7.0
5.00	37.0
4.75(pK_a)	50.0
4.40	70.0
4.00	86.0
3.70	93.0
3.00	98.0

From Liewen and Marth 1985

and other foods. The antimicrobial action of propionates is similar to that of benzoate in the undissociated form. The piCa of propionate is 4.87 and at a pH of 4.0, 88% of the compound is undissociated, whereas at a pH of 6.0 only 6.7% remains undissociated (Jay 1986b). Because these compounds do not have a tendency toward dissociation, they are useful in low-acid foods. Dziezak (1986) suggested 0.2–0.4% levels of propionates to retard mold growth on syrups, blanched apple slices, figs, cherries, blackberries, peas, and lima beans. The compound seems to act as a fungistat rather than a fungicide according to Jay (1986b).

Sorbic Acid ($CH_3CH{=}CHCH{=}CHCOOH$) and Its Salts Sorbic acid is a monocarboxylic acid and its potassium salt forms (both GRAS status) are used to preserve foods. According to Liewen and Marth (1985), a German chemist, A.W. Hoffmans, first isolated sorbic acid from the pressed unripened berry of the rowan or mountain ash tree in 1959. For further information on sorbic acid, see review of Liewen and Marth (1985).

With use of the sorbates, it is also the undissociated molecule that provides antimicrobial properties. Table 6.7 shows the effect of pH on sorbic acid dissociation and its pK_a of 4.75 (Sofos and Busta 1980). It appears that the upper pH limits for sorbates to be effective as antimicrobial agents are around 6.0–6.5, whereas for propionate and benzoates, they are 5.0–5.5 and 4.0–4.5, respectively (Liewen and Marth 1985).

This provides a basis for the traditional uses of sorbates in cottage cheese, baked goods, beverages, syrups, fruit juices, wines, jellies, jams, salads, pickles, margarine, and dried sausages. Work has also explored the use of sorbate in fresh produce, fresh fish and poultry, and yeast-raised and baked goods (Robach 1980).

Table 6.8 (Liewen and Marth 1985) shows the suggested concentration of sorbic acid for a number of foods. As indicated earlier, a number of environmental factors including more acid pH, lower a_w, lower temperature, higher CO_2, the type and number of microbial flora, and food components all have important effects on the efficiency of sorbate and should be considered in its use on a specific food product.

Table 6.8 Typical concentration (%) of sorbic acid used in various food products

Cheeses	0.2–0.30
Beverages	0.03–0.10
Cakes and pies	0.05–0.10
Dried fruits	0.02–0.05
Margarine (unsalted)	0.05–0.10
Mayonnaise	0.10
Fermented vegetables	0.05–0.20
Jams and jellies	0.05
Fish	0.03–0.15
Semimoist pet food	0.1–0.3
Wine	0.02–0.04
Fruit juices	0.05–0.20

From Liewen and Marth 1985

Robinson and Hills (1959) worked with preserving apple juice (cider), peach slices, and fruit salad (pineapple and citrus slices) that were subjected to temperatures of 37.8–54.4 °C for 5 min in the presence of sodium sorbate. The apple juice, peach slices, and fruit salad had a final concentration of sodium sorbate of 0.06%, 0.048%, and 0.12% by weight on the basis of 16 ounces net contents, respectively. These treatments greatly increased the storage life of these products at storage temperatures of 22.8 and 10 °C. In the cider study, 0.06% sorbate with a 37.8 °C heat treatment for 5 min destroyed 50% of the initial yeasts, molds, and bacteria in the cider as well as improved storage life to 14 days at room temperature. At 48.9 °C there was a 99% reduction in microbial counts, and storage life was increased to 23 days at 21.1 °C. Similar results were shown with peach slices and fruit salad, with peach slices showing a 92-day shelf life before gas formation in most treatments. The 10 °C storage temperature gave slightly better results than the 22.8 °C storage samples. Although the flavor of these samples was rated as good, refrigerated/chilled temperatures would have improved the results of this work. Interactions of sorbates with other preservatives and other preservation methods are also discussed later in this chapter.

Malic, Succinic, and Tartaric Acids Malic acid ($C_4H_6O_s$) was reported by Gardner (1966) in the following fruits, apples, apricots, bananas, cherries, grapes, orange peel, peaches, pears, plums, and quinces, and in vegetables, broccoli, carrots, peas, potatoes, and rhubarb. In his somewhat short list, malic acid is reported in all fruits and vegetables except avocados and mushrooms (Table 6.5) (Gardner 1966).

Succinic acid ($C_4H_60_4$) and succinic anhydride ($C_4H_40_3$) are white crystals and powder and white crystals, respectively. Succinic acid is found in a number of fruits and vegetables (Table 6.5) and in asparagus, rhubarb, and sugar beets as well according to Beuchat and Golden (1989).

Tartaric acid ($C_4H_60_6$), which is a white crystal and imparts a bitter tart flavor sensation, is present in a number of fruits and tomatoes (Table 6.5).

The antimicrobial activity of these natural organic acids appears to be the result of a decrease in pH. Yeasts and some bacteria are target populations. Because they have

not been studied in any detail for use on MPR fruits and vegetables, they should be considered in products where they are naturally present and possibly in others.

Phenolic acids are reported (Yildiz 2010) to have excellent preservative and antioxidant properties naturally in cherries, pomegranate arils, quinces, rose hips, parsnip, eggplant, and artichoke.

6.4.2 Indirect Antimicrobials

This category of antimicrobials is summarized by primary use and most susceptible organisms in Table 6.9 taken from Jay (1986b).

6.4.2.1 Medium-Chain Fatty Acids

Medium-chain fatty acids, that is, those containing 12–18 carbon atoms, have been known since the early part of this century to exhibit antimicrobial properties (Branen et al. 1980). This is an additional benefit of these compounds which are used primarily as emulsifying agents in foods. Some organisms may be killed by fatty acids, but the antimicrobial action of fatty acids is more static than cidal (Neiman 1954). The undissociated form of the fatty acid molecule is thought to be responsible for antimicrobial activity against yeasts and gram-positive bacteria. Therefore, compounds will react in the same manner as organic acids to changes in pH. One would

Table 6.9 Some GRAS indirectly antimicrobial chemicals used in foods

Compound	Primary use	Most susceptible organisms
Butylated hydroxyanisole (BHA)	Antioxidant	Bacteria, some fungi
Butylated hydroxytoluene (BHT)	Antioxidant	Bacteria, viruses, fungi
t-Butylhydroquinone (TBHQ)	Antioxidant	Bacteria, fungi
Propyl gallate (PG)	Antioxidant	Bacteria
Nordihydroguaiaretic acid	Antioxidant	Bacteria
Ethylenediaminetetraacetic acid	Sequestrant/stabili	Bacteria
Sodium citrate	Buffer/sequestrant	Bacteria
Laurie acid	Defoaming agent	Gram-positive bacteria
Monolaurin	Emulsifier	Gram-positive bacteria, yeasts
Diacetyl	Flavoring	Gram-negative bacteria, fungi
D- and L-Carvone	Flavoring	Fungi, gram-positive
Phenylacetaldehyde	Flavoring	Fungi, gram-positive
Menthol	Flavoring	Bacteria, fungi
Vanillin, ethyl vanillin	Flavoring	Fungi
Spices/spice oils	Flavoring	Bacteria, fungi

From Jay 1986a

expect greater antimicrobial activity as the pH is lowered. Concentrations needed for inhibition usually are in the 10–1000 mg/ml range (Branen et al. 1980).

The gram-negative bacteria and molds seem to be less susceptible than gram-positive bacteria to the antimicrobial properties of the various fatty acids. For control of gram-positive bacteria, the most effective chain length for saturated fatty acids is 12 carbons; the most effective monounsaturated fatty acid is $C_{16:1}$ (palmitic); and the best polyunsaturated is $C_{18:12}$ (linoleic) (Beuchat and Golden 1989). Kabara (1983) has reviewed the antimicrobial activity of fatty adds. Also see Kabara (1981) for food grade chemicals to use in designing food preservative systems.

For use in MPR foods, medium-chain fatty acids could be most helpful in fruits and vegetables that are slightly acidic and do not yield well to other preservatives. In addition, there are other factors aside from pH and acidity that control effectiveness of these compounds. Neiman (1954) has found that antagonists such as starch, cholesterol, and serum albumen reduce antimicrobial action of fatty acids. The effectiveness of these compounds may also rest on their solubility or lack of solubility in water. To assist in solving this problem, fatty acids could be added to dressings or condiments and should be considered as a method to extend shelf life of MPR fruits and vegetables. Other preservation hurdles might also be included in such a system to improve the effectiveness of fatty acids.

6.4.2.2 Fatty Acid Esters of Polyhydric Acids

Fatty acid esters of sucrose and other polyhydric alcohols are used primarily as emulsifiers, but they also provide antimicrobial properties. They can be manufactured or occur naturally in plants (Beuchat and Golden 1989). Monolaurin, a glycerol monoester, is a satisfactory compound to be used against gram-positive organisms or yeasts. Combining monolaurin with other food additives such as citric acid, polyphosphoric acid, EDTA, and BHT or with other physical food preservation hurdles such as refrigeration or heating could increase the effectiveness of monolaurin against the more difficult to control gram-negative organisms (Branen et al. 1980). Lipid esters will have to be tested in food products to gain insights on their functionality in a specific situation. For more information concerning these preservatives, see the above listed references and Jay (1986b) and Kato (1981).

6.4.2.3 Other Indirect Antimicrobials

According to Jay (1986b), many other compounds including those found in Table 6.9 have some antimicrobial properties (also see Marth 1966). The literature does not cover these compounds for use in MPR fruits and vegetables, but applications should be sought where capability and functional properties could be used to advantage. This would be especially true with spices and flavoring agents used in MPR fruits and vegetables. Beuchat and Golden (1989) have covered in detail other natural antimicrobials such as pigments and related compounds, humulones and lupulones, hydroxycinnamic acid derivative, oleuropein, caffeine, theophylline and theobromine, and phytoalexins, and their article should be read for more detail than can be provided here.

6.4.2.4 Sugar and Salt

Sugars such as sucrose, glucose, fructose, etc. exert antimicrobial properties at concentrations much higher than are normally used in MPR fruits or vegetables. Some yeasts and molds can grow in sugar solutions of 60% sucrose which could rarely be used in MPR products (Jay Jay 1986b).

Salts may be used in MPR vegetables but at relatively low levels. Conner et al. (1986) found NaCl concentrations in cabbage juice at 7.2% caused a decrease in strains of *L. monocytogenes,* Scott A, and LCDC 81861. The levels of salt in MPR vegetables should be studied to determine if salt concentration alone could lower microbial activity. In most instances it would be expected that the levels of sugar or salt added to MPR fruits and vegetables alone would not be effective antimicrobial agents; however, they might be linked with other preservative hurdles and provide antimicrobial protection. See also the section on water activity (a_w) for more information.

6.4.2.5 Antibiotics

A great number of antibiotics are mentioned in the literature as antimicrobials. They include nisin, natamycin, tetracyclines, subtilin, and tylosin. Nisin produced by *Streptococcus lactis* is usually referred to as an antibiotic but probably should be classified as a bacteriocin because it has no therapeutic value in human or veterinary medicine, feedstuffs, or growth promotion (Banks et al. 1989). Nisin and natamycin have been approved for food use in many countries, and recently nisin has been approved in the United States by the US Food and Drug Administration (USFDA) for use in processed cheese spreads. The tetracyclines, that is, chlortetracycline and oxytetracycline, have been studied widely for their use in fresh foods, and natamycin has been suggested as a food fungistat (Jay 1986b).

Jay (1986b) has summarized the considerations one should make in deciding on the use of antibiotics on foods or MPR fruits and vegetables. These were developed, because of the general resistance of consumers to the use of antibiotics in foods to preserve them. See below:

1. The antibiotic agent should kill, not inhibit the flora, and should ideally decompose into innocuous products, or be destroyed, on cooking for products that require cooking.
2. The antibiotic should not be inactivated by food components or products of microbial metabolism.
3. The antibiotic should not readily stimulate the appearance of resistant strains.
4. The antibiotic should not be used in foods if used therapeutically or as an animal feed additive.

Nisin, along with subtilin, and tylosin have been used as adjuncts to the use of heat in canning many vegetables. The concept includes giving canned low-acid foods the typical F_0 3-min or “bot cook” heat treatment, and then the added antibiotic

will prevent more economic or pathogenic spoilage by attacking the more heat-resistant thermopiles. F*q* is the time in minutes required to destroy a stated number of organisms with a known *z* at temperature *T*.

MPR fruits and vegetables may need a mild pasteurization temperature, but this will not prevent the outgrowth of the *Bacillus* and *Clostridium* species if temperature abuse should occur. Banks et al. (1989) have studied pasteurization temperatures in conjunction with use of pH, acidity control, and preservatives. Nisin was used in *Bacillus* spore cocktails at levels of 0, 125, 250, and 5000 IU ml^{-1}. Addition of nisin alone to the heating medium did prevent germination and outgrowth of spores. As the inoculum concentration increased to 10^6 spores/mT^1, the efficacy of nisin decreased, and as the pH was decreased from 6.0 to 4.2, the effectiveness of nisin increased.

According to Wagner and Moberg (1989), to ensure successful application of nisin as a preservative, the food should be acidic in nature to provide stability of the antimicrobial during processing and storage, and the spoilage organism to be controlled should be gram-positive, be nisin sensitive, and not contain nisinase. If nisin is depleted from the food system, its protection could be lost.

It appears nisin could be helpful in mildly heated refrigerated fruits and vegetables where heating could induce germination and outgrowth of spores. This might reduce the potential for growth of psychrotrophic and mesophilic aerobic spore formers and their resultant spoilage of food. Little research has been conducted on the use of antibiotics to provide antimicrobial activity in MPR fruits and vegetables, and it is an area that should be investigated.

Although gas—controlled atmosphere (CA) and modified atmosphere (MA)—treatments in storages or through packaging provide antimicrobial protection and can logically be covered under antimicrobials, special emphasis for this area in MPR fruits and vegetables is presented in the section on gas preservation in this chapter.

6.4.2.6 Antioxidants

The US Food and Drug Administration (FDA) in 21 Code of Federal Regulations (CFR) 170.3(0)(3) (Anon. 1992) has defined antioxidants as substances used to preserve food by retarding deterioration rancidity or discoloration due to oxidation. In MPR fruits and vegetables, there are several types of oxidative reactions in which electrons are removed from atoms or molecules to lead to a reduced form. These reactions cause browning reactions, discoloration of endogenous pigments, loss or changes of product flavor or odor, changes in texture, and loss of nutritional value from destruction of vitamin A, C, D, or E and essential fatty acids such as linoleic acid. These changes are important in most MPR fruits and vegetables. Special problems arise in seed crops and lipid-containing vegetables such as avocado leading to the possible development of rancid off-flavors and toxic oxidation products (Dziezak 1986).

As seen in Fig. 6.7, there are four categories of chemical structures used to stabilize foods. They are (1) the free radical interceptors such as BHA, BHT, etc., which are usually used for oil- and lipid-containing foods and are very insoluble in

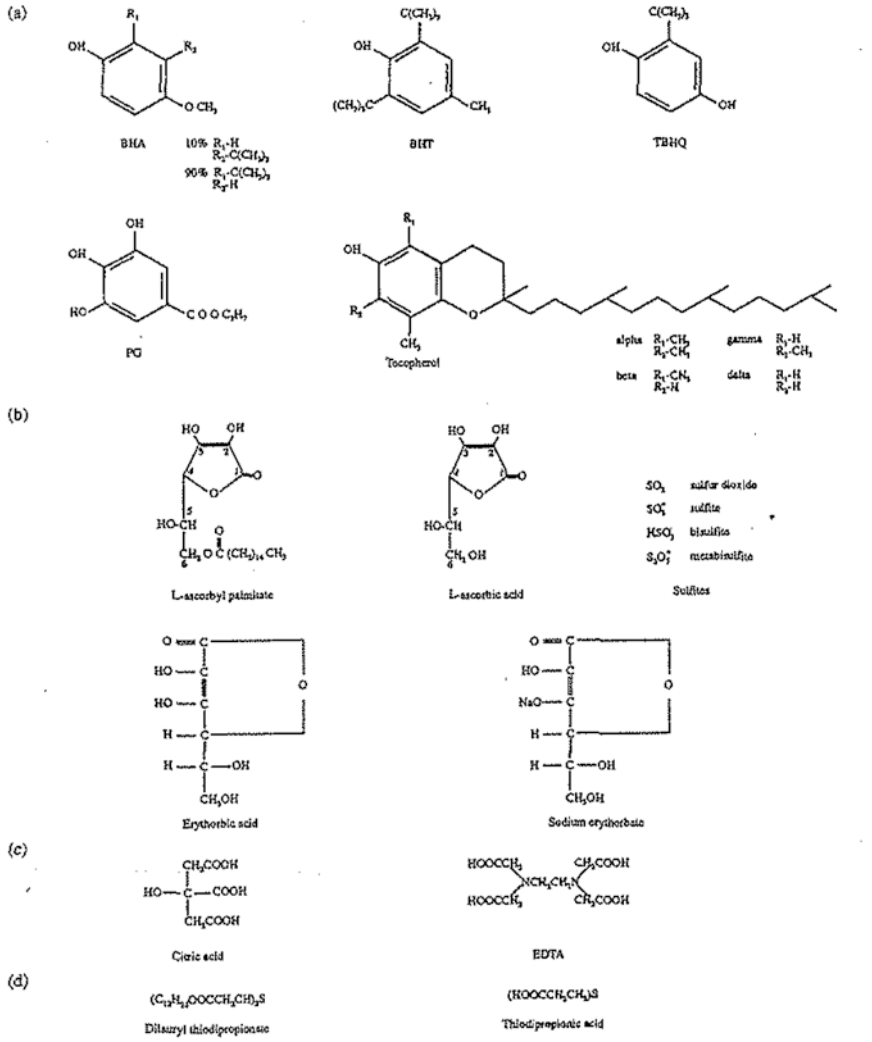

Fig. 6.7 Chemical structures of several antioxidants and their synergists: **a** free radical interceptors, **b** reducing agents, **c** chelating agents, and **d** secondary antioxidants (From Dziezak 1986)

H_2O; (2) the reducing agents such as ascorbic acid and isomer erythorbic acid and related compounds which are used extensively to transfer hydrogen ions; (3) chelating agents such as citric acid and EDTA; and (4) the "secondary" antioxidants including dilauryl acid, thiodipropionate, and thiodipropionic acid. The most important compounds used in stabilizing MPR fruits and vegetables are reducing agents and certain GRAS chelating agents that are not actually antioxidants but are important in preventing oxidative reactions in fruits and vegetables. With the recent restrictions of the use of sulfites on fruits and vegetables to be served raw or sold

raw to consumers or presented to customers as fresh (21 CFR 182.3739, 182.3766, 182.3862, 182.3798, 182.3637, and 182.3616) (Anon. 1992), there can be little doubt these regulations relate to like-fresh MFR foods. Much research is being conducted to find suitable substitutes for sulfites for all fresh and MPR fruits and vegetables. The most promising substitutes may be combinations of the ascorbic acid derivatives with citric or other organic acids.

6.4.2.7 L-Ascorbic Acid

L-Ascorbic acid (vitamin C) and its various neutral salts and other derivatives have been leading GRAS antioxidants for use on fruits and vegetables and their juices to prevent browning and other oxidative reactions (Bauernfemd and Pinkert 1970). Sapers et al. (1989a) have recently reported on a number of ascorbic acid derivatives, PPO inhibitors, and complexing agents used to control enzymatic browning. The L-ascorbic acid isomer erythorbic acid is covered later in this section. In its removal of oxygen from food, ascorbic acid is oxidized to form dehydroascorbic add. Ascorbic add is usually added together with citric acid which tends to maintain more acid pH levels and also acts as a chelating agent on such enzymes as copper-containing PPO (Whitaker 1972a). Ascorbic add is a moderately strong reducing compound, is acidic in nature, forms neutral salts with bases, and is very water soluble. The product may be added to foods as tablets or wafers, dry premixes, liquid sprays, or as a pure compound. It is important to add the ascorbic acid as late as possible during processing or preservation to maintain highest levels during the shelf life of the food commodity. If the goal is to maintain a vitamin C level in fruit and vegetable juices of about 30 mg/8 fl. oz., then 3 oz. of crystalline ascorbic acid need to be added to 100 gallons of juices (Bauernfemd and Pinkert 1970). Potter (1968) suggested that levels of ascorbic acid dissolved in a sugar syrup should be 0.05–0.2% and given adequate time to penetrate could keep peaches from darkening for 2 years at 0 °F. Sapers et al. (1990), working with the problem of penetration of ascorbic acid, erythorbic acid, or their sodium salts in combination with citric acid, found apple plugs and potato plugs were best infiltered with 34 and 108 kPa pressure, respectively. Storage life was increased 3–7 days for apple plugs and dice and 2–4 days for potato plug under refrigerated temperatures of 4 °C. Because low levels such as 100 ppm of ascorbic acid may induce prooxidative effects, levels such as 2000 ppm are suggested to prevent these reactions (Cort 1982).

6.4.2.8 Erythorbic Acid

Erythorbic acid and its salt, sodium erythorbate, are GRAS and are strong reducing agents; they act as oxygen scavengers, thus reducing molecular oxygen. Erythorbic acid is the D-isomer of ascorbic acid but has no vitamin C activity. Most research suggests L-ascorbic acid and erythorbic acid have about equal antioxidant properties; thus L-ascorbic acid might be used only where vitamin C addition is a necessity.

At today's prices (1992) L-ascorbic acid is about five times more expensive than erythorbic acid.

The use of erythorbic acid with citric acid has been often suggested as a substitute for sulfites. This combination is used at retail to inhibit oxidative rancidity and discoloration in salad vegetables, coleslaw, apples, and frozen seafood. Erythorbic acid or sodium erythorbate can suppress browning reactions in frozen fruits and should be helpful in MPR fruits.

See Sapers et al. (1989a), Sapers et al. (1989b), and Sapers et al. (1990) for information on use of ascorbic acids and their derivatives to prevent browning reactions in fruits and vegetables. The 1989b article covers some uses of ascorbic acid-2-phosphate, ascorbic add-2-triphosphate, and ascorbic add-6 fatty acid ester as novel browning inhibitors. These substances have not yet been approved by the USFDA.

6.4.2.9 Sulfites

Sulfites are increasingly under fire for use in raw fruits and vegetables. As indicated earlier in this section, sulfites are no longer GRAS for fruits and vegetables served raw, sold raw, or presented to the consumer as raw. Foods containing a detectable level of a sulfiting agent defined as 10 ppm, regardless of source, must declare the sulfite and its content on the ingredient label (21 CFR Part 182.3862) (Anon. 1992). It is likely that more and more regulatory restrictions will be applied to the use of sulfites in foods globally because of the sulfite allergies in a significant portion of our population. They are not recommended for MPR fruits and vegetables.

6.4.2.10 Chelating Agents

As seen in Fig. 6.7, chelating agents are not antioxidants but work as synergists with antioxidant preservatives. They complex with prooxidative agents such as prooxidative copper and iron ions through an unshared pair of electrons in their molecular structures which provides the complexing or chelating action. The best known chelating agents for use on fruits and vegetables that are GRAS are citric acid and EDTA. Sapers et al. (1989b) report on papers describing *non-GRAS* chelating agents such as cyanide, diethyldithiocarbamate, 2-mercaptobenzothiazole, and azide which inhibit PFO by interacting with its prosthetic group and polyvinylpyrrolidone which bonds the phenolic substances, preventing their conversion to quinones.

Friedman (1991, Personal Communication) has been testing an acidic polyphosphate, Sporix, on precut apples, potatoes, broccoli florets, snow pea pods, and other fruits and vegetables. It is soluble in water and has a pH of 2.0. The recommended dip for these commodities is a 0.5% solution, Sporix-ascorbic acid combinations were synergists in reducing browning in the juice of Granny Smith apples and the cut surface of Red Delicious and Winesap apples (Sapers et al. 1989b). According to Friedman (1991, Personal Communication) the compound is allowed on fruits and vegetables in Taiwan, Korea, and Japan and is being considered in the United States by USFDA.

6.4.2.11 Citric Acid

Citric acid (covered earlier) is also a chelating agent that is GRAS and used synergistically with ascorbic or erythorbic acids and their neutral salts to chelate prooxidants which might cause rancidity and inactivate enzymes such as PPO that cause browning reactions. Suggested usage levels for citric acid are typically 0.1–0.3% with the appropriate antioxidant at 100–200 ppm (Dziezak 1986), Citric acid can also be used as a chelating agent in many other foods.

6.4.2.12 EDTA

EDTA is another chelating agent permitted as a chemical preservative. The major compounds approved as additives by FDA are calcium disodium EDTA (21 CFR 172.120) and disodium EDTA (21 CFR 172.135) (Anon. 1992). The former compound can be used in potato salad (100 ppm), pickled cabbage (220 ppm), pickled cucumbers (220 ppm), nonstandardized dressings (750 ppm), salad dressing and sauce (75 ppm), as well as in many other food products. The intended usage of calcium disodium EDTA is to promote color, flavor, and texture retention and as a preservative. Disodium may be used as a preservative at 75 ppm in nonstandardized dressing, French dressing, mayonnaise, salad dressing, and sauces. It might be expected that sauces, dressings, and the like would be added to MPR fruit and vegetable salads. Highly stable complexes are formed by the sequestering action of the EDTA compounds on iron, copper, and calcium. The maximum chelating efficiency occurs at the higher pH values where the carboxyl groups are dissociated (Dziezak 1986).

6.4.2.13 Miscellaneous Chemical Preservatives

Dehydroacetic Acid
Dehydroacetic acid ($C_7H_60_4$) (21 CFR 172.130) (Anon. 1992), a pyranose structured compound, or its sodium salt may be used as a preservative for cut or peeled squash to control molds. The use levels should be no more than 65 ppm of the acid remaining on or in the squash. This compound can also be used on other fruits and vegetables in Mexico and other countries.

6.4.2.14 Chlorine Compounds (Cl_2)

These compounds are normally used in connection with washing MPR fruits and vegetables and are sometimes the primary preservation agent (see Chap. 2 for details.)

6.4.2.15 Antifungal Agents for Fruits and Vegetables

This area is not covered in depth because most postharvest dips have been stopped in Europe and the United States and there are very stringent rules on application time before harvest; however, benomyl, a fungicide in foods for human consumption such as raisins and concentrated tomato products, is allowed up to levels of 50 ppm (CFR 21193.30), (Anon. 1992), as a result of application to the growing of grapes and tomatoes in the United States. Thiabendazole, an important fungicide, is allowed a tolerance of 3 ppm in or on milled wheat fractions (except flour) resulting from applications to growing wheat. Other antifungal agents should be checked out in consultation with regulatory authorities even though they are normally added to the skin or peel of the fruit or vegetable. MPR fruits and vegetables should have much lower levels than fresh or raw products that are not partially processed

6.4.3 Combinations of Chemical Preservatives

Chemical preservatives/antimicrobials in combination are currently utilized in the food industry, and this area has been addressed by Davidson and Parish (1989), Scott (1989), Banks et al. (1989), Oscroft et al. (1989), and Aguilera and Parada (1991). A good example of combination treatment in the food industry is the combination of potassium sorbate and sulfur dioxide to preserve sparkling wines. Data showing additive synergistic or antagonist results from combinations of preservatives (Fig. 6.6) have not been studied in detail for MPR fruits and vegetables although there have been studies such as those of Banks et al. (1989) investigating heat and chemical preservatives to improve stabilized "pasteurized/chilled" recipe dishes. Most of the preservative interaction studies appear to have used microbial "cocktails" *studies* on fish, meats, poultry, and dairy products. These studies have centered on type E *C. botulinum*, *S. aureus*, *Salmonella typhimurium*, *L. monocytogenes*, and *Yersinia enterocolitica* control in the abovementioned substrates, using salt concentrations with sodium nitrite and phenolic antioxidants with sorbates (Scott 1989). Restaino et al. (1982) studied the synergistic antimicrobial effects between potassium sorbate and lactic, citric, phosphoric, or hydrochloric acids on growth of *Yersinia enterocolitica, Salmonella pseudomonas,* and lactic acid bacteria in trypticase soy broth and APT broth. They found organic acids, specifically citric and lactic, potentiate the antimicrobial action of potassium sorbate. There need to be many additional combination studies of chemical preservatives for MPR fruits and vegetables.

6.5 Gas and Controlled/Modified Atmosphere Preservation

The use of gases, vapors, and controlled/modified atmosphere for preservation does not include packaging factors (Chap. 4) and the gas mass transfer (Chap. 5) taking place in the tissues of MPR foods. With fresh fruits and vegetables, voluminous

research information is found to reduce O_2 levels, increase CO_2 levels, and reduce ethylene as methods to improve storage and shelf life. A symposium chaired by Blanpied (1987) gives a good summary of the postharvest work conducted in this area until 1987.

However, this chapter necessarily covers mainly the antimicrobial and antioxidant properties of gaseous substances used with packaging to extend shelf life of MPR fruits and vegetables. The area is receiving much attention by research laboratories and industry as a way to preserve MPR fruits and vegetables.

One of the earlier workers to study gas exchange and the use of bactericidal and enzymicidal gases to keep fruits and vegetables like-fresh and extend storage life was Kramer et al. (1980). This preservation and shelf life extension technique consisted of replacement of intratissue gases in particulate raw foods with one or more gases, in appropriate sequence. The idea was to stabilize the product and extend the like-fresh shelf life of the foods at ambient or refrigerated temperatures. Kramer et al. (1980) felt the new preservation method was an extension of CA/MA storage used for fresh products. However, there are several differences between normal CA/MA storages and the gas exchange process. The atmosphere changes are slower in normal CA/MA storages, usually days or minutes, as compared to gas exchange; times of treatment are relatively short, and additional gases such as carbon monoxide (CO), ethylene oxide (EO), and SO_2 were used in the gas exchange system as compared with N_2, O_2, and CO_2 modifications used in CA/MA storages. Also, only the edible portion or a minimally processed portion of the fruit or vegetable is treated in the gas exchange method. Emphasis in the gas exchange method is placed on antimicrobial properties of the gases and their efficacy in inhibiting enzyme activity. These are also the objectives in the preservation of MPR fruits and vegetables.

Some of the problems with the gas exchange treatment were related to the gases selected for use. Such gases as EO should be used in a very low a_w atmosphere, and unfortunately its by-products are ethylene glycol (EG) and ethylene chlorohydrin (ECH), which show mutagenicity at certain concentrations. FDA regulations 21 CFR Part 193.20 (Anon. 1992) state there should not be over a 50 ppm residue of EO in whole and ground spices. The content of carbon monoxide (CO), a product of combustion gases (21 CFR 193.65) (Anon. 1992), should not be over 4.5% by volume in gas treatments. The use of CO is banned in France.

The concept of using gas exchange preservation treatments on MPR fruits and vegetables has merits if safe and suitable bactericidal and enzymicidal gas can be found or linked with other preservation hurdles.

The most important gases and vapors to be discussed in detail are CO, CO_z, EO, propylene oxide (PO), SO_2, and ozone. Propane, helium, N_2, and combustion gases 21 CFR 184.1655, 184.1355, 184.1540, and 193.65 (Anon. 1992), respectively, will not be covered. Antimicrobial effectiveness of a gas can be related to the food substrate, other processing and preservation methods, and the microorganisms and enzymes present.

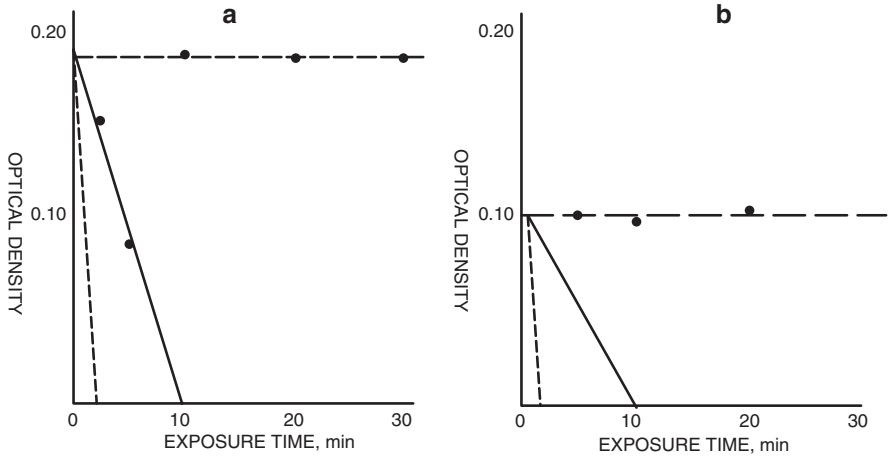

Fig. 6.8 Polyphenol oxidase activity following exposure to CO (——), ethylene oxide (-----), or sulfur dioxide gas (——). **a** Potato plugs and **b** apple plugs (From Kaffezakis et al. 1969)

6.5.1 Carbon Monoxide

As indicated earlier there are safety problems with the use of this gas. CO at the 1% level has been shown to inhibit yeasts and molds and prevent postharvest decay in fruits and vegetables (Aharoni and Stadelbacher 1973). However, treatments of apple and potato plugs with pure CO were not able to reduce counts of *Escherichia coli* ATCC II 229, a standardized suspension of *Clostridium botulinum* 62A, and *Staphylococcus aureus* ATCC 6538 (Kaffezakis et al. 1969). These workers also studied the enzymicidal activity of CO and showed CO was effective in slowing down PPO activity (Fig. 6.8). In a two-step gas treatment with SO_2 followed by CO, potato strips after 60 days at room temperature showed 93.7% and 99.9% of the PO and PPO inactivated (Kramer et al. 1980).

6.5.2 Carbon Dioxide

This gas has antimicrobial properties that kill or inhibit various microorganisms and that depend on the gas concentration, temperature of incubation, age of the cells used, and the aw of the microbial medium. Most work has been conducted on meat and meat products, and these results showed a 10% level of CO_2 usually gives about 50% inhibition on the basis of total counts after a given incubation time (Wagner and Moberg 1989). Huxsoll and Bolen (1989) have recently reviewed CA and MA storage of MPR foods. Table 6.10 shows the effects of SO_2, CO_2, and temperature on peroxidase, PPO, and PE. It appears that there is less effect of these gases on the texture-related enzymes such as PPE than PPO (Kramer et al. 1980).

Table 6.10 Results of SO_2 followed by CO_2 treatment[a] of potato strips, stored under CO_3 at 21 and 4 °C for 45 days

	Results	
Determination	21 °C	4 °C
Aerobic count (g)	10	0
Anaerobic count (g)	0	80
Color, visual	Slightly white	Normal
Color, Hunter Lab	85.9. 1.7. 14.2	84.3. 2.8. 13.1
Time until color change (h)	>5	>5
pH	5.1	5.2
Soluble solids (%)	4.5	3.5
Texture (Ibf/100 g)	787	717
Odor (sniff)	Normal	Normal
Free liquid (ml/100 g)	9.4	12.2
Total SO_2 (ppm)	104	328
Free SO_2 (ppm)	0	0
Peroxidase inactivation (%)[b]	79.2	87.4
Polyphenol oxidase inactivation (%)[b]	100.0	100.0
Pectinesterase inactivation (%)[b]	54.6	62*4

[a]The product was prepared as follows: pretreatment, treated with 100% SO*, 5 min, 5 psi evacuated, 5 min, 26 in Hg flushed with 100% CO* 10 min peeling; steam treatment, evacuated, 5 min, 25 in Hg
Vacuum broken with 10% SO_2 in CO_2/5 s, vacuum reduced to 20 in Hg evacuated, 5 min, 25 in Hg
Vacuum broken with 100% CO_2/15 s, vacuum reduced to 0 in Hg
[b]Percent reduction from activity before treatment (From Kramer et al. 1980)

The same results for PG were reported by Puri (1980). It is likely the enzymicidal properties shown in Table 6.10 are mainly due to SO_2 since CO_2 was used as a flushing and carrier gas, but such results could not be determined from the data supplied.

6.5.3 Sulfur Dioxide

SO_2 discussed earlier under sulfites in the section on antioxidants is also well known to be effective against molds, yeasts, and bacteria (Dziezak 1986). In the fruit and vegetable realm, it has been used to control microorganisms on soft fruits, fruit juices, wines, pickles, leafy greens, and the like. However, the sulfite compounds have been banned on fruits and vegetables to be sold or consumed (21 CFR 182, 3862) (Anon. 1992). Nonetheless, earlier Kramer et al. (1980) reported good results with SO_2 combined with CO_2 (Table 6.10), but because of the great sensitivity of a small part of the population to SO_2, we should assume more and more pressure will be brought to bear to reduce all uses of SO_2. Therefore, finding alternatives to SO_2 use on fruits and vegetables is an important research area in the development of MPR fruits and vegetables (Sapers et al. 1989a, Sapers et al. 1990).

Table 6.11 D-values for ethylene oxide sterilant of some foodborne microorganisms

Organisms	D[a]	Concentration (mg/L)	Temperature[b]	Condition
C. *botulinum 62A*	11.5	700	40	47% RH
C. *botulinum 62A*	7.4	700	40	23% RH
C. *sporogenes*				
AT CC 7955	3.25	500	54.4	40% RH
B. coagulans	7.0	700	40	33% RH
B. coagulans	3.07	700	60	33% RH
B. stearothermophilus				
ATCC 7953	2.63	500	54.4	40% RH
L. brevis	5.88	700	30	33% RH
M. radiodurans	3.00	500	A	40% RH

[a]In minutes
[b]*C.* (From Jay 1986a)

6.5.4 Ethylene Oxide

Ethylene oxide (C_2H_40) (21 CFR Part 193.200) (Anon. 1992) has been used primarily to reduce microbial contamination and insect infestation in dried foods such as spices with maximum allowed levels at 50 ppm. There have been concerns that the toxicity of this compound or its reaction products, particularly in a_w situations $\leq$0.80. See Table 6.11 for effectiveness of this gas on several microorganisms at relative low a_w. Attempts to use this gas at higher a_w on fresh apple and potato plugs containing *E.* coli, *S. aureus*, and *C. botulinum* showed EO was not as effective as SO_2 as an antimicrobial (Fig. 6.9) (Kaffezakis et al. 1969). As an enzyme inhibitor, the gas appears to have little effect on PPO activity (Fig. 6.8).

6.5.5 Propylene Oxide

Propylene oxide (C_3H_6O), which exists as a gas, has not been studied to the same extent as EO. Its effectiveness is increased by higher temperatures and concentrations and lower a_w. Bacteria are more resistant to this gas than molds and yeasts. There have been few reports of toxicity of this compound, and its reaction products and most applications have been to dried foods such as starch, cocoa, gums, spices, and processed nutmeats (Wagner and Moberg 1989). Propylene oxide CFR 21 Part 380 (Anon. 1992) is a food additive permitted in the United States up to levels of as high as 700 ppm in glace fruits and dried prunes. All other allowed products—cocoa, gums, processed nutmeats (except peanuts), processed spices, and starch—can have maximum levels of 300 ppm.

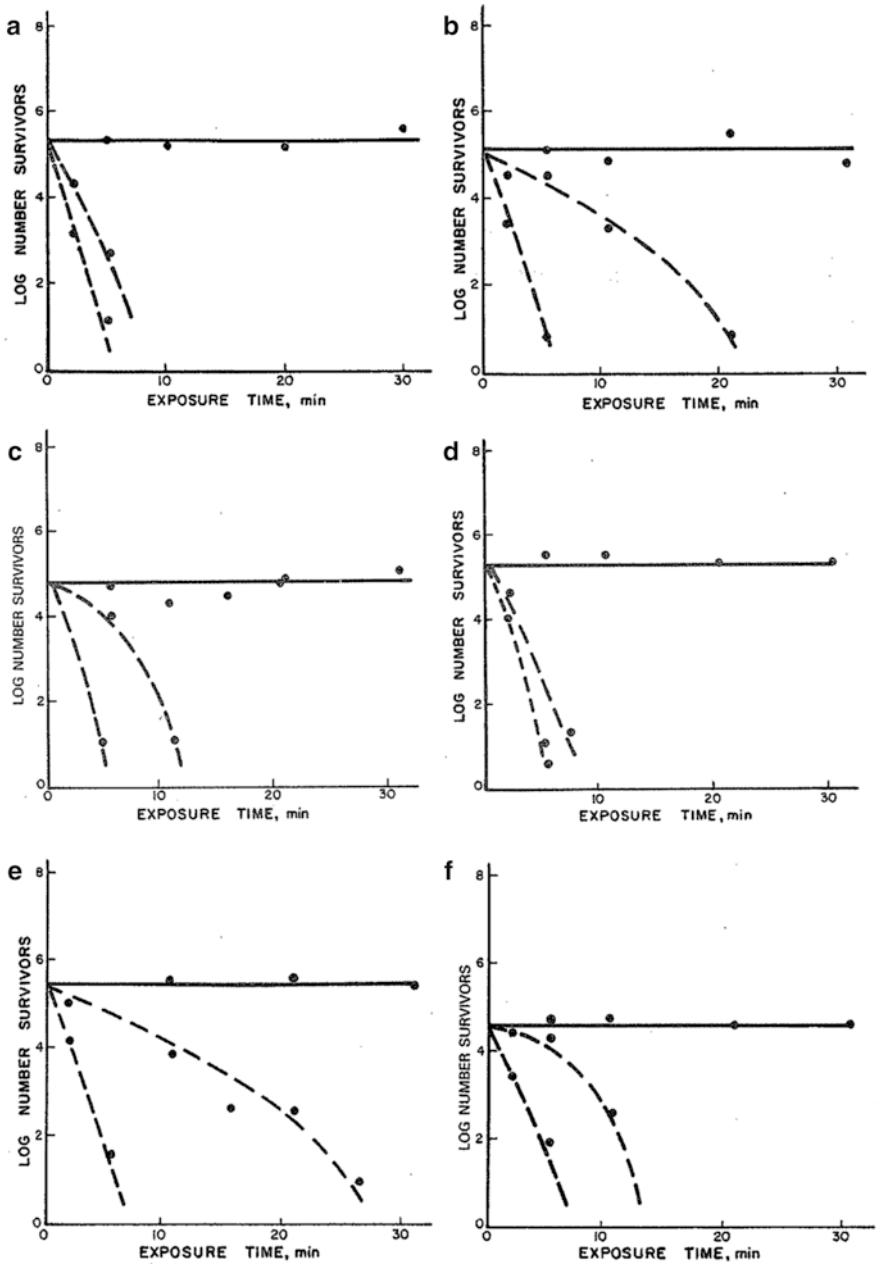

Fig. 6.9 Survivor curves for microorganisms exposed to carbon monoxide (—→—) (CO), ethylene oxide (——) (C_2HO), or sulfur dioxide (---→---) (SO_2) gas: **a** *E. coli* on apple plugs. **b** *S. aureus* on apple plugs. **c** *C. botulinum* on apple plugs. **d** *E.coli* on potato plugs. **e** *S. aureus* on potato plugs. **f** *C. botulinum* on potato plugs (From Kaffezakis et al. 1969)

6.5.6 Ozone

Bacteria are more susceptible to ozone (0_3) (21 CFR 184.1563) (Anon. 1992) than yeasts and molds, with bacterial spores 10–15 times more resistant than vegetative cells. It appears that ozone attacks many vital constituents of microbial cells, but actual cause of death is not known (Wagner and Moberg 1989). It is usually used to sterilize bottled water. The US Good Manufacturing Practices (GMPs) allow maximum residual level at time of bottling of 0.4 mg of ozone/L of bottled water. Its use in fruit and vegetable juices has been limited because of the oxidative properties of the gas.

6.5.7 Other Vapors

Acetaldehyde vapors have been used to control postharvest pathogens of fruits and vegetables, and vapors from 0.25% to 20% applied for 0.50–120 min at room temperature killed tested organisms. The organisms that cause postharvest decay in order of most sensitivity to acetaldehyde to least sensitivity were *Erwinia carotovora*, *Pseudomonas fluorescens*, *Botrytis cinerea*, *Monilinia fructicola*, *Rhizopus stolonifer*, and *Penicillium expansum.* Low levels of acetaldehyde can control pathogens in strawberries without injuring the product (Stadelbacher and Aharoni 1971).

Ethanol (C_2H_5OH) vapors have been used in preservation because of their desiccant and denaturant properties (Jay 1986b).

6.6 Cold Preservation

The cold preservation/refrigeration/chilled storage during distribution and retailing is a necessary and required step in MPR fruits and vegetables. This is based on the idea that refrigerated temperatures slow down most microbial growth and are effective to reduce enzyme activity. Freezing of foods as indicated earlier, although effective in reducing microbial and enzyme activity, may change some of the like-fresh qualities of the fruit or vegetable.

Most of the metabolic reactions of plant or human pathogens in fruit and vegetable tissue are enzyme catalyzed. The concern in MPR fruits and vegetables of enzyme activity makes cold temperatures (the refrigerated chain) an absolute necessity for these products. The rate of enzyme-catalyzed reactions is controlled to a great extent by temperature. With every rise in temperature of 10 °C (in the biological important ranges), there is a twofold increase in rate of reaction. This is known as the temperature coefficient (Q_{10}). On the other hand, every 10 °C reduction in temperature gives a similar decrease in the rate of biological activity. This means that the refrigeration hurdle is broad based and is a continuing factor in the preservation of MPR fruits

and vegetables. As discussed in Chaps. 7 and 9, the low-temperature growing psychrotrophs are destructive microbes in refrigerated foods as compared with mesophiles and thermophiles.

In the past, temperatures below about 6 °C were considered safe from food poisoning bacteria. However, with the advent of MPR foods exhibiting fairly long shelf life, much more attention has been given to microorganisms that grow below about *6* °C such as *C. botulinum* type E and nonproteolytic B and F strains, strains of *V. parahaemolyticus*, and *Y. enterocolitica* (Jay 1986a). See also Chaps. 7 and 9 for information regarding the above organisms and strains of hydrophila that are associated with gastroenteritis. Another foodborne disease caused by *L. monocytogenes,* a facultative anaerobe, has also been associated with low temperatures of 1 °C up to 45 °C with optimum at 30–37 °C and has become a major safety problem in MPR fruits and vegetables.

For fruits and vegetables, there is a great deal of variation in ideal refrigerated temperatures. Some workers such as Jay (1986a) prefer to call temperatures between 10 and 15 °C chill temperatures and temperatures between 0–2 °C and 5–7 °C refrigeration temperatures. Since the composite Appendix Tables IIIA, IIIB, and IIIC (Anon. 1989a) give ideal temperature values from −1.7 to 21.1 °C, it is not possible to draw a distinct line between refrigerated and chilled temperatures because it depends to a great extent on the commodity studied. Chill-sensitive fruits that are sliced, diced, etc., such as citrus fruits and vegetables such as cucumbers and tomatoes, are considered to be MPR fruits or vegetables by definition.

Appendix Tables 1, 2, and 3 (Anon. 1989a) do not attempt to predict shelf life and safety for the commodities listed because of the minimal processing that may take place and could make the usual storage period different than that published data for intact items. Shelf life and safety data for MPR fruits and vegetables have not yet been suitably developed in the public domain.

The preservation method required for all MPR fruits and vegetables that is emphasized throughout this text (Fig. 1.2) has been refrigeration. A major problem associated with MPR fruits and vegetables is the possibility of temperature abuse during the time interval after preservation and packaging during distribution, transportation, storage, retailing, or wholesaling before use by the ultimate consumer. MPR fruits and vegetables are normally classified as extended shelf life (ESL) foods and under best conditions should have a time-temperature indicator (TTI). The field has been studied extensively with mathematical analysis of the relationships between TIIs and chemical and sensory quantity attributes and the remaining shelf life of food products (Taovkis and Labuza 1989; Wells and Singh 1988, etc.). The Anon. (1991a) reference lists 23 recent patents, 21 commercial indicators (cold chain monitoring, partial and full history), and about 50 references that cover TTI devices for refrigerated and frozen foods. The field is much too extensive to cover in this section on cold preservation. One system described by LaGrenade et al. (1986) consists of (1) indicator labels printed in a bar code format that contain polymer compounds that change color as a result of accumulated temperature exposure, (2) a handheld microcomputer with optical wand for reading the indicator label, and (3) software for data analysis and telecommunications. The accumulated time-

temperature readings for fruit juices, vegetable juice, and fruit punch showed good correlations with objective and subjective color and flavor changes in these products at 3 °C. These techniques are especially useful for manufacturers and for storage and transportation operators and perhaps the consumer to track location and extent of temperature abuse in MPR products (Fields and Prusik 1986). For more information on TTIs, also see Anon. (1991b) and Labuza and Breene (1989).

6.7 Preservation Using Irradiation

The term "irradiation of food" refers primarily to electromagnetic radiation. The electromagnetic spectrum can be separated on the basis of wavelength with the shortest wavelengths considered the most damaging to biological systems (Fig. 6.10) (Jay 1986c).

6.7.1 Infrared Heating

This type of direct heating of food shown in Fig. 6.10 located on the electromagnetic spectrum above 8000 A units is characterized by low penetration but can produce rapid surface cooking of the food which is highly undesirable in MPR fruits and vegetables unless carefully controlled. Brennan et al. (1976) suggested the results of infrared treatment can result in rapid sealing and browning of the outer layers. The process if used properly would tend to seal in volatile flavors and water which could be lost in preparation operations such as peeling, dicing, etc. This sort of heating would have to be combined with other forms of heat, for example, to get heat transfer to the center of the piece or be combined with other types of preservation hurdles for application to MPR foods. Heat has to be carefully managed to preserve the like-fresh quality of MPR fruits and vegetables.

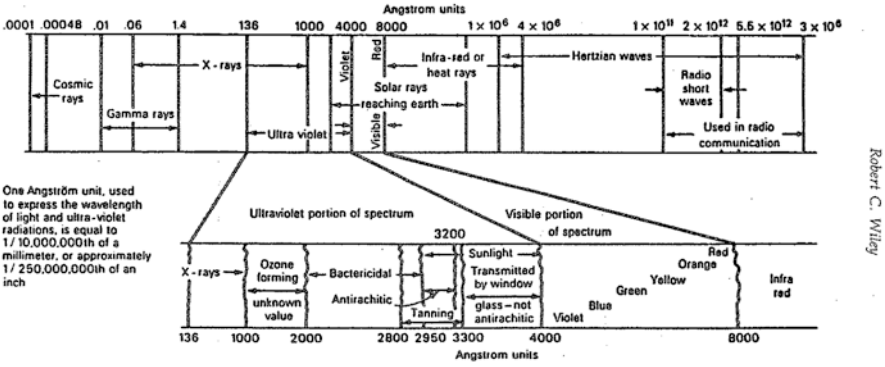

Fig. 6.10 Spectrum chart (From Jay 1986c)

6.7.2 Microwave

Microwave energy, which causes intermolecular friction, yields a heating effect and lies on the electromagnetic spectrum between the infrared and the radiofrequency section (Fig. 6.10): The use of in-depth heat treatment in MPR fruits and vegetables alone is limited because of possible effects on the like-fresh quality required in these types of foods. Microwave energy could be used in providing mild heat treatments in a hurdles system with other preservation methods. The cost of this type of heating as compared with steam, hot water, and the like would have to be considered.

6.7.3 Ultraviolet Light

Ultraviolet (UV) light, which has the most effective wavelength of about 2600 A (Fig. 6.10), is nonionizing and is primarily absorbed by proteins which eventually causes cell death. Poor penetration of UV light limits its uses to surface treatment of food products (particularly meats and bakery products) before packaging and to processing surface and storages. It is more likely to be used in storage rooms to prevent surface mold growth on room surfaces and products (Kader 1986). Treatment of fresh fruits and vegetables with up to 1 kGy (100 k rad) was approved by the USFDA in 1986. Of course, this is lower than the treatments to inactivate most enzymes which range from 1,100,000 to 100,000,000 rads (Jay 1986c). According to Kader (1986) 1152 reports on ionizing energy use on fruits and vegetables have been published in the last 30 years.

6.7.4 Ionizing Radiation

There are a number of forms of ionizing energy that are approved for foods which are derived from radionuclide and machine sources. The only radionuclide sources permitted are cobalt-60 and cesium-137, both of which emit gamma rays and have good penetrating ability. There are many different types of electron beam generators or electron accelerators that produce x-rays or electron beams. X-rays have some physical characteristics similar to those of gamma rays, but the electron beams are somewhat different (Anon. 1989b). All of these treatments can be called "cold sterilization" if the levels of treatment are high enough, since very little heat is produced (Desrosier and Rosenstock 1960). These preservation methods have been selected for their *inability* to produce significant radioactivity in treated foods. For complete details for food processing applications for ionizing energy, see Anon. (1989b) which summarizes much of the research of the late Dr. Eugen Wierbicki, his colleagues, and a host of experts.

A great deal of research has been conducted relating to the use of ionizing radiation for postharvest handling of fresh fruits and vegetables. An excellent review of this area has been published by Kader (1986). Treatment of fresh fruits

Table 6.12 Relative tolerance of fresh fruits and vegetables to ionizing radiation stress at doses <1 kGy

Relative tolerance	Commodities
High	Apple, cherry, date, guava, longan, mango, muskmelon, nectarine, papaya, peach, rambutan, raspberry, strawberry, tamarillo, tomato
Moderate	Apricot, banana, cherimoya, fig, grapefruit, kumquat, loquat, lychee, orange, passion fruit, pear, pineapple, plum, tangelo, tangerine
Low	Avocado, cucumber, grape, green bean, lemon, lime, olive, pepper, sapodilla, soursop, summer squash, leafy vegetables, broccoli, cauliflower

From Kader 1986

and vegetables with up to 1k Gy (100k rad) was approved by the USFDA in 1986. Of course, this is lower than the treatments to inactivate most enzymes, which ranges from 110,000 to 100,000,000 rads (Jay 1986c). According to Kader (1986), 1152 reports on ionizing energy use on fruits and vegetables have been published in the last 30 years.

The research to date suggests that ionizing energy has potential for some fresh fruits and vegetables but also has potential limitations. Kader (1986) suggests that this form of preservation should be considered as a supplement to refrigeration or some other postharvest procedure, and this idea fully supports the use of the hurdle or barrier concept for preservation/extension of shelf life.

The major biological problem in using ionizing energy on fruits and vegetables is that they are particularly sensitive to stress of all kinds (Kader 1986), and the unit operations used to prepare MPR fruits and vegetables would only increase these problems. The cutting, slicing, dicing, and shredding increase the respiration and ethylene production of MPR fruits and vegetables; therefore, considerable research has yet to be conducted to determine those commodities that can benefit from ionizing energy. It would be expected that the same relative tolerance to ionizing radiation stress that is found for intact fresh fruits and vegetables would also be found for MPR fruits and vegetables though these forms may be less tolerant (Table 6.12). From the table it is apparent that fruits are much more tolerant to ionizing radiation than vegetables such as broccoli, leafy greens, cucumbers, and the like.

In addition there are many other factors that affect the radiation processing quality of MPR fruits or vegetables, and they include production area and cultivar, season and climate, quality of irrigation water, maturity at harvest, maturity and quality at time of processing, processing and preservation hurdles, and postharvest handling including careful control of refrigeration. Much of this information has not been developed for MPR fruits and vegetables treated by ionizing energy or combined preservation methods.

There is a social problem associated with the use of ionizing energy to preserve MPR fruits and vegetables, and it relates to the safety issue brought to consumers' attention by anti-irradiation advocacy groups. It should be emphasized that most food scientists agree that food irradiation can prolong the shelf life of many foods and does not cause safety or health problems. Kantor (1989) in his article, "The great food irradiation controversy," covers in succinct form the advantages and disadvan-

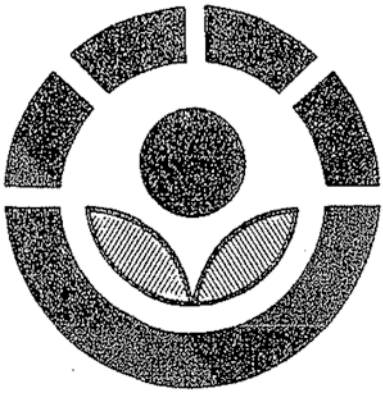

Fig. 6.11 Logo to be used on irradiated foods (From Kader 1980)

Table 6.13 Irradiation: the consumer's view from Waterfield 1991

Appearance	%	Color	%
Worse	14.6	Worse	8.8
Same	42.2	Same	58.4
Better	42.2	Better	32.2
Do not know	1.1	Do not know	0.5
Freshness		Storage life	
Worse	12.7	Worse	17.9
Same	43.8	Same	27.8
Better	41.4	Better	40.0
Do not know	2.1	Do not know	14.3
Taste		Nutrition	
Worse	20.4	Worse	2.2
Same	41.9	Same	17.6
Better	27.6	Better	4.4
Do not know	10.1	Do not know	75.8
Firmness		Overall quality	
Worse	18.9	Pleased with irradiated strawberries	80.1
Same	41.9		
Better	38.8	Pleased with nonirradiated strawberries	67.2
Do not know	0.5		

tages of using ionizing radiation and some of the consumer issues. Kantor (1989) feels there may be an uncertain future for irradiation that has to be faced by both industry and the government. There is an apparent reluctance by industry to use the irradiation logo shown in Fig. 6.11 (Kader 1986).

In the *Packer*, which is a national weekly business newspaper of the fruit and vegetable industries, Waterfield (1991) has reported public opposition to the use of irradiated produce, and the lack of retail initiative has severely hampered marketing of irradiated fresh fruits and vegetables, but they have not entirely vanished from the scene. Table 6.13 gives a relatively recent viewpoint of consumer's reactions to irradiated and nonirradiated strawberries.

In a 1990 survey of consumers who were asked to try irradiated and nonirradiated strawberries, here's how the irradiated produce fared.

It appears that consumers are about equally divided on the question of whether irradiated strawberries are higher in quality, and data also imply considerable education is required relating to the nutritional quality of the treated and untreated strawberries.

There is considerable reluctance by the industry to gamble with ionizing energy as a hurdle to preserve MPR fruits and vegetables because of consumer doubts concerning its safety; however, a commercial concern in Mulberry, Florida, is continuing to irradiate fruits and vegetables to reduce the use of chemicals and pesticides and reduce spoilage (Waterfield 1991). One anti-irradiation advocacy group has reported a consumer survey that showed 93% of the respondents expressed concern about irradiation, with 59% saying they are either "extremely" or "very" concerned (Anon. 1991a).

The issue seems to boil down to market-driven and safety aspects of irradiated MPR fruits and vegetables. Research and utilization of ionizing energy as hurdles for MPR fruits and vegetables seem to rest on suitable and stable markets for these products. This controversy must be satisfactorily resolved in the future.

6.8 Reduction of Water Activity

This preservation method is well known and is based on the desiccation of food to a_w levels that will not support the growth of vegetative microbial cells. According to Jay (1986a) the approximate minimum a_w for the growth of the major groups of microorganisms is 0.9 for most spoilage bacteria, 0.88 for most spoilage yeast, and 0.80 for most spoilage molds. Many MPR fruits and vegetables have an a_w of 0.98 or above and are therefore very sensitive to reduction in a_w as a means of controlling microbial and enzyme activity. This means the use of reduction of a_w as a preservation measure for MPR fruits and vegetables must be carefully controlled to preserve the like-fresh quality demanded of the product.

This method is primarily involved in removing moisture from the food product by some sort of dehydration or by adding an ingredient with a high osmotic pressure which will form a complex with the water in the product (Huxsoll and Bolen 1989). If reduction of a_w is used as the sole preservation method for fruits and vegetables, the process will dehydrate them. Although this system is considered to be a conventional hurdle in many food products, the use of fl_w probably does not hold promise for MPR fruits and vegetables because of possible loss of the like-fresh character. Huxsoll and Bolen (1989) suggested a_w reduction by application of osmotic agents will result in products with undesirable flavor characteristics such as too sweet or too salty because high concentrations of sugars and salts are needed for osmotic dehydration.

The use of a_w reduction coupled with other preservation methods has been well covered by Scott (1989) and Lazar (1968). The former author has covered preservatives, pH, heat, and other interactions with a_w reduction, whereas the latter author utilized a_w reduction with freezing. So far little application and little reporting of a_w reduction as a single preservation method have been used for MPR fruits and vegetables because of troublesome quality problems in the like-fresh characteristics of the products, primarily crispness and turgidity. It is not likely that this method of preservation will find much use with MPR fruits and vegetables as defined by most workers.

6.9 Oxidation-Reduction Potential

The oxygen tension/atmosphere surrounding a food, whether it is aerobic or anaerobic or variations thereof, has a great effect on the growth of microorganisms. This type of preservation relates closely to controlled (CA) and modified atmosphere packaging (MAP) (Chaps. 2 and 7), and various types of gas preservation (this chapter). These preservation methods may be included with other hurdles to preserve/maintain the like-fresh quality of MPR fruits and vegetables.

Oxidation will occur in atoms or groups of atoms when electrons are removed, whereas reduction occurs with the addition of electrons to a different atom or group of atoms, both reactions being simultaneous (Lindsay 1985). Oxidation can also be accomplished by the addition of oxygen to atoms or group of atoms.

The element or compound that loses electrons is generally considered to be oxidized, whereas the elements /substrates that gain electrons become reduced. In these cases a substance that easily gives up electrons is a satisfactory reducing agent, whereas the one that easily takes up electrons is a satisfactory oxidizing agent (Jay 1986a).

As electrons are transferred from one compound to another, a potential difference is developed between the two reactive compounds, and this is known as oxidation-reduction potential which is expressed by the symbol E_h. The potential difference can be measured instrumentally and expressed as positive, negative, or neutral millivolts (mV). (Christian 1980). A substance that becomes more highly oxidized will have a more positive electrical potential, whereas the substance that becomes more reduced will have a more negative electrical potential.

In MPR fruit processing, it is important to maintain reducing (more anaerobic) conditions that are less favorable for aerobic growing conditions for molds and yeasts, which are major problems in these products. However, this opens the threat of *C. botulinum* under strongly anaerobic conditions.

The oxidoreductases tend to catalyze both oxidation and reduction of substrates (Whitaker 1972b). However, most of these enzymes catalyze oxidation of the substrate, and these include PPO (o-diphenol activity), PPO (hydroxylation reaction), catalase, PO, and lipoxygenase which collectively or individually affect color and flavor of MPR fruits and vegetables and intact fruits and vegetables as with highly

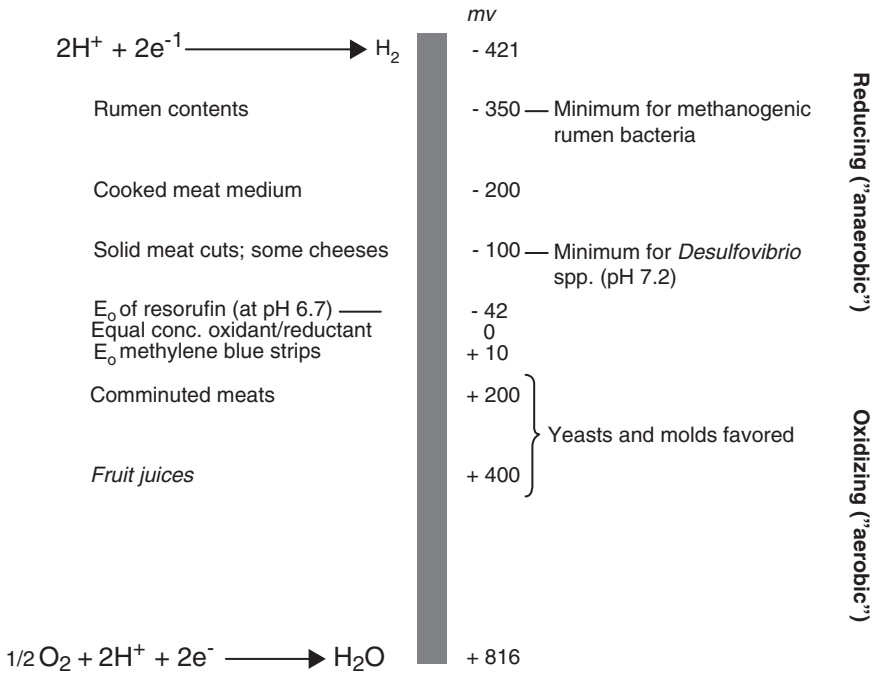

Fig. 6.12 Schematic representation of oxidation-reduction potentials relative to the growth of certain microorganisms (From Jay 1986a)

negative E_h values in most vegetable products that have a pH <4.6. Figure 6.12 shows the oxidation-reduction potentials for growth of several types of microorganisms and the foods in which they will reproduce {Jay 1986a). Those compounds that tend to maintain reducing conditions in fruits and vegetables are ascorbic acid and reducing sugars. Oxygen of course would oxidize a system and tend to maintain aerobic conditions in the food or package but may also increase respiration. For more on oxidation-reduction, see Jay (1986a), well if not properly controlled by preservation methods. It appears that a great deal of research remains to be conducted relative to *Of* R, control of microorganisms, and enzymes to preserve and extend shelf life of MPR fruits and vegetables.

6.10 Preservation by Combined Methods

Recently a symposium was held to discuss the use of combined preservation methods to provide safety and satisfactory shelf life for foods (Aguilera and Parada 1991). The hurdle technology suggested included heating, a_w control, chilling, pH control, O/R potential, preservatives, and competitive flora (Leistner 1978, 1987, 1991).

The hurdle concept must be carefully applied to MPR fruits and vegetables, and the issue is still subject to much research and governmental regulation. Selected hurdles must maintain safety and like-fresh quality and extend shelf life of the product.

In Ibero-American countries, losses of fruits and vegetables range between 35% and 40% because of lack of satisfactory preservation facilities. Welte (1991) reports that combined method techniques that seem to imply mainly a_w and pH reduction for products such as pineapples, papayas, bananas, mangos, peaches, potatoes, carrots, etc. can prolong shelf life longer than 1 year and still retain their like-fresh characteristics. The preservation parameters given were 0.92 ≪ 0.97 $a_{w/}$ and pH control to 3.5. The use of minimal processes combined with proper packaging was shown to be much more complicated than those used for fresh intact nonprocessed fruits and vegetables. One unique application to extend storage life of oranges whose rinds are chill sensitive is to peel the fruit and store in a 5% O_2, 5% CO_2, and 90% N_2 gas mixture at 0 °C. The peeled fruit was equal to the control in sensory and other quality characteristics. This is a good example of a combination preservation method of low temperature and modified gaseous atmospheres to improve shelf life quality (Mannapperuma and Singh 1990).

Earlier sections of this chapter have introduced the concepts of one or more preservation methods in combination or combinations of preservation steps within a major preservation category such as combined chemical preservatives and multiple heat treatments at different stages of the processing and packaging cycle. Scott (1989) has reported the interaction of factors to control the microbial spoilage in refrigerated foods not specifically MPR fruits and vegetables. One is directed to her article which more specifically covers interactions of various preservation systems including water activity (a_w), pH, preservatives, temperature during storage, and modified atmosphere packaging. In the latter case, a warning should be reemphasized that vacuum packaging utilizing MAP has the potential with minor temperature abuse to induce the growth and production of *C. botulinum* toxin.

Scott (1989) comments that there is information available on some interactions of preservation methods found in the literature and reported earlier in this chapter, but there is a great need for studies of multiple variables (more than two) which might involve pH, a_w, preservatives, strains of pathogen, storage temperature, etc. This makes for very complicated experiments, and it appears at the present time, much of the work that is being conducted on preservation by combined methods is proprietary and found only in research institutes and industry laboratories.

The use of multiple preservation methods for MPR fruits and vegetables is even more complex because enzymes or living biological plant systems have to be dealt with as well as microorganisms, both pathogens and spoilage types, to extend shelf life and provide like-fresh quality. This is a difficult task and challenge to the food industry in the years to come.

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Chapter 7
Packaging and Preservation Methods of Minimally Processed Produce

Zehra Ayhan

7.1 Introduction

Ready-to-eat/use products are a rapidly growing sector in the market because of increased consumer demand for fresh, healthy, convenient, and additive-free prepared products. Especially minimally processed or fresh-cut products are becoming rapidly growing sector of the horticultural industry (Soliva-Fortuny and Martin-Bellaso 2003). However, freshly prepared food items are highly perishable and prone to major spoilage mechanisms of enzymatic discoloration, moisture loss, and microbial growth.

Good manufacturing practices along with appropriate packaging materials are required to control these spoilage mechanisms (Ayhan 2011). The challenge is to develop and apply treatments effective on pathogens on the surface and in subsurface areas of fresh and fresh-cut produce without compromising sensory quality through the shelf-life (Beuchat 2007). This chapter reviews the different packaging and preservation methods applicable to minimally processed produce to maintain quality and safety and increase the shelf-life.

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7.2 Packaging Technologies and Materials

7.2.1 Packaging Technologies

7.2.1.1 Modified Atmosphere Packaging (MAP)

The basic concept of the MAP is simply replacement of air in a package headspace with a specific gas or gas mixture (Yuan 2003; Farber et al. 2003; Tas and Ayhan 2005). MAP has become a widely used food preservation technique especially for fresh-cut fruits and vegetables to extend shelf-life by reducing water loss, inhibition of cut-surface browning by low O_2 and high CO_2, lowering respiration rates and ethylene biosynthesis by low O_2 and high CO_2, delayed tissue softening, and retarded microbial growth (Gorny 2003; Wang 2006).

The success of MAP depends on an achievement of a balance between minimally processed product respiration rate and film permeability to maintain an acceptable equilibrium atmosphere within the package which is important for delaying ripening/maturation/ senescence and thus extending the product shelf-life. Achievement of this goal is dependent on product and package parameters as shown in Table 7.1 (Ayhan 2011).

MAP technology seems straightforward since it uses permeable film and the product respiration rate at a specific temperature to alter the concentration of oxygen and carbon dioxide around the product. However, the complexity of this packaging system is always underestimated (Jobling 2001). In the new MAP era, it is necessary to show that MAP does not only deal with gas mixture and films. The new thought is to incorporate the functional elements such as active and intelligent packaging to solve the food safety issues. Intelligent and active packaging technologies called "interactive packaging" have been introduced for use in conjunction with existing packaging technologies such as MAP (Yuan 2003).

The effects of MAP and different parameters have been extensively investigated for various fresh-cuts, and some examples from the literature are listed in Table 7.2.

Table 7.1 Product and package parameters effective on the success of MAP of fresh-cuts

Product factors	Packaging film factors
Respiration rate of the product at the selected storage temperature	Oxygen, carbon dioxide, and water vapor permeability of selected polymeric packaging materials at the selected storage temperature per unit thickness of material
Respiratory quotient of the product at the selected storage temperature	Effect of relative humidity on film permeability to oxygen and carbon dioxide
Mass of the product to be placed inside the package (or free volume inside the package)	Total surface area of the sealed package
Oxygen and carbon dioxide concentrations necessary to achieve optimum reduction of product aerobic respiration rate	Seal integrity of the package
Product initial maturity and microbial quality	

Table 7.2 Shelf-life of selected minimally processed fruits and vegetables under different MAP conditions

Produce	Atmosphere	Packaging material	Storage temperature	Shelf-life	References
Pomegranate arils	Air; 100%N_2;70%O_2–10%CO_2	BOPP	5 °C	18 days	Ayhan and Esturk (2009)
Fresh-cut broccoli	5%O_2–10%CO_2	Microperforated PP bags	5 °C	12 days	Fernandez-Leon et al. (2013)
Orange segments	20%O_2–10%CO_2; 80%O_2–10%CO_2	PP tray with CPP/OPP lid	4 °C	10 days	Karacay and Ayhan (2010)
Grapefruit segments	Air; 20%O_2–10%CO_2; 70%O_2–20%CO_2	PP tray with CPP/OPP lid	4 °C	< 10 days 10 days	Karacay and Ayhan (2010)
Carrot slices	Air; 5% O_2–10% CO_2; 80% O_2–10% CO_2	PP trays sealed with PP-based film	4 °C	7 days for passive MAP and high O_2, 2 days for low O_2	Ayhan et al. (2008)
MP butternut squash	5% O_2–5% CO_2	OPP	4 + 1 °C	2 weeks	Lucera et al. (2012)
Mandarin segments	Passive MAP	PP/EVOH/PP cups sealed with microperforated film	3 + 1 °C	3 weeks	Del-valle et al. (2009)
Fresh-cut carrots	Passive and active MAPs (10%O_2–10%CO_2)	Microperforated PP-based bags	4 °C	2 weeks	Mastromatteo et al. (2012)
Minimally processed grapes	Passive MAP	OPP, biodegradable polyester films, nylon/polyolefin film	5 °C	35 days	Del Nobile et al. (2009b)
Fresh-cut cime di rapa	10%O_2–2%CO_2 (MAP1) 8%O_2, 2% CO_2 (MAP2)	OPP bags	5 °C	14 days (MAP1) 9 days (MAP2)	Conte et al. (2011)
Fresh-cut zucchini	Air; 5% CO_2–5% O_2; 10% CO_2–15% O_2	OPP bags	5 °C	9 days	Lucera et al. (2010)

The effect of passive and active MAP (low and high oxygen concentration) on quality and shelf-life of pomegranate arils was searched by Ayhan and Esturk (2009). The pomegranate arils packaged with air, 100% nitrogen, and enriched oxygen (70% O_2 and 10% CO_2) kept physical and chemical quality attributes and were sensorially acceptable for 18 days using BOPP at 5 °C. However, the shelf-life was suggested as 15 days for low oxygen MAP (5% O_2, 10% CO_2) due to sensory quality.

The effect of MAP was searched for orange and grapefruit segments by Karacay and Ayhan (2010a, b). The authors applied air, low oxygen, and high oxygen using PP trays with CPP/OPP lids at 4 °C. Orange segments had a shelf-life of 10 days under low (20%O_2, 10% CO_2) and high oxygen (80%O_2, 10%CO_2) MAP. However, grapefruit segments had a shelf-life of less than 10 days under air and low oxygen (20% O_2, 10% CO_2) and 10 days under high oxygen (70% O_2, 20% CO_2) MAP.

Modified atmosphere packaging (5% O_2 and 10% CO_2) using microperforated PP bags maintained freshness and quality properties for fresh-cut broccoli at 5 °C for 12 days compared to unpackaged control in air. The decrease in functional compounds (chlorophyll, carotenoid pigments, vitamin C, total phenol content, and intact glucosinolates) and quality characteristics (overall acceptance, odor, weight loss, and color) was significantly lower in MAP than in control samples (Fernandez-Leon et al. 2013).

MAP is widely applied to minimally processed vegetables to retard the detrimental effects occurred by cutting process; however, MAP could lead to formation of off-flavors, and low O_2 and high CO_2 can promote anaerobic fermentation and growth of potential pathogens. Therefore, MAP should be carefully applied for MP fruits and vegetables considering significant parameters of produce, packaging materials, and storage conditions.

7.2.1.2 Active Packaging

Active packaging is a manipulation of the environment in the package to enhance food quality and safety and extend the shelf-life. Active packaging systems are divided into active-scavenging systems (absorbers) and active-releasing systems (emitters) (De Kruijf et al. 2002). The active packaging systems are oxygen and ethylene scavengers, moisture absorbers, ethanol and carbon dioxide emitters, and antimicrobial-releasing systems (Powers and Calvo 2003). Fruits and vegetables can be packaged using ethylene absorber and humidity regulators to increase the effectiveness of MAP. Antimicrobial-releasing films could be used to control the surface microorganisms.

Ethylene accelerates senescence and softening, increases chlorophyll degradation, and reduces shelf-life of fresh and minimally processed fruits and vegetables (De Kruijf et al. 2002). The application of MAP combined with ethylene absorber/adsorber could be more beneficial to control product metabolism and increase shelf-life of fruits and vegetables than application of MAP individually. An ethylene scavenger system involves the inclusion of a small sachet which contains an appropriate scavenger in the package. The sachet material is highly permeable to ethylene

which diffuses through the sachet. The reacting component inside the sachet is commonly potassium permanganate (KMO_4) which oxidizes or inactivates ethylene (Floros et al. 1997). Another ethylene removing system is based on the use of finely dispersed minerals (zeolite, active carbon, pumice, etc.) to absorb ethylene. These minerals could be incorporated in polyethylene bags which are used to package fresh fruits and vegetables (De Kruijf et al. 2002). These minerals not only absorb ethylene but also alter the permeability of the film so that ethylene and CO_2 diffuse more rapidly and O_2 enters more readily than through pure polyethylene (Esturk et al. 2014; De Kruijf et al. 2002).

Ethylene absorbers can especially extend the shelf-life of climacteric fruits such as apples, kiwifruit, apricot, bananas, mango, cucumber, tomato, and avocados and vegetables such as carrots, potatoes, and asparagus (De Kruijf et al. 2002). Esturk et al. (2014) successfully applied LDPE bags with ethylene adsorber (8% Tazetut® masterbatch, an inorganic product containing 50% of various aluminosilicate minerals (zeolite)) to broccoli florets and increased the shelf-life up to 20 days at 4 °C.

Fresh-cut tomatoes were packed in PP trays containing ethylene absorbent pads with potassium permanganate and heat sealed with perforated PP film, composite film, and BOPP film and stored at 0 °C and 5 °C for 10 days under active MAP (12–14 kPa O_2 and 0 kPa CO_2). The use of ethylene absorbent within packages did not improve shelf-life of tomatoes which is attributed to short storage period. Quality attributes of sliced tomatoes were better preserved at 0 °C for 10 days independent of the films tested. Increase in storage temperature from 0 °C to 5 °C in perforated and high permeability films increased yeast and mold growth by 3 log, while a slight increase was observed at 0 °C (Gil et al. 2002).

Oxygen absorbers are successfully used with nonrespiring products such as meat and pastries (Brody et al. 2001). The use of oxygen absorbers for respiring products is risky since reduction of oxygen in fresh and minimally processed fruits and vegetables below tolerance limit may lead to anaerobic conditions resulting in off-flavor and growth of anaerobic microorganisms. Charles et al. (2003) tested generic mathematical model to predict the gas exchange dynamics in an active MAP with an oxygen absorber using commercial iron-based scavenger for tomatoes in LDPE bags. The authors validated the model on real conditions that oxygen absorbers had a strong influence on reduction of the transient period duration (50 and 100 h with and without oxygen absorber, respectively) and elimination of CO_2 peak.

Excess CO_2 could be removed by using CO_2-permeable pack placed inside a modified atmosphere package. CO_2 absorbers include $Ca(OH)_2$, activated charcoal, and magnesium oxide. The amount of CO_2 absorbent required depends on the excess CO_2 level, the desired CO_2 level, and the shelf-life period (Schlimme and Rooney 1994).

Moisture regulators can prevent the growth of yeast and bacteria for foods with high water activity like minimally processed fruits and vegetables (De Kruijf et al. 2002). Shirazi and Cameron (1992) studied the humidity-controlling capacity of dry sorbitol, xylitol, NaCl, KCl, and $CaCl_2$ enclosed in polyethylene pouches in modified atmosphere packaged tomatoes. The study showed that the storage life of tomatoes was extended from 5 days to 15–17 days at 20 °C with the use of sodium

Table 7.3 Examples of different types of active packaging applications for fresh produce

Type of active packaging	Fresh-cut produce	Effect	References
LDPE bags with ethylene adsorber	Broccoli florets	Increased shelf-life up to 20 days at 4 °C	Esturk et al. (2014)
PP trays with ethylene absorbent pads ($KMnO_4$)	Fresh-cut tomatoes	Had no improvement in shelf-life	Gil et al. (2002)
PE pouches with moisture regulators (dry sorbitol, xylitol, NaCl, KCl, $CaCl_2$)	Tomatoes	Extension of storage life of tomatoes from 5 days to 15–17 days at 20 °C with NaCl	Shirazi and Cameron (1992)
LDPE bags with oxygen absorber (iron-based scavenger)	Tomatoes	Strong influence of oxygen absorbers on reduction of transient period duration and elimination of CO_2 peak	Charles et al. (2003)

chloride due to prevention of surface mold development. Examples of different types of active packaging applications are summarized in Table 7.3 for some fresh produce. This area still needs further research.

7.2.1.3 Intelligent Packaging

Intelligent packaging is a novel interactive packaging system carrying out intelligent functions such as detecting, sensing, recording, tracing, and communicating in order to inform consumers about the quality and safety of the food inside the package and warn about possible problems. There are basically two types of intelligent packaging. The first one is based on measuring the condition of the package outside such as time-temperature indicators. The second one is dependent on measuring the quality of the product inside the package like gas indicators, freshness indicators, and biosensors. In the latter case, there is direct contact with the food or with the headspace, and there is always need for a marker, which is indicative of the quality and/or safety of the packed food (De Jong et al. 2005; Ayhan 2012).

The new concept is to incorporate new packaging technologies such as active and intelligent packaging with MAP in order to monitor gas change, storage temperature, and quality of packaged product and maintain the initial gas level during the whole storage time (Ayhan 2010). Indicators or sensors in the form of a package label or printed on packaging films can monitor changes in the gas composition, storage temperature, and quality of the product (Table 7.4).

The success of the modified atmosphere packaging is dependent on the storage at low temperature and cold chain management for fresh and minimally processed fruits and vegetables. The main factor responsible for most problems in MAP in a commercial situation is the temperature. Unfortunately, the cool chain for fresh produce is not always continuous throughout the marketing system (Jobling 2001). Thus, improper storage temperatures or temperature abuses during storage lead to shorter shelf-life than expected.

Table 7.4 Potential intelligent packaging systems for MP produce

Intelligent packaging system	Use of purpose
TTIs	To provide temperature history of the MA-packaged product during storage and transportation to make sure proper handling during transportation and storage
Freshness indicators	To indicate lack of freshness or the spoilage of the product, in addition to temperature abuse or package leaks
Gas indicators (O_2, CO_2)	To determine gas level in the headspace of MA package To monitor maturity stage of fruits To determine improper sealing and package leakage and quality deterioration of MA packages

An intelligent packaging device such as time-temperature indicator (TTI) can provide temperature history of the MA-packaged product during storage and transportation. TTIs are placed outside the pack and can be defined as small measuring devices that show a time- and temperature-dependent irreversible color change (De Jong et al. 2005). TTIs may also be used as freshness indicator to estimate the remaining shelf-life of perishable products (Yam et al. 2005; Riva et al. 2001). These indicators are already commercialized for products which require cold chain such as MA products. However, the application of TTIs should be extended for all MA products to make sure proper handling during transportation and storage.

The correct gas mixture in MAP maintains high quality with extended shelf-life for individual products. However, the initial gas composition in the package headspace often changes as a result of the activity of the food product, the nature of the package, or the environmental conditions. For example, respiration of fresh produce, gas generation by spoilage microorganisms, or gas transmission through the packaging material or package leaks may result in changes of the gas composition inside the package. MAP application can be combined with intelligent packaging indicators such as gas indicators or sensors to monitor changes in the gas composition (Yam et al. 2005). Most of the indicators are based on the color change principle due to chemical or enzymatic reactions. These indicators are in contact with gas atmosphere and work based on the gas change in the package.

Gas indicators especially oxygen and carbon dioxide indicators could be useful at MAP applications. Another application of the gas indicators is to determine package leakage which not only causes change in internal atmosphere but also microbial contamination from the environment. Oxygen indicators can be used to determine improper sealing and quality deterioration of modified atmosphere packages (Smiddy et al. 2002). Carbon dioxide indicators are used to monitor carbon dioxide level in MAP systems. A leakage will result in a decrease in the CO_2 level in high CO_2 packages, and this change can be monitored by leakage indicators (Ayhan 2010).

An ideal indicator for the quality control of packaged food is to indicate lack of freshness or the spoilage of the product, in addition to temperature abuse or package leaks (De Kruijf et al. 2002). A freshness indicator determines the quality of

packaged foods by measuring the specific by-products formed through the deterioration process in the food and therefore inside the package (Fig. 7.1). Most of the freshness indicators are based on the detection of volatile compounds such as CO_2, diacetyl, amines, ammonia, and hydrogen sulfide produced during aging of foods (De Jong et al. 2005; De Kruijf et al. 2002). The ripeSense indicator shows consumers whether a tray of pears is ready to eat or not in terms of maturity, based on a color change on the label, which is triggered by the aroma components produced by the fruits (Fig. 7.2) (De Jong et al. 2005). Critically, these indicators need to be validated before commercial use for each produce.

Fig. 7.1 Freshness indicator (Fresh-Check®)

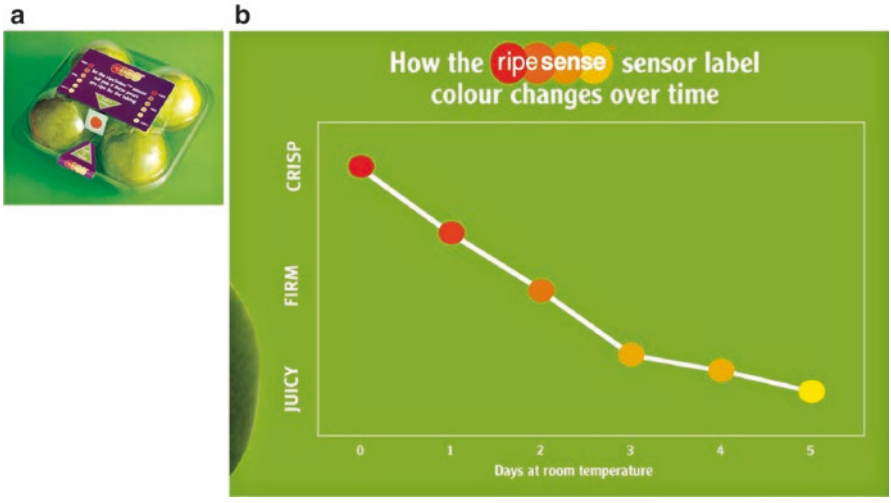

Fig. 7.2 (**a**, **b**) "ripeSense®" indicator showing maturity of the pears: *red color* indicating crispness and *yellow color* indicating juiciness (ripeSense Ltd.)

Table 7.5 Biodegradable materials tested for fresh-cut products

Fresh-cut product	Biodegradable material	References
Minimally processed lettuce	Polyester-based biodegradable films	Del Nobile et al. (2008a)
Head lettuce, cut and whole broccoli, tomatoes, and sweet corn	Laminate of chitosan-cellulose/ polycaprolactone	Makino and Hirata (1997)
Fresh-cut cantaloupe	Chitosan/methyl cellulose film	Sangsuwan et al. (2008)
Minimally processed table grapes	Polyester-based biodegradable films	Del Nobile et al. (2008b)
Ready-to-eat sweet cherries	Coextruded polyester	Conte et al. (2009)
Fresh-cut zucchini	Coextruded polyester	Lucera et al. (2010)
Asparagus, baby corn, and Chinese cabbage	Banana/chitosan films	Pitak and Rakshit (2011)

7.2.2 New-Generation Packaging Materials

7.2.2.1 Biodegradable Materials

The use of biodegradable materials in food packaging is still limited due to high gas permeability and low mechanical properties; however, these materials could be applied in packaging for respiring products to reduce the waste problems. The extensive use of petroleum-based packaging films has resulted in serious ecological problems due to their nonbiodegradability. Limited applications of bio-based films in ready-to-use fruit and vegetables have been reported in the literature (Table 7.5).

Del Nobile et al. (2008a) tested two types of polyester-based biodegradable films against OPP to prolong the shelf-life of minimally processed lettuce. Microbial shelf-life in both biodegradable films was determined longer than that of OPP. Del Nobile et al. (2006) also tested two high gas permeability polyolefins (PF1 and PF2) commercially used for MP products and biodegradable film (BF, a ternary biodegradable mixture of three biodegradable polyesters) to package iceberg and Romaine lettuces. Products in PF1 had the lowest respiration activity, whereas fresh-cut product packed in PF2 and BF films showed similar respiration activity probably due to similar gas barrier properties. Biodegradable film tested was suggested for fresh-cut lettuce as an alternative to commercially available materials in terms of senescence level and color variation.

The laminate of chitosan-cellulose/polycaprolactone was found suitable for MA packaging of head lettuce, cut and whole broccoli, tomatoes, and sweet corn based on computer simulation using the respiration rate equations in the temperature range of 10–25 °C. It is reported that the biodegradable laminate had a permeability value comparable to that of LDPE film which is commonly used for MP vegetables (Makino and Hirata 1997). Effect of chitosan/methyl cellulose film with and without vanillin as a natural antimicrobial agent was studied on microbial and quality characteristics of fresh-cut cantaloupe and pineapple against commercial stretch film at 10 °C for 20 days. Chitosan/methyl cellulose and vanillin containing films

showed inhibitory effect against *E. coli* and *S. cerevisiae*. Vanillin was more effective on the microorganisms in a low pH fruit. Quality attributes of both fruits were acceptable; however, vanillin film significantly reduced the ascorbic acid content in pineapple (Sangsuwan et al. 2008). The antimicrobial activity of vanillin against bacteria and yeast and molds was dependent on time of exposure, concentration, and target organisms (Fitzgerald et al. 2004; Rupasinghe et al. 2006).

Two polyester-based biodegradable films including a monolayer film based on blend of biodegradable polyesters (NTV1, thickness of 18 μm) and multilayer coextruded film based on a blend of biodegradable polyesters (NTV2, thickness of 25 μm) were tested for minimally processed table grapes stored at 5 °C for a period of 30 days. There was no significant difference reported between two biodegradable films in terms of quality decay of the product probably due to similar oxygen permeability values (Del Nobile et al. 2008b).

A biodegradable coextruded polyester (COEX, 35 μm) and oriented polypropylene (OPP, 20 μm) were used to package ready-to-eat sweet cherries under ordinary and modified atmosphere packaging. OPP showed the best performance under ordinary atmosphere in delaying quality loss due to lower water loss and more controlled respiration rate. However, both biodegradable and OPP films had similar effects on the fruit due to counterbalanced effects between dehydration and respiratory activity (Conte et al. 2009). The same materials also tested for fresh-cut zucchini under passive and active MAP. OPP film (90 μm in thickness) under both atmospheres showed slightly better performance in extending the shelf-life compared to COEX. The product in biodegradable COEX maintained overall quality better under active atmosphere comparing to air (Lucera et al. 2010).

Banana/chitosan films made by mixing banana flour with chitosan were found to protect asparagus, baby corn, and Chinese cabbage against *S. aureus*. The presence of starch in the composite film provided water solubility and sealable bags, while the presence of chitosan gained antimicrobial property (Pitak and Rakshit 2011).

Biodegradable materials could be used to generate a suitable modified atmosphere around packaged products and might replace nonbiodegradable plastics commonly used in MAP. The application of eco-friendly biodegradable films is currently limited for minimally processed products due to high conversion cost and small amounts available. This area still needs further research and development.

7.2.2.2 Microperforated Materials

In passive MAP systems, establishment of gas equilibrium is based on respiration rate of the produce and gas permeability of the packaging materials. The time to reach steady-state level is longer in this traditional approach comparing to active MAP. The oxygen permeability of current packaging materials is also insufficient for highly respiring produce leading to eventual oxygen depletion and off-flavor formation. These problems could be solved by applying active MAP using microperforated packaging materials. However, perforation level and gas composition are significant for the design of efficient microperforated active MA packages (Rodov et al. 2007).

The use of perforated films provides holding a modified atmosphere and allowing enough oxygen to partially satisfy the metabolic processes (Zanderighi 2001). Microperforation technology depends on numerous, smaller, and precise holes in the range of 50–60 μm in diameter for delivering the targeted atmosphere. The number of microperforations designed for any specific product depends on product's respiration rate, the desired balance between oxygen and carbon dioxide in the package, the diameter of holes, and thickness and permeability of the packaging material (Greengras 1999). The size and shape are two important attributes of perforated films which should be specific to the product to be packaged. It was reported that no stationary conditions compatible with any MA can be found for continuous film; however, with perforated film, it is possible to find a stationary state where a constant MA is maintained inside the pack (Zanderighi 2001).

Perforated films may have potential to provide adequate fluxes of O_2 and CO_2 for different produces with medium to high respiration rates (Bai et al. 2003). Del-Valle et al. (2003) presented theoretical predictions for the exchange of gases and vapors through perforated films. A model based on the diffusivity of gases on air considering the pore dimensions was used to preselect a suitable package with microperforations for mandarin segments. Selected quality indicators and sensory evaluation showed that the optimum equilibrium-modified atmosphere packaging for clementine mandarin segments was 19.8% O_2 and 1.2% CO_2 (Del-Valle et al. 2009).

Microperforated PP (30 μm with 2, 7, 12, and 20 micro-holes with a diameter of 70 μm) and non-perforated OPP (20, 40, 60, and 80 μm in thickness) films were tested for fresh-cut butternut squash under passive and active MAP (5% O_2 and 5% CO_2). Results of this study showed that microperforated films did not match respiratory requirements of fresh-cut butternut squash leading to high O_2 headspace and visible mold growth. OPP with the thickness of 40 μm showed better performance with providing 2 weeks of shelf-life under active and passive MAP at 4 °C (Lucera et al. 2012). Lucera et al. (2011) tested the same materials for fresh-cut broccoli florets and had the best performance using microperforated PP (MP-PP) films with the thickness of 7 and 20 μm in controlling mass loss, wilting, and sensory quality compared to other microperforated and non-perforated PP films (with the thickness of 20, 40, and 80 μm). The shelf-life of fresh-cut broccoli in the MP-PP-20 and MP-PP-7 was approximately 14 and 20 days, respectively, comparing to the shelf-life of product in OPP-20 (9 days) and unpackaged product (6 days) at 5 °C. These studies showed that mass transport properties of the packaging films have significant influence on headspace gas concentration and thus the selection of appropriate packaging materials can maintain quality characteristics of the fresh-cut products.

Microperforated PVC (totally permeable to O_2 and CO_2) and three P-Plus films (P-Plus 240, 160, 120) made of PP with different permeabilities were tested for minimally processed broccoli and cauliflower both under dark and light. P-Plus-120 with the lowest O_2 permeability film was the best for cauliflower under dark conditions. However, all packages gave similar results in both light and dark for broccoli (Olarte et al. 2009).

Fresh-cut "Gala" apple slices were packaged in multilayered polyolefin film (with OTR and CO_2TR of 14.1 pmols s^{-1} m^{-2} Pa^{-1} and 94 pmols s^{-1} m^{-2} Pa^{-1}, respectively) establishing high CO_2 and low O_2 and ultra microperforated (MP) film

(with OTR and CO_2TR of 94 pmols s^{-1} m^{-2} Pa^{-1} and 69 pmols s^{-1} m^{-2} Pa^{-1}) establishing high CO_2 and high O_2 atmosphere and stored at 5 °C for 21 days. Fruits in MP packages had less juice loss and better textural and sensory properties which are attributed to lower ethylene concentration and high O_2 and CO_2 levels (Cliff et al. 2010).

Recently developed, microperforated films with very high gas transmission rates are now commercially available and used for maintaining aerobic equilibrium-modified atmosphere (EMA) (e.g., 5–15%O_2 and 5–15%CO_2) for highly respiring produce such as broccoli, cauliflower, carrot, mushroom, and spinach. However, microperforated films are relatively expensive, permit moisture and odor losses, and may allow for the ingress of microorganisms into the sealed package during wet handling situations (Day 2003).

7.2.2.3 Nanomaterials

Application of nanotechnology in food packaging is considered highly promising since this technology could improve safety and quality of food while reducing the use of valuable raw materials and the generation of packaging waste. Nanotechnology is applicable in food packaging to improve packaging performances such as gas, moisture, UV and volatile barriers, mechanical strength, heat resistance and flame retardancy, and weight (Jamshidian et al. 2010; Garcia et al. 2010; Silvestre et al. 2013). Most of the research papers deal with lowering gas permeability with addition of nanoparticles into polymers which are applicable to nonrespiring products. However, there are some limited applications of nanomaterials in the literature for respiring products.

Polyvinyl chloride (PVC) film coated with nano-ZnO powder was tested to package fresh-cut "Fuji" apple at 4 °C for 12 days, and the results were compared with the uncoated PVC (control). Nanocoated PVC film reduced fruit decay rate, slowed down ethylene production, maintained brix and titratable acidity, and inhibited the enzyme activity (PPO and POD). Authors claimed that nano-PVC provided more oxygen and less carbon dioxide inside the package compared to control, indicating low respiration in the nanopackages (Li et al. 2011).

Fresh-cut yam was coated by chitosan including nano-$CaCO_3$ and stored at 10 °C. The results revealed that titratable acidity, vitamin C content, and L value of the fresh-cut yam coated with chitosan-nano-$CaCO_3$ were higher than the product coated with only chitosan. The weight loss and total phenolic content of the product coated with chitosan-nano-$CaCO_3$ were reported less than that of chitosan coated. Chitosan incorporated with nano-$CaCO_3$ was suggested for use in fresh-cut yam to prolong the shelf-life (Zisheng et al. 2009).

Titanium dioxide (TiO_2)-coated oriented PP (OPP) bags were applied on the fresh-cut lettuce inoculated with *E. coli*. The number of *E. coli* cells of the cut lettuce packaged in TiO_2-coated bags exposed to UV light decreased from 6.4 log CFU/g to 4.9 log CFU/g on the first day of storage; however, there was only 0.3 log CFU/g reduction in the product packaged in uncoated bags. The results showed that

Table 7.6 Nanomaterials tested for different types of fresh-cut produce

Type of nanomaterial	MP produce	Main effect	References
PVC coated with nano-ZnO	Fresh-cut apple	Reduced fruit decay, slowed down respiration rate and ethylene production, inhibited enzyme activity	Li et al. (2011)
iPP with $CaCO_3$ nanoparticles	Apple slices	Limited oxidation and microbial growth, shelf-life of 10 days	Avella et al. (2007)
TiO_2-coated OPP bags	Fresh-cut lettuce	Reduced microbial contamination (*E. coli*)	Chawengkijwanich and Hayata (2008)
Cellulose-based absorbent pads with nanosilver	Fresh-cut melons	Controlled spoilage microorganisms, retarded senescence, reduced brix	Fernandez et al. (2010)
Chitosan with nano-$CaCO_3$	Fresh-cut yam	Higher vitamin C, L value and titratable acidity, less weight loss, and total phenolic content	Zisheng et al. (2009)
Chitosan-nano-SiOx coating	Fresh-cut bamboo shoots	Reduction in respiration rate and ethylene production, less enzymatic activity, lower browning index	Zisheng and Li (2010)
PE with nano-powder (Ag, TiO_2, Kaolin)	Fresh strawberry	Reduced fruit decay, controlled brix and MDA, inhibited enzyme activity, and kept vitamin C	Yang et al. (2010)

TiO_2-coated film could reduce the microbial contamination on the surface of fresh-cut products with reducing the risk for microbial growth (Chawengkijwanich and Hayata 2008). The list of the some applications of nanomaterials is presented in Table 7.6.

It might be very risky to use nanomaterials with low gas permeability for respiring products such as fruits and vegetables, which require high permeability materials. Nanomaterials could be an alternative to regular synthetic materials if they are produced with high gas permeability for respiring products. The material permeability and product respiration rate should match to obtain gas equilibrium inside the package for successful modified atmosphere package (MAP) applications (Ayhan 2013).

7.3 Preservation Methods

7.3.1 Chemical Treatments

Chemical treatments of fresh-cut fruits and vegetables mostly involve surface treatments by dipping fresh-cuts into solutions containing antimicrobials, antioxidants, calcium salts, or functional ingredients to improve overall quality and increase the shelf-life. Dipping treatments with organic acids are mostly used to reduce browning reactions, maintain firmness, and improve sensory quality (Lucera et al. 2012).

The use of natural antimicrobial agents like plant essential oils (EOs) is getting more attention to improve microbiological stability and increase the shelf-life of fresh-cuts due to increased awareness of consumer for natural products (Oms-Oliu et al. 2010). However, the limited solubility, the negative impacts on the organoleptic properties of food, and their variable activity in food due to interactions with food components are the major difficulties of plant essential oils (Gutierrez et al. 2008). EOs have been proposed for several fresh-cut fruits to control the microbial growth. The addition of 0.02% citrus, mandarin, cider, lemon, and lime EOs to MP fruit mix inhibited the growth of naturally occurring microbial flora and reduced the growth rate of inoculated *S. cerevisiae* and increased the shelf-life without compromising the sensory quality (Lanciotti et al. 2004). Dipping apple slices into vanillin (0.18%, w/v) inhibited 37% and 66% of the microbial growth on "Empire" and "Crispin" apple slices, respectively, after 19 days of storage (Rupasinghe et al. 2006). The application of carvacrol and cinnamic acid (0.015%, v/v) was effective in reducing microbial growth on fresh-cut kiwifruit and honeydew melon with no detrimental effect on sensory properties. However, the carvacrol in the range of 0.075–0.225% (v/v) had undesirable effect on color and odor of kiwifruit (Roller and Seedhar 2002). The potential applications of different EOs may need further research for fresh-cut produce.

Chlorine is most practicable and an efficient antimicrobial at low cost and widely used in fresh-cut industry; however, there is a concern about the presence of chlorine by-products or chlorine residue. The hypochlorous acid is the active component to kill bacteria by disrupting cell walls. In the USA, rinsing with potable water after chorine treatment is obligatory (Varoquaux and Mazollier 2002; Klaiber et al. 2005). Cooled hypochlorite solutions containing 50–200 ppm free chlorine are widely used with suggested contact time less than 5 min (Baur et al. 2004). 10 ppm available chlorine in washing water can inactivate vegetative organisms, but higher concentrations are required in commercial applications since available chlorine is lost by interaction with organic matter, amino acids, proteins, and other amine compounds in the tissues and juices of commodities. Washing with chlorine (100 ppm available chlorine) had significant effect on the quality and shelf-life of dry coleslaw mix by reducing respiration rate and microbial loads leading to improved sensory scores at 4 °C for 9 days (Cliffe-Byrnes and O'Beirne 2005).

Chlorine dioxide gas is reported as a strong oxidizing and sanitizing agent and tested on different MP fruits and vegetables focusing on the efficacy of ClO_2 on pathogens such as *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella typhimurium*, spoilage microflora, and quality parameters (Han et al. 2004; Singh et al. 2002; Du et al. 2002, 2003; Lee et al. 2004; Gomez-Lopez et al. 2007, 2008). ClO_2 does not form significant amounts of chlorinated by-products as chlorine does (Gomez-Lopez et al. 2009). Gaseous ClO_2 treatment reduced initial microbial count for MP lettuce and cabbage, but enhanced the growth of surviving microorganisms before the third day of storage comparing to untreated samples with ClO_2 (Gomez-Lopez et al. 2008). Gaseous ClO_2 was also tested for grated carrot with 1-day enhancement in shelf-life without affecting respiration rate and sensory attributes (Gomez-Lopez et al. 2007).

Among the applications of chlorinated water (200 mg/l free chlorine), ozone treatment (1 mg/l ozone), and tap water washing for shredded iceberg lettuce, chlorinated water was the most effective one in terms of reduction in microbial load and maintenance of sensory quality. This study suggested the use of a more efficient ozone application technology to obtain comparable results to chlorine (Baur et al. 2004). Klaiber et al. (2005) tested cold (4 °C) and warm water (50 °C) with chlorine (200 mg/l chlorine) for minimally processed carrots (peeled and trimmed) and reported that comparable antimicrobial efficacy was obtained by chlorine in combination with thermal treatment. However, sensory properties of the product were slightly affected.

Ozone as a potential alternative to chlorine could be used for sanitation of fresh produce since ozone does not leave hazardous residue on food (Khadre et al. 2001). FDA approved the use of ozone as an antimicrobial agent for processing of horticultural produce (Alothman et al. 2010). Ozone has been shown to inhibit the growth of spoilage bacteria and yeast at concentrations of 0.15–5.00 ppm (Jay et al. 2005).

The effect of ozone treatment was investigated on total phenolic, flavonoid, and vitamin C contents of fresh-cut pineapple, banana, and guava. Total phenol and flavonoid contents of pineapple and banana increased significantly when exposed to ozone at a flow rate of 8 ml/s for 20 min; however, this positive effect was compromised by vitamin C reduction of the fruits tested (Alothman et al. 2010). The ozone treatment was effective in extending the shelf-life of oranges, apples, grapes, raspberries, and pears (Skog and Chu 2001; Alothman et al. 2010).

There are some other chemicals such as benzylaminopurine also tested for MP produce. Fresh-cut broccoli florets were treated with 6-benzylaminopurine (BAP) in the range of 0–15 ppm for 10 min at 6 ± 1 °C. Treatment with 10 ppm BAP showed significant effect on delaying chlorophyll degradation and yellowing with higher retention of protein and ascorbic acid content (Siddiqui et al. 2011).

Since browning is one of the major problems for fresh-cut surfaces, reducing agents which prevent browning such as citric acid, ascorbic acid, isoascorbic acid, sodium erythorbate, thiol-containing amino acids such as N-acetylcysteine and glutathione, oxalic acid, and 4-hexylresorcinol have been investigated (Soliva-Fortuny et al. 2002; Dong et al. 2000; Oms-Oliu et al. 2006, 2010; Rojas-Graü et al. 2006; Son et al. 2001). Dipping apple slices into antioxidant solution of 1% ascorbic acid and 1% citric acid reduced the initial oxygen respiration rate for about 10 h of storage and inhibitory effect of CO_2 on the respiratory activity of the packaged product (Rocculi et al. 2006).

The use of sulfites as antibrowning agent on fruits and vegetables is banned in the USA. Ascorbic acid and 4-hexylresorcinol (HR) and a mixture of both on apple slices were tested as possible alternatives to sulfites. L-cysteine solution was found effective as a browning inhibitor for minimally processed vegetables (lettuce and cabbage) in preventing darkening caused by ClO_2 (Gomez-Lopez et al. 2008). It is reported that the mixture significantly inhibited the browning of vacuum-packed apple slices for up to 8 weeks during 0.5 °C storage (Luo and Barbosa-Canovas 1996), but the use of vacuum packaging for respiring fresh-cuts is not a common and practical application.

Among the chemical treatments for preventing tissue softening of fresh-cut produce, calcium treatments are most popular. Although calcium chloride has been one of the most frequently used calcium salt, it is reported to impart the taste of the products. Thus, the other calcium salts especially calcium lactate, calcium propionate, and calcium ascorbate have been investigated as alternative sources of calcium (Dong et al. 2000; Gorny et al. 2002; Alandes et al. 2006; Oms-Oliu et al. 2010).

The effectiveness of these chemicals used for fresh-cut surfaces could be improved by incorporation of these compounds into edible coatings. Application of edible coatings to deliver active substances is one of the major advances to preserve the fresh-cut fruits and vegetables.

7.3.2 Coating

Edible coatings provide a semipermeable gas barrier against oxygen, carbon dioxide, and moisture between food and the surrounding atmosphere and reduce respiration, water loss, solute migration, and oxidation reaction rates in fresh-cuts (Valencia-Chamorro et al. 2011; Mastromatteo et al. 2011; Chiumarelli et al. 2011). Edible coatings retard moisture loss by providing water vapor barrier and minimize microbial contamination in fresh-cut products (Perera and Baldwin 2001). The coatings are also carriers of a wide range of food additives such as antibrowning agents, antimicrobials, nutrients, colorants, flavors, and spices to improve quality and safety and increase the shelf-life of minimally processed produce (Lee et al. 2003; Chiumarelli et al. 2011; Colla et al. 2006; Rojas-Graü et al. 2007; Mastromatteo et al. 2011; Cagri et al. 2004; Pranoto et al. 2005). The use of edible coatings may also reduce overall packaging requirement and waste disposal problems to some extent (Valencia-Chamorro et al. 2011). However, application of edible coating itself may not provide the desired extended shelf-life. There are different types of coating applied to various minimally processed fruits and vegetables with different effects (Table 7.7).

Alginate films are hydrophilic and thus poor moisture barriers; however, calcium incorporation makes alginate films water insoluble and reduces the water vapor permeability (Olivas et al. 2007). Alginate-based edible coatings retarded moisture loss and significantly increased crispiness in minimally processed lettuce (Tay and Perrera 2004). Alginate coatings can be easily removed by washing, although they are edible. There are different studies indicating the positive effect of sodium alginate on preservation of quality parameters of fresh-cut papaya, apple, and melon with extended shelf-life (Olivas et al. 2007; Oms-Oliu et al. 2008; Rojas-Graü et al. 2008; Tapia et al. 2008).

Alginate-based edible coatings were tested to preserve the quality of minimally processed "Gala" apples. Apple wedges were coated with alginate, alginate-acetylated monoglyceride-linoleic acid, and alginate-butter-linoleic acid. The alginate coatings increased the shelf-life without resulting in anaerobic respiration, minimized weight loss, prevented loss in firmness, and decreased the browning. Flavor volatiles like

Table 7.7 Different types of coatings applied to MP produce and their effects

Type of coating	MP produce	Effects	References
Alginate-based coatings	MP lettuce	Retarded moisture loss and significantly increased crispiness in minimally processed lettuce	Tay and Perrera (2004)
Na alginate-based coatings	Fresh-cut papaya, apple, and melon	Extended the shelf-life	Olivas et al. (2007), Oms-Oliu et al. (2008), Rojas-Graü et al. (2008), Tapia et al. (2008)
Alginate-based coatings	Apple wedges	Increased shelf-life, minimized weight loss, prevented firmness loss, decreased browning	Olivas et al. (2007)
Coating with calcium caseinate, carboxymethyl cellulose and whey protein isolate (WPI)	Apple slices	Reduced surface discoloration and ethylene production	Brancoli and Barbosa-Canovas (2000)
Whey protein concentrate (WPC) and WPI	Apple cubes	Delayed browning, WPC was more effective in reducing weight loss than WPI	Sonti et al. (2003)
Coating with carrageenan or WPC in combination with antibrowning agents (ascorbic acid, citric acid, oxalic acid)	Apple slices	Had a shelf-life of 2 weeks at 3 °C. WPC-$CaCl_2$ was the most effective method in terms of sensory quality	Lee et al. (2003)
A coating with whey protein isolate and beeswax	Apple slices	Inhibited browning but not effective in moisture loss	Perez-Gago et al. (2003)
Coating with cassava starch or sodium alginate w or w/o glycerol	Fresh-cut mango	Reduced respiration rate, cassava w/o glycerol was more effective in reducing weight loss and maintaining mechanical properties and color	Chiumarelli et al. (2011)
Trehalose	Apple slices	Reduced browning, decreased weight loss and organic acid loss, retarded growth of yeast and molds and aerobic bacteria, no significant effect on firmness	Albanese et al. (2007)
Coating with pea protein candelilla wax and sorbitol	Broccoli heads	Effective on reducing the rate of vitamin C loss and firmness, had no effect on reducing the mass loss and chlorophyll content	Kowalczyk et al. (2010)
Coating with hydroalcoholic solution and grapefruit seed extract solution	MP kiwifruit	Inhibited microbial growth	Mastromatteo et al. (2011)

(continued)

Table 7.7 (continued)

Type of coating	MP produce	Effects	References
Alginate-based coatings incorporated with malic acid and essential oils of cinnamon, palmarosa, and lemongrass	Melon pieces	Prolonged microbiological shelf-life by more than 21 days, odor and taste affected by cinnamon oil, firmness affected by lemongrass, coating with 0.3% of palmarosa oil maintained quality parameters, and inhibited native flora and *S. enteritidis*	Raybaudi-Massilia et al. (2008)
Chitosan and pectin coating with microencapsulated trans-cinnamaldehyde	Fresh-cut papaya	Extended the quality and the shelf-life up to 15 days at 4 °C, no negative effect on flavor	Brasil et al. (2012)
Chitosan coating enriched with bioactive compounds and essential oils	Fresh-cut broccoli	Significant reduction on mesophilic and psychrotrophic population, no negative effect on sensory attributes	Alvarez et al. (2013)
HPMC or chitosan (CH) with or without bergamot essential oil	Table grapes	CH coatings had the most effective antimicrobial activity and the greatest inhibition on the respiration rates	Sanchez-Gonzalez et al. (2011)
Coating with 1% of chitosan-nano-SiOx complex	Fresh-cut bamboo	Reduced the respiratory rate and ethylene production rate, delayed increase in enzymatic activity	Zisheng and Li (2010)

hexanol and trans-2-hexenal were more pronounced in fatty acid-containing coatings, but the effect of these volatiles on flavor is not determined (Olivas et al. 2007).

Coating with calcium caseinate, carboxymethyl cellulose, and whey protein isolate (WPI) reduced surface discoloration and ethylene production of apple slices (Brancoli and Barbosa-Canovas 2000). Coating with calcium caseinate, carboxymethyl cellulose, and whey protein concentrate (WPC) was effective on delaying browning of apple slices (Le Tien et al. 2001). Synergistic effect was noted when edible coatings were used with antibrowning agents. Apple slices coated with carrageenan or WPC in combination with antibrowning agents such as ascorbic acid, citric acid, and oxalic acid had a shelf-life of 2 weeks at 3 °C. WPC with ascorbic acid plus $CaCl_2$ was the most effective preservation method in terms of sensory quality (Lee et al. 2003). A coating including WPI and beeswax inhibited browning of apple slices but was not effective in moisture loss (Perez-Gago et al. 2003). WPC and WPI delayed browning and texture decay for apple cubes. Coating with WPC was shown to be more effective in reducing weight loss than coating with WPI (Sonti et al. 2003).

Fresh-cut mangoes were pretreated with citric acid and coated with either cassava starch or sodium alginate with or without glycerol. All coatings tested reduced the respiration rate, but dipping in citric acid and coating with cassava starch without glycerol were more effective in reducing weight loss and maintaining mechanical properties and color compared to untreated fresh mangoes or control samples (Chiumarelli et al. 2011).

Broccoli heads were coated with pea protein, candelilla wax, and sorbitol and stored at 4 °C for 21 days. Coating was effective on reducing the rate of vitamin C loss and firmness loss, but had no effect on reducing the mass loss and chlorophyll content. The coated samples were significantly less yellow than the uncoated ones (Kowalczyk et al. 2010).

Trehalose as an edible coating is mainly used to preserve aroma and color of dried fruits. The role of trehalose in decreasing biological activity in vegetables is well known, having its properties of water replacement, glass transformation, and chemical stability. The effect of trehalose on the weight loss, color (WI and h°), and firmness of minimally processed apple slices was investigated by Albanese et al. (2007). Coating with trehalose reduced browning significantly, decreased weight loss and organic acids loss, and retarded growth of yeast and molds and aerobic mesophilic bacteria; however, there was no difference in terms of firmness between coated and uncoated apple samples.

Edible films and coatings containing antimicrobials such as organic acids, essential oils, or natural plant extracts have been shown effective in controlling microbial spoilage, enhancing safety, and extending the shelf-life of fresh-cut horticultural products (Valencia-Chamorro et al. 2011; Campos et al. 2011; Raybaudi-Massilia et al. 2008; Cagri et al. 2004; Franssen and Krochta 2003). The combination of coating with hydroalcoholic solution and grapefruit seed extract solution inhibited microbial growth on minimally processed kiwifruit (Mastromatteo et al. 2011).

Edible alginate-based coatings incorporated with malic acid and essential oils of cinnamon, palmarosa, and lemongrass and their main active compounds prolonged

microbiological shelf-life by more than 21 days for melon pieces; however, odor and taste were significantly affected by incorporation of cinnamon oil, and firmness was significantly affected by lemongrass. Coating including 0.3% of palmarosa oil was acceptable by the panelists and was suggested as a good preservation technique for maintaining quality parameters, inhibiting growth of natural flora, and reducing *S. enteritidis* population for fresh-cut melon (Raybaudi-Massilia et al. 2008).

Hydroxypropylmethylcellulose (HPMC) or chitosan (CH) with and without bergamot essential oil was applied to table grapes. CH coatings containing bergamot oil had the most effective antimicrobial activity and the greatest inhibition on the respiration rates (Sanchez-Gonzalez et al. 2011). Chitosan (1%) with lemon essential oil (3%) applied to strawberry enhanced the antifungal activity in vitro tests and during 5 °C storage in strawberries inoculated with *Botrytis cinerea.* However, chitosan did not have significant effect on the physicochemical quality of strawberries (Perdones et al. 2012).

Chitosan as a natural antimicrobial preservative is limited to food products with low protein and NaCl content such as fruits and vegetables. Chitosan coating with lactic acid/Na-lactate was tested for strawberry and mixed lettuce. A clear antimicrobial activity of chitosan coating was demonstrated for both products; however, the coating was not applicable for lettuce due to bitter taste formation. The antimicrobial effect of chitosan on strawberry lasted for 12 days at 7 °C (Devlieghere et al. 2004).

The use of essential oils as food preservatives is still limited due to their high volatility and negative impact on flavor since effective antimicrobial doses generally exceed sensorial acceptable levels. These problems could be overcome by encapsulation of essential oils. Cyclodextrins are successfully applied for encapsulation of cinnamaldehyde (Brasil et al. 2012).

Incorporation of microencapsulated trans-cinnamaldehyde into multilayered edible coating made of chitosan and pectin was effective in extending the quality and the shelf-life up to 15 days at 4 °C for fresh-cut papaya. Encapsulated cinnamaldehyde had no negative effect on the fruit flavor (Brasil et al. 2012). Application of chitosan coating enriched with bioactive compounds (bee pollen, ethanolic extract of propolis, pomegranate dried extract, and resveratrol) and essential oils (tea tree, rosemary, clove, lemon, oreganum, and *Aloe vera*) resulted in a significant reduction on mesophilic and psychrotrophic population of fresh-cut broccoli. Sensory attributes of broccoli were not affected by the enriched coatings (Alvarez et al. 2013).

The effects of nano-coating (1% of chitosan-nano-SiOx complex) on the quality and physiology of fresh-cut bamboo shoots were investigated at 4 °C for 8 days. The coating reduced the respiratory rate and ethylene production rate, delayed increase in enzymatic activity, and maintained high L value and low level of browning index (Zisheng and Li 2010).

Active coating incorporated with silver-montmorillonite (Ag-MMT) nanoparticles improved the shelf-life of fresh-cut carrots in OPP packages. The combination of calcium alginate coating with Ag-MMT controlled microbial growth and maintained sensory quality better than the coating itself and prolonged the shelf-life up to 70 days (Costa et al. 2012) which is quite long for a fresh-cut. The use of coatings as carriers of silver represents a viable approach to avoid the direct contact of

nanoparticles with food due to safety concern (Incoronato et al. 2010, 2011; Gammariello et al. 2011). Previous surveys revealed that nanotechnology in food packaging was perceived as less problematic than nanotechnology in foods by consumers (Siegrist et al. 2007, 2008). Thus, negative consumer perception through nanoparticles as part of food should be taken into consideration.

7.3.3 Irradiation

Ionizing radiation is considered as one of the best methods to eliminate pathogenic and spoilage microorganisms with keeping nutritional properties and sensory quality of foods (Ahn et al. 2005; Bidawid et al. 2000). Low-dose gamma irradiation to ensure microbial safety and extend the shelf-life of minimally processed fruits and vegetables has been gaining importance in industry, restaurants, etc. (Lee et al. 2006; Prakash et al. 2000; Zhang et al. 2006; Lu et al. 2005; Baskaran et al. 2007). Irradiation doses are permitted for fresh produce up to 1 kGy by FDA in the USA, and 1 kGy is permitted for mushroom in Korea (Ahn et al. 2005; Baskaran et al. 2007).

Application of low-dose gamma irradiation for different types of minimally processed fresh-cuts is listed in Table 7.8. The effect of gamma irradiation up to 2 kGy was investigated on microbial and physicochemical qualities of cut Chinese cabbage under modified atmosphere packaging. Irradiation at 1 kGy or above (1-2 kGy) with

Table 7.8 Effect of gamma irradiation on different MP produce

Dose of irradiation	MP produce	Main effect	References
Up to 2 kGy	Cut Chinese cabbage	1–2 kGy with MAP enhanced the microbial quality with no significant effect on the quality attributes for 3 weeks at 4 °C	Ahn et al. (2005)
2 kGy	Ready-to-use cucumber, spinach	Reduction in *S. typhimurium* and *L. ivanovii* by 4 log	Lee et al. (2006)
1 kGy	Seasoned burdock	Reduction in *S. aureus* by 4 log	Lee et al. (2006)
1.7 kGy	MP watercress	Reduction in *Salmonella* spp. by 4 log	Martins et al. (2004)
1 kGy	MP watercress	Increase in shelf-life by 36 h	Martins et al. (2004)
1 kGy	Fresh coriander leaves	Improved the microbiological safety	Anu Kamat et al. (2003)
1 kGy	Potato cubes	Effective on the optimization of quality characteristics	Baskaran et al. (2007)
2 kGy	MP carrots	Texture, sensory, and microbial quality were effectively maintained for 14 days at 5 °C	Chaudry et al. (2004)
3 kGy	Precut carrots	Negative effect on appearance and flavor attributes	Chaudry et al. (2004)
1 kGy	Fresh-cut celery	Had a shelf-life of 6 days at 4 °C	Lu et al. (2005)

modified atmosphere packaging was suggested to enhance the microbial quality with no significant effect on the quality attributes for 3 weeks at 4 °C (Ahn et al. 2005). It is reported by Lee et al. (2006) that low-dose irradiation can reduce the risk of pathogenic bacteria in ready-to-use vegetables like cucumber, blanched and seasoned spinach, and seasoned burdock. *S. typhimurium* and *L. ivanovii* inoculated into cucumber and spinach were reduced by 4 log using 2 kGy of irradiation, and *S. aureus* inoculated into burdock was reduced by 4 log using 1 kGy.

Minimally processed watercress inoculated with *Salmonella* spp. had 4 decimal reduction by 1.7 kGy of irradiation, and the shelf-life was increased by 36 h when the product was exposed to 1 kGy (Martins et al. 2004). Low-dose gamma irradiation (1 kGy) improved the microbiological safety of fresh coriander leaves (Anu Kamat et al. 2003).

Effect of low-dose irradiation (1 kGy) applied after application of 0.33% citric acid and 0.55% potassium metabisulfite mixture was effective on the optimization of quality characteristics of potato cubes under modified atmosphere for 4 weeks (Baskaran et al. 2007). Chaudry et al. (2004) reported that texture, sensory, and microbial quality of minimally processed carrots packaged in PE were effectively maintained for 14 days at 5 °C by irradiation dose of 2 kGy. However, 3 kGy was not suggested due to the negative effect on appearance and flavor attributes of precut carrots. Fresh-cut celery γ-irradiated with 1 kGy dose had a shelf-life of 6 days at 4 °C (Lu et al. 2005).

Increase in antioxidant and phenolic content of some vegetables after radiation process is reported for cabbage (Ahn et al. 2005), carrot (Arvanitoyannis et al. 2009), and fresh cilantro leaves (Fan et al. 2003a). This increase is attributed to free radicals generated by radiation process which may trigger stress responses in vegetables leading to increased antioxidant synthesis (Fan et al. 2003b). However, this is not the case for all vegetables processed with irradiation. Microbiological methods based on the use of direct epifluorescent filter technique (DEFT) and aerobic plate count (APC) were suggested to screen irradiation treatment indicating hygienic status of vegetables (Araujo et al. 2009).

Some researchers claimed tissue softening by irradiation for some fruits and vegetables (Rastogi 2005) like diced tomatoes (Prakash et al. 2002) and potatoes (Rastogi and Raghavarao 2004). Radiation-induced depolymerization of cell wall components such as pectin, cellulose, and hemicellulose could cause decreased firmness and softening in plant tissues (Prakash et al. 2000, 2002). WHO (1999) reported that irradiation process did not demonstrate any short-term or long-term toxicity based on a large number of toxicological studies.

7.3.4 Hurdle Technology

MAP and cold storage are usually not sufficient to extend the shelf-life of fresh-cut produce due to the excessive physiological stress and increased susceptibility. Processing operations like cutting and slicing result in microbial spoilage and reduce the shelf-life significantly. Hurdle technology involves the use of several

preservation techniques in combination to reduce the intensive use of one preservation technique resulting in a lower impact on the sensory quality and extend the shelf-life (Gupta et al. 2012; Ahn et al. 2005).

Edible coatings containing antimicrobial agents in conjunction with MAP and cold storage are alternatives as a hurdle technology to reduce the deleterious effects of minimal processing (Mastromatteo et al. 2011; Del Nobile et al. 2009a, b). Peeled kiwifruit was coated with sodium alginate combined either with hydroalcoholic solution or with grapefruit seed extract solution and packaged with OPP under active and passive MAP and stored at 4 °C for 15–21 days. The coatings controlled dehydration and respiration of kiwifruit both in passive and active MAP. The shelf-life of minimally processed product was 2.7 days under active MAP; however, the combination of coating with passive and active MAPs prolonged the shelf-life up to 12 and 13 days, respectively (Mastromatteo et al. 2011).

Citric acid treatment (8.4 g/l), gamma irradiation (0.7 kGy), modified atmosphere packaging (18% O_2 and 4% CO_2), and low temperature storage (10 °C) were applied as hurdles to extend the shelf-life of French beans by 1 week with acceptable sensory and nutritional quality. Citric acid was reported to reduce radiation-induced softening of French beans (Gupta et al. 2012).

The combination of washing, dipping into hydroalcoholic solution, coating with sodium alginate, and packaging with active (10% O_2 and 10% CO_2) and passive atmospheres using microperforated PP film was applied to fresh carrots. It is reported that the coating treatment enhanced the product quality by preventing dehydration and microbial proliferation and controlling respiration under both passive and active MAPs. The combination of dipping and coating controlled microbial growth better than dipping alone and prolonged the shelf-life by 12–13 days which is only 2 days for uncoated samples at 4 °C (Mastromatteo et al. 2012).

Pineapple and mango fruit chunks preserved by hurdle technology using pH, mild heat treatment (blanching in syrup at 85 °C for 5 min), preservatives (340 mg potassium metabisulfite/kg and 413 mg sodium benzoate/g), and packaging (PP pouches) provided acceptable sensory and microbiological qualities up to 30 days at 27 °C and 60 days at 2 °C. *Papaya* chunks had a shelf-life of 90 days at 2 °C with higher level of preservatives (Vijayanand et al. 2001).

The combined use of a potassium sorbate treatment with active MAP (5%O_2 and 5% CO_2) using 40 μm OPP improved shelf-life of fresh-cut butternut squash to 22 days at 4 °C. However, application of MAP without dipping into potassium sorbate had a shelf-life of 2 weeks under both active and passive MAP (Lucera et al. 2012).

7.4 Conclusions/Suggestions

MAP technology can be integrated with active or interactive packaging using new-generation packaging materials to improve the control over the package atmosphere to achieve superior product quality and safety. MAP by itself is a dynamic system that is normally not controlled. Integration of MAP with active and intelligent

packaging devices would possibly provide the control on the MAP system. Sensors adapted in the modified atmosphere package could detect gas levels, and also the sensor could release gas to compensate for gas loss in the package. Thus, the initial gas mixture could be maintained in the modified atmosphere package during the storage time. However, the complexity of the integrated system should be considered for the respiring foods. Low-dose irradiation combined with hurdle technology seems promising for MP produce if not constrain freshness.

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Part II
Commodities

Chapter 8
Postharvest Quality and Safety of Fresh-Cut Vegetables

Mustafa Erkan and Işılay Yıldırım

8.1 Introduction

Fruits and vegetables are an essential part of the human diet. They provide many important vitamins and minerals needed to sustain a healthy body. Consumption of fruits and vegetables has long been shown to help ward off illness and protect against certain diseases. On the other hand, it is essential to avoid microbial contamination of fresh intact and fresh-cut produce to assure their safety. Outbreaks associated with fresh fruits and vegetables have nearly tripled since 1973 (Gagliardi et al. 2003). The increase in outbreaks coincides with increased intake of fruits and vegetables (Osman et al. 2006). Among the others, fresh-cut horticultural products stand out as convenient novel foods that fit the many needs of a modern lifestyle as they combine technical content with an innovative food concept (Olivas and Barbosa-Canovas, 2005).

Fresh-cut fruits and vegetables, initially called minimally processed or lightly processed products, are those that have been trimmed and/or peeled and/or cut into 100% usable product that is bagged or prepackaged and kept at refrigerated storage (IFPA 2006). In particular, fresh-cut products attract consumers because they are fresh, nutritious, reasonably priced, and ready to eat. As a consequence, a wide assortment of minimally processed fruits has been developed to meet consumer's needs for "quick" and convenient products and to benefit from fruit's healthy image (Ahvenainen 1996). With the busy lifestyles, consumer tends to use less time for preparing meals. Some health conscious consumers prefer eating fruits and vegetables (FV) and prefer a ready-to-eat salad than preparing it themselves. As a result, the maintenance of the quality of fresh-cut produce has become more challenging to the food industry (Sonti 2003; Lamikanra 2002).

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The fresh-cut vegetable industry was first developed to supply hotels, restaurants, catering services, and other institutions. For the food service industry and restaurants, it presents a series of advantages including a reduction in the need of man power for food preparation, a reduced need to have special systems to handle waste, and the possibility to deliver in short notice specific forms of fresh-cut products (Watada et al. 1996).

More recently, it has expanded considerably to include food retailers as a response to consumer's demand for fresh, healthy, and convenient foods (Wiley 1994; Watada et al. 1996). Minimal processing gives additional value to fresh-cut products in terms of convenience and time saving, although several hurdles are encountered due to the difficulty in preserving their freshness during prolonged periods. Processors of fresh-cut fruit face numerous difficulties not commonly encountered during fresh-cut processing of vegetable products. Especially with those fruits that undergo ripening, the results in maintaining quality and extending the shelf life are far from satisfactory (Beaulieu and Gorny 2001).

Fresh-cut products, in fact, are characterized by a shorter shelf life than their whole counterparts, because of higher susceptibility to microbial spoilage and increased respiration rate and ethylene production, which is stimulated by wounding of the tissue; in fact, the process operations (i.e., cutting, splicing, etc.) form lesions in the tissue that determine enzymatic browning, texture decay, rapid microbial growth, weight losses, and undesirable volatile production, thus reducing the shelf life (Chien et al. 2007).

Fresh-cut fruits, including melons, have become increasingly more popular with consumers in the United States. The demand for fresh-cut fruits has risen dramatically in the last two decades. Consumers want and need foods that are convenient and eaten quickly. With increased dining away from home, fresh-cut products are playing an ever-increasing role in the United States' food service sector. In 2006, 27% of fresh-cut produce in the United States was sold in the food service sector, while 73% was sold in retail. Fresh-cut produce sales increased in value from US$3.3 billion in 1999 to US$15.5 billion in 2007 (Cook 2009).

Bagged salads and cut vegetables showed a growth trend in 2008, while sales in fresh-cut fruits declined. Fresh-cut organic salads are now being mainstreamed across the United States and satisfying consumer desire for healthy food and preservation of the environment. They are now widely available in restaurants and retail outlets (James and Ngarmsak 2010). Sales show that for fresh-cut salads, consumers will pay for the convenience of fresh-cut, if quality is perceived to be better than or equal to uncut product (Beaulieu and Lea 2003).

Fresh-cut vegetables for cooking include peeled baby carrots, baby corn, broccoli and cauliflower florets, cut celery stalks, shredded cabbage, cut asparagus, stir-fry mixes, and cut sweet potatoes. Fresh-cut herbs are also marketed widely (James and Ngarmsak 2010).

8.2 Factors Affecting the Production of High-Quality Fresh-Cut Vegetables

Quality is influenced by maturity at harvest and the methods of harvesting, storage times, temperatures, and extent of handling. Produce should ideally be harvested during the cooler part of the day, on the day of processing, in order to minimize storage and handling and thus increase fresh-cut shelf life. Fruit should be physically mature, with flavor developing for best taste. Field workers must use the appropriate harvesting equipment and protective containers in order to prevent damage to produce during harvesting (James and Ngarmsak 2010).

The ripeness stage at processing influences both the shelf life and the eating quality of fresh-cut products (Gorny et al. 2000). Beaulieu and Gorny (2001) pointed out that minor physiological differences in initial fruit quality appear to translate to substantial differences in the quality of cut products. Ripeness stage at processing was shown to be important in many processes that results in changes in the quality of fresh-cut products as respiration rate (Soliva-Fortuny et al. 2004), ethylene production (Soliva-Fortuny et al. 2002, 2004), appearance (Soliva-Fortuny et al. 2004), color (Soliva-Fortuny et al. 2002), and texture change (Soliva-Fortuny et al. 2002, 2004). In general, when selecting less mature fruits for processing, an extension in shelf life can be achieved due to firmness retention and less changes in appearance, but the amount and composition of volatiles and consequently the aroma/flavor can be unsatisfactory (Gorny et al. 2000; Beaulieu and Lea 2003; Beaulieu et al. 2004).

8.3 Processing Steps for the Production of Fresh-Cut Vegetables

A general flow diagram for the production of fresh-cut vegetables is depicted in Fig. 8.1. The first step is the selection of raw material, it is self-evident that vegetables or fruit intended for pre-peeling and cutting must be easily washable and peelable, and their quality must be first class. The results revealed that not all varieties of a particular vegetable can be used to manufacture prepared vegetables. The correct choice of variety is particularly important in the case of carrot, potato, rutabaga, and onion. For example, carrot and rutabaga varieties that give the juicier grated product cannot be used in the production of grated products that need to have a shelf life of several days, whereas poor color and flavor become problems if the variety of potato is susceptible to browning (Cabezas-Serrano et al. 2009). Furthermore, the results showed that climatic conditions and cultural practices, including the use of fertilizers and the harvesting conditions, can also significantly affect the "behavior" of vegetables during minimal processing (Ahvenainen 1996).

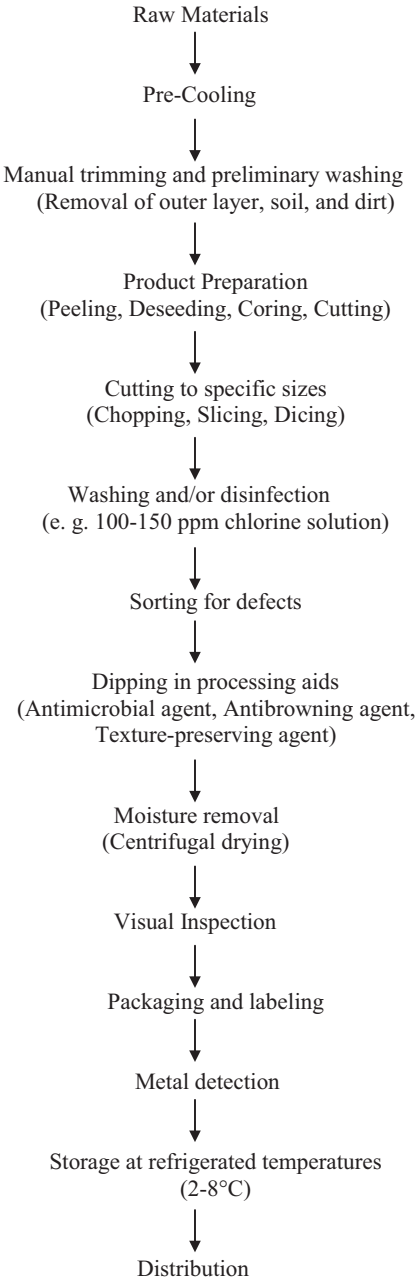

Fig. 8.1 General flow diagram for the production of fresh-cut vegetables (Modified from Francis et al. 1999; James and Ngarmsak 2010)

After the selection of raw materials, steps of fresh-cut processing involve peeling, trimming, and deseeding fresh produce and cutting it to specific size segments. Many studies confirm that cutting and shredding must be performed with sharp blades. Sharp blade slicing and rotary cutting of lettuce were both superior to either dull

blade slicing or chopping. Carrots cut with a razor blade were more acceptable from both a microbiological and a sensory point of view than carrots cut using various commercial slicing machines. It is clear that slicing with dull knives impairs the retention of quality because it ruptures cells and releases tissue fluid to a great extent. Mats and blades that are used in slicing operations can be disinfected, for example, with a 1% hypochlorite solution. A slicing machine must be installed securely because vibrating equipment may impair the quality of sliced surfaces (Ahvenainen 1996). The impact of different types of cuts and storage temperatures on the quality of stored, fresh-cut radish was investigated by Saavedra del Aguila et al. (2006). Two types of cuts (sliced and shredded), and three storage temperatures (1, 5 and 10 °C), were studied during 10 days. Twelve hours after processing, shredded roots had produced 0.04, 0.11, and 0.17 ng kg^{-1} s^{-1} of C_2H_4 at 1, 5 and 10 °C, respectively. On the tenth day, whole roots stored at 1 °C showed the lowest respiration rate (1.59 g kg^{-1} s^{-1} of CO_2), while the highest rate was observed in shredded roots stored at 10 °C (7.42 g kg^{-1} s^{-1}). Furthermore, shredded radishes had lower soluble solids during storage compared to other cut types. Temperatures between 1 and 5 °C are recommended for maintenance of quality in fresh-cut radishes (Saavedra del Aguila et al. 2006).

Washing is one of the most important steps during the processing of produce. The objective of the washing or sanitizing step is to remove dirt, pesticide residues, and microorganisms responsible for quality loss and decay. Additionally, this step is used to precool cut produce and remove cell exudates adhering to product cut surfaces, thus impeding microbial growth and discoloration, respectively (Saltveit 2003). Ideally, produce should be cooled to remove field heat prior to storage or processing. Cooling extends the shelf life of the final fresh-cut product. Large fresh-cut processing operations make use of any of a number of technologies such as chilled water baths, forced air cooling, vacuum cooling, and packing with ice-water mixtures, for removing field heat from fresh produce (James and Ngarmsak 2010).

The use of effective sanitizing agents during the washing of produce is necessary to ensure product safety. In minimally processed vegetables, such as shredded carrot, chlorine solutions have been widely employed by the industry for sanitization purposes. Water containing 50–200 mg l^{-1} of chlorine with a contact time of 1–2 min is widely used to sanitize fresh-cut produce on a commercial scale (Beuchat and Brackett 1990; Parish et al. 2001). However, reduced microbiological efficiency coupled with sensorial changes and the eventual formation of carcinogenic chlorinated compounds has demonstrated the need for alternative decontamination methodologies. On the other hand, the use of chlorine in the processing of minimally processed products has been banned in some European countries (Day 2001; James and Ngarmsak 2010).

Washing after cutting had a great impact on physicochemical measures of quality in sliced green pepper (Toivonen and Stan 2004; Szabo et al. 2003). It was suggested that this effect was mediated by the removal of stress-related compounds produced as a response to the wound injury. For example, conventional processing practices of fresh-cut iceberg lettuce normally include a washing step in cold, chlorinated water – commonly not before, but after cutting/slicing/shredding (Delaquis et al. 1999). However, as recently demonstrated under conditions of industrial practice,

prewashing trimmed, cored iceberg lettuce heads in cold, chlorinated tap water prior to shredding and subsequent washing of the shredded produce in cold tap water represents a feasible alternative to conventional washing (Baur et al. 2005).

The newest tendency is called the immersion therapy. Cutting a fruit while it is submerged in water will control turgor pressure, due to the formation of a water barrier that prevents movement of fruit fluids while the product is being cut. Additionally, the watery environment also helps to flush potentially damaging enzymes away from plant tissues. On the other hand, UV-C light has been also used while cutting fruit to cause a hypersensitive defense response to take place within its tissues, reducing browning and injury in fresh-cut products. Another alternative could be the use of water-jet cutting, a noncontact cutting method which utilizes a concentrated stream of high-pressure water to cut through a wide range of foodstuffs (Allende et al. 2006a).

As another step of the process, fresh-cut produce can be optionally dipped in a solution of an acidulant/antioxidant blend consisting of a combination of ascorbic acid/citric acid, for example, or in an anti-softening agent such as calcium chloride (James and Ngarmsak 2010). After dipping treatments, excess water or liquid associated with the produce must be removed prior to packaging of fresh-cut products. Water in the finished product encourages mold growth and the growth of other microorganisms resulting in rapid deterioration of texture. Various manual and mechanical methods have been developed for the removal of excess water from fresh-cut vegetables (air-drying, spin basket, conveyor shakers, etc.) (James and Ngarmsak 2010).

A metal detector is generally positioned at the end of the line in automated operations and bags of products that pass through the detector as a control measure to test for metal contaminants. Finished product containers are labeled with a *use by* date to alert customers of the optimum product shelf life. Other company codes may be printed on the package (James and Ngarmsak 2010).

8.4 Quality Changes

8.4.1 Textural and Cell Wall Changes

Appearance and texture changes are two fundamental criteria determining the acceptability of fresh-cut vegetables. The understanding of the processes leading to these changes is essential in developing better approaches to minimizing them and, hence, improving quality and shelf life for the consumer. One common issue is that much of the biochemistry of appearance and textural changes has been, by and large, studied in whole plant or tissue systems. The consequences of the cutting operation and post-cutting processes in fresh-cut products have not been extensively studied (Toivonen et al. 2008). In essence, appearance and texture changes are very tightly linked to tissue deterioration and as such can and are used as measures of freshness and quality decline in fresh-cut research and industry (Cantwell and Suslow 2002).

Hence, these two characters are probably the most interesting quality attributes with which fresh-cut processors are currently concerned in relation to maximizing shelf life.

Accelerated loss of texture is considered one of the main factors that limit the shelf life of fresh-cut tissues (King and Bolin 1989; Beaulieu and Gorny 2001). Decreased turgor due to water loss has also been suggested as a cause of tissue softening (Beaulieu and Gorny 2001) in fresh-cut fruit.

Many factors can influence the intensity of the wound response in fresh-cut fleshy tissues. Characteristics of the raw material such as cultivars (Varoquaux et al. 1996), stage of physiological maturity (Gorny et al. 2000), chemical composition (Varoquaux et al. 1996), and enzyme activity especially those related to phenolic metabolism (Babic et al. 1993) are important factors. The extent of cutting operations can have a great impact of keeping the quality of the product (Abe and Chachin 1995; Ahvenainen 1996).

Firmness loss is immediate and faster in wounded tissues due to cell rupture and loss of tissue integrity (Miller 1992). Cell wall enzyme activity may be accelerated by wounding (Miller 1992; Karakurt and Huber 2002), which may induce extensive softening in fresh-cut tissues. The enhanced cell wall enzyme activity, thus, causes depolymerization of pectic and hemicellulosic polyuronides, which may result in further textural changes in fresh-cut tissues (Ergun 2006).

The expression of polygalacturonase (PG), beta-galactosidase (beta-gal), and pectinesterase (PE) genes all increased within the first 6 h after wounding of tomato fruits, but, in most cases, returned to pre-wounding levels after 24 h. The activities of these three enzymes were also examined in intact (control) and wounded tomato fruit tissues 24 and 48 h from wounding. In early breaker fruits, the increase in activities of PG and beta-gal associated with normal ripening were both retarded resulting in wounded fruits having reduced activities compared to the controls, while PE activity actually declined 24 h after wounding. In ripe tomato fruits, PG and PE activities both decreased 48 h after wounding, but beta-gal was unaffected (Thanh Tu and Tucker 2003).

Furthermore, microbial increase can have an impact on the texture changes. On this point, pectolytic bacteria have been identified by different authors as responsible for the texture losses in vacuum-packaged carrot slices (Buick and Damoglou 1987) and in a number of other minimally processed vegetables (Manvell and Ackland 1986).

Longer times of stream treatment resulted in higher values of crispness coefficient (CC) in the fresh-cut lettuce (Rico et al. 2008). Firming effects of heat treatments could be linked to the action of heat-activated pectin methylesterase (PME) (Bartolome and Hoff 1972; Garcia et al. 1996). Previous work on this matter showed that heat shock alone or combined with calcium lactate better maintained textural properties of fresh-cut lettuce than nonthermal treatments (Martin-Diana et al. 2005a, b, 2006b; Rico et al. 2006, 2007a). Another possible reason of higher CC can be due to the temperature of the treatment, causing a higher retention of water in the vegetable. The heating of the air within the lettuce tissue and the subsequent cooling and contraction of this air would have absorbed

the surrounding water into the lettuce, therefore increasing the moisture and turgor of the cells (Martin-Diana et al. 2006b).

In addition, Barry-Ryan et al. (2000) and Bolin and Huxsoll (1991a) reported an increase in firmness for shredded carrots and lettuce, respectively. According to these authors, the firmness increases during storage due to the drying out of the vegetables and may be partially caused by lignin production.

For climacteric fruit, initial fruit firmness is considered to be a good indicator of fruit maturity for determining the shelf life of cut products (Beaulieu and Gorny 2001). It was therefore reasonable to assume that the initial firmness could also be used both as index of maturation and of potential shelf life for fresh-cut tomatoes. Indeed the assessment of firmness has been included in the quality evaluation of fresh-cut tomato by many authors (Hong and Gross 1998; Artes et al. 1999; Gil et al. 2001; Wu and Abbott 2002).

Firmness of fresh-cut fruits (fleshy tissue) is most commonly measured using puncture tests (Bolin and Huxsoll 1989; Varoquaux et al. 1990; Abe and Watada 1991; Hong and Gross 1998; Luna-Guzmán and Barret 2000; Gorny et al. 2002; Karakurt and Huber 2002). Compression tests (Abe and Watada 1991; Artes et al. 1999; Beaulieu et al. 2004) and shear tests (Rosen and Kader 1989) are also reported for few products.

Calcium treatments have been used to extend the shelf life of fruit and vegetables. Calcium helps to maintain the vegetable cell wall integrity by interacting with pectin to form calcium pectate. Calcium is reported to maintain firmness by cross-linking with cell wall and middle lamella pectins (Grant et al. 1973; Rico et al. 2007b). Thus, fruit and vegetables treated with calcium generally remain firmer than controls during storage (Luna-Guzman et al. 1999; Martin-Diana et al. 2006b; Rico et al. 2007b).

Saftner et al. (2003) using 40 mM Ca propionate, $CaCl_2$, and chelate on "honeydew" pieces increased tissue Ca content by more than double, inhibiting firmness loss. However, Ca sources have different behaviors and different beneficial effects on firmness maintenance. A better response with Ca lactate (2.5%, w/w) compared with $CaCl_2$ (2.5%, w/w) was found by Luna-Guzmán and Barret (2000). In "Amarillo" melon, despite using equal Ca concentrations (0.18 g, 100 mL^{-1}) supplied by different Ca salts (lactate, carbonate, and propionate), the Ca tissue contents and their effect on retarding softening delays were different (Aguayo et al. 2008). Also Aguayo et al. (2008) reported an increase in Ca levels, mainly of bound Ca, in "Amarillo" melon pieces treated with $CaCl_2$, propionate, lactate, and carbonate for 1 min combined with heat treatment (60 °C).

8.4.2 Effects of Minimal Processing on Nutrients of Vegetables

Few studies have been conducted on the effect of minimal processing on the maintenance of the nutritional quality of vegetables. Therefore, there is a need for more information on the effects of minimal processing and type of package on the contents of other nutrients in these products. The evaluation of the influence of fresh-cut processing is still a key factor in finding those technological conditions

necessary to preserve the content, activity, and bioavailability of naturally occurring antioxidants and other health-promoting constituents of fruits and vegetables. Research into the health benefits of fruits and vegetables needs to identify optimum conditions for maintaining these compounds after harvest and after minimal processing operations (Gil and Kader 2008).

8.4.2.1 Titratable Acidity

The contents of titratable acidity were not affected by the storage period and did not present statistical differences among treatments for carrot and green pepper. Benedetti et al. (2002) found a decrease from 0.08 to 0.06 mg in 100 g of citric acid in sliced green peppers packed in polystyrene trays and stored for 10 days under temperatures of 5 and 10 °C, and Carlin et al. (1990) reported a decrease in malic acid in grated carrots packed under modified atmosphere (10% O_2 and 40% CO_2) for 10 days at 10 °C. Kakiomenou et al. (1996) studied the sensory alterations in minimally processed carrots and demonstrated that there was an increase in organic acids resulting in a reduction in the values of texture, characterized by softening of the tissues during storage. Gomez and Artes (2005) found that modified atmosphere packaging (MAP) also affected the degradation of organic acids. In this study, oriented polypropylene (OPP) treatment showed no change in total organic acids content, probably through preservation of oxidation by its lower O_2 concentration.

8.4.2.2 pH

In an experiment with cut cantaloupe melons stored at 4 °C, by 14 days, Lamikanra and Watson (2003) evaluated the biochemical changes compared to stored whole melons at 20 °C. No significant changes were observed in titratable acidity, pH, °Brix, and organic acid content over a period of 14 days. At 20 °C, lactic acid was by far the dominant acid present after 2 days, differently from the fresh fruit in which oxalic, citric, malic, and succinic acids were dominant. The results found in this research differed from those reported by Abdul-Raouf et al. (1993), who found a pH decrease with minimally processed mixed carrot, lettuce, and cucumber salad packed under modified atmosphere (3% O_2 and 97% N_2) and stored under temperatures of 5, 12, and 21 °C. García-Gimeno and Zurera-Cosano (1997) observed, in a mixed salad made of carrots, lettuce, and purple cabbage, a pH decrease from 7.0 to 4.0 after 9 days of storage at 15 °C, concluding that this reduction could be related to the higher concentration of CO_2 found at this temperature.

8.4.2.3 Vitamin C (Ascorbic Acid)

In a study conducted by Benedetti et al. (2002), sliced green peppers are packed in expanded polystyrene trays and wrapped in PVC (polyvinyl chloride) film stored at 5 °C for 10 days. This packaging treatment did not show a protective effect on the

vitamin C retention as accepted. The vitamin C was lost through exudates that occurred from the green pepper. Teles et al. (2000) reported that on the 13th storage day at 5 °C, minimally processed cabbages packed in polyolefin plastic films at modified atmosphere (10% O_2, 5% CO_2, and 85% N_2) had 80% of the vitamin C found at the beginning of the study.

Myojin et al. (2008) studied about the changes in the ascorbic acid content of shredded vegetables during storage at 10 °C for 7 days under air or nitrogen gas. The percent of ascorbic acid in vegetables remaining after 7-day storage under air ranged from 45% (Japanese radish) to 97% (cabbage). In case of storage under nitrogen gas, the retention percentage ranged from 74% (green pepper) to 95% (red cabbage). In the case of green pepper, the ascorbic acid content decreased to 65–74% of the original content during 7-day storage under air or under nitrogen ($p < 0.05$). In red and white cabbages, however, the ascorbic acid content did not decrease during storage.

8.4.2.4 Sugars

Gomez and Artes (2005) found that MAP had significant effects on the celery stick sugar content. After cold storage in air, fructose and glucose showed a decrease probably because fructose was consumed or transformed to glucose by the initial reaction of the glycolysis pathway and then consumed. Total sugar content decreased for air but not for MAP, possibly indicating a lower respiration rate which would retard loss of sugars.

8.4.2.5 β-Carotene

The content of β-carotene in the carrot packed under MA presented the lowest values during the storage period. The contents of β-carotene decreased continuously during the storage at 1 °C in peeled carrots packed in polymeric film bags, with approximately 33% of the initial content after 28 days. Between the 14th and 21st days, there was a reduction in the β-carotene content in the treatment under air (Li and Barth 1998). Hussein et al. (2000) reported a reduction in contents of β-carotene in vacuum-packed green peppers on the tenth day of storage.

In another study, minimally processed carrot and green pepper were put into BOPP/LDPE (biaxially oriented polypropylene/low-density polyethylene) plastic bags, which were sealed under atmospheric air, vacuum, and MA (2% O_2, 10% CO_2, 88% N_2) and stored at 1 °C. The contents of β-carotene decreased slightly during the storage period for the minimally processed carrot and green pepper (Pilon et al. 2006).

8.4.2.6 Phenolic Content and Antioxidant Capacity

Heredia and Cisneros-Zevallos (2009) have shown that wounding increases the phenolic content and the antioxidant capacity of carrot tissue. Similar response to wounding was reported for fresh-cut potatoes, lettuces, and onions (Kang and

Saltveit 2002; Martinez et al. 2005; Mateos et al. 1993; Tudela et al. 2002). Ferreres et al. (1996) studied the impact of perforated films on flavonoids of shredded onions, after 7 days of storage, and found that the content of all anthocyanins decreased. Ewald et al. (1999) found that the greatest loss of flavonoids in onion took place during the preprocessing step, where the onion was peeled, trimmed, and chopped before blanching. In a study conducted by Pérez-Gregorio et al. (2011), onions were minimally processed to produce fresh-sliced onions, which were packed either in closed plastic cups or under vacuum conditions, taking into account the effect of light exposure. In general, after storage in the dark, a slight increase in flavonols was observed, whereas a clear decrease in the relatively low amounts of anthocyanins was evident. However, the best performance was obtained when the more transparent polystyrene cups were stored under the light. Both types of flavonoids increased, with an enhanced increase of total flavonols by 58% and an increase in total anthocyanins of 39% (Pérez-Gregorio et al. 2011).

Myojin et al. (2008) studied about the changes in the total phenol content of shredded vegetables stored at 10 °C for 7 days under air or under nitrogen. The percent of total phenolics remaining in vegetables after 7-day storage under air ranged from 72% (lettuce) to 132% (red cabbage), with an average of 100%. In the case of storage under nitrogen, the percent of total phenolics remaining ranged from 83% (lettuce) to 123% (white cabbage), with an average of 99%. In the case of red and white cabbages, the total phenol content significantly increased during 7-day storage ($p < 0.05$) regardless of air or nitrogen, but that of lettuce significantly decreased after 7-day storage under air ($p < 0.05$). The apparent increase of polyphenols in cabbages may be due to deglycosylation of anthocyanins.

Besides the presence of O_2 in the package, the PAL activity is conditioned by different abiotic and biotic stresses, for example, as a response to wounding and ethylene, generally resulting in elevated levels of soluble phenolics (Cisneros-Zevallos 2003). Martinez et al. (2005) verified higher quercetin concentration in chopped samples of onion packed under MA than in whole bulbs, after long-term refrigerated storage at 4 °C (11–30 days). Heredia and Cisneros-Zevallos (2009) found that the synthesis and accumulation of stress-induced phenolic compounds in carrot tissues are dependent upon the intensity of wounding. Similar results were obtained by Tudela et al. (2002), who studied the induction of antioxidant flavonol biosynthesis in fresh-cut potatoes. Fresh-cutting induced the biosynthesis of three flavonols: quercetin 3-rutinoside, quercetin 3-diglucoside, and quercetin 3-glucosylrutinoside. Mateos et al. (1993) found that extractable PAL activity and phenolic content were more pronounced with cut lettuce than with the undamaged product.

Anthocyanins were also studied by Ferreres et al. (1996) in shredded red onions, with regard to their changes during storage in perforated films.

Light is also known to induce the synthesis of flavonols in fresh-cut potatoes (Tudela et al. 2002). Higashio et al. (2005), who studied the effect of UV illumination on the content of flavonoids in fresh-cut onion, confirmed that irradiation induced the synthesis of quercetin in this vegetable. These works show that, like wounding, the exposure of fruits and vegetables to light (visible or UV) causes a stress signal that enhances the health benefit properties of these products (Cisneros-Zevallos 2003).

8.4.3 Chlorophyll Loss and Color Change

Studies on chlorophyll loss in fresh-cut product have predominately produced evidence for breakdown induced by oxygen radical oxidation of the chlorophyll molecule (Brown et al. 1991; Toivonen et al. 2008). Chlorophyll breakdown in parsley leaves has in one case been shown to involve pheophytin accumulation (Yamauchi and Watada 1991) and in another case to involve two breakdown intermediates, pheophytin and an unknown believed to be C13^2-hydroxychlorophyll a (Amir-Shapira et al. 1987). In broccoli, there are numerous reports strongly supporting the hypothesis that peroxidase- and/or chlorophyll oxidase- and/or lipoxygenase-mediated chlorophyll breakdown are important in whole product storage and packaged fresh-cut product (Zhang et al. 1994; Funamoto et al. 2002, 2003; Costa et al. 2005, 2006). Spinach leaf chlorophyll loss has been associated with peroxidase-mediated breakdown as well (Yamauchi and Watada 1991). In a final example, excised cabbage discs show chlorophyll losses strongly associated with lipoxygenase activity and concomitant fatty acid degradation (Cheour et al. 1992; Toivonen et al. 2008).

8.4.4 Color Preservation of Fresh-Cut Vegetables

8.4.4.1 Enzymatic Browning and Control

Fresh-cut produce deteriorates faster than intact produce because of internal and external browning of the cut surface. Browning detracts from the appearance of the slices and reduces their marketability. Physical damage during the peeling and cutting process also causes an increase in respiration rates, biochemical changes, and microbial spoilage, which often result in degradation of color, texture, nutrient, and flavor of the produce (Buta et al. 1999).

Being deprived of their natural protecting matrices, the phenolic compounds are exposed to oxygen during processing and may therefore be oxidized to quinones. Various classes of phenolic compounds, showing a great diversity of structures such as catechins, hydroxycinnamic acid derivatives, and anthocyanins, have been found to contribute to nonenzymatic and enzymatic browning of foods (Pati et al. 2006). Basically, enzymatic browning can be defined as an initial enzymatic oxidation of phenolic compounds into slightly colored o-quinones, catalyzed by polyphenol oxidase (PPO). Although PPOs are localized in plastids, their phenolic substrates are mainly located in the vacuole so that enzymatic browning only occurs when this subcellular compartmentation is lost (Rigal et al. 2000).

Various approaches to control the extent of browning have been investigated. In general, enzymatic browning can be avoided by thermal inactivation of PPO, but heat can cause unwanted softening of the tissues. Instead of blanching, chemical additives have been used to prevent enzymatic browning (Tortoe et al. 2006).

Reducing agents, antioxidants, and enzymatic inhibitors prevent browning by chemically reducing the o-quinones to colorless diphenols. Acidulants, such as citric,

oxalic, malic, or phosphoric acid, can also inhibit PPO activity by reducing pH and/or chelating copper in a food product (Ibrahim et al. 2004). Sapers (1993) reduced darkening of potatoes by using a dip treatment with ascorbic acid, $CaCl_2$, and citric acid. Calcium has an important role in maintaining quality of fruits and vegetables in respect to structural integrity of membranes and cell walls (Poovaiah 1986). Calcium binds anionic groups of all membranes to form bridges between structural components, thereby maintaining cell permeability and compartmentation and structural integrity (Conway and Sams 1984). Izumi and Watada (1994) reported the effects of calcium treatments on the shelf life extension of fruit and vegetables. These effects are reduced respiration, suppression of ethylene production, increased firmness retention, and reduced incidence of physiological disorders and decay. Dip treatment with ascorbic acid retards enzymatic browning in freshly prepared vegetables (Roura et al. 2003). Heat treatments are effective as a nonchemical means of improving postharvest quality for a variety of horticultural products. A brief heat shock (90 s at 45 °C) disrupts the wound-induced increase in PAL activity, delaying and diminishing the accumulation of phenolic compounds and tissue browning (Loaiza-Velarde et al. 1997). Murata et al. (2004) showed that the heat shock treatment is useful for prolonging the shelf life of cut lettuce, repressing the induction of PAL activity and phenolic accumulation during storage, and preventing tissue browning.

The intensity of damage in the production of minimally processed cut romaine lettuce directly affects the physiological response of the samples and the rate of change in PAL activity during the first day of storage (Pereyra et al. 2005). As browning greatly reduces the visual quality of cut lettuce, it is important to prevent browning reactions. The techniques used to retard browning include low storage temperatures (Bolin and Huxsoll 1991b), modified or controlled atmospheres (MA or CA) with low O_2 and/or high CO_2 concentrations (Heimdal et al. 1995; Mateos et al. 1993), chemical additives (McEvily et al. 1992), or the use of brief heat shocks (Loaiza-Velarde et al. 1997; Saltveit 1998, 2000).

Bolin and Huxsoll (1991b) found that dipping salad-cut lettuce in 0.5 g/100 g ascorbic acid increased the shelf life by about 10%. On the other hand, Moreira et al. (2008) reported that mild heat shocks at 50 °C reduced the enzymatic browning of romaine lettuce. Mild heat shock treatment is a new method developed to control browning reactions. The ease with which a heat shock can be administered to lettuce and the lack of an offensive chemical residue make this technique an attractive alternative to preserve fresh-cut lettuce (Saltveit 2000). Loaiza-Velarde et al. (1997) reported that dipping lettuce in water at 45–55 °C extends the shelf life and visual quality of minimally processed lettuce by inhibiting the activity of PAL.

8.4.4.2 Other Color Changes

White Blush

One of the important criteria is whitening or white blush formation caused by drying at the surface of the peeled and sliced carrots (Emmambux and Minnaar 2003; Klaiber et al. 2005). A mechanism proposed for white discoloration on peeled

carrots is related to physical and physiological responses to wounding. The physical response is reflected as a color change because of the reversible surface dehydration and physiological response as a result of the activation of phenolic metabolism and the production of lignin resulting in an irreversible color change (Cisneros-Zevallos et al. 1995; Emmambux and Minnaar 2003). The definitive demonstration that lignification is important to "white blush" formation was provided by Howard et al. (1994). They showed that steam treatment inhibited PAL and syringaldazine oxidase activities in minimally processed carrots and these declines in activity could be correlated with reduced accumulation of soluble phenolic compounds and lignin in treated carrots. Steam-treated carrots were also shown to have significantly reduced levels of "white blush" (Howard et al. 1994). Since the physical appearance and lignification are intensified with increased roughness of the processing, the use of fine abrasives to polish the carrot surface results in less "white blush" (Bolin and Huxsoll 1991a). Similarly, the use of sharp cutting implements will also reduce the wound response and lignin accumulation (Tatsumi et al. 1993; Bolin and Huxsoll 1991a). In addition, "white blush" can be controlled with treatments that alter tissue pH and hence enzyme activity (Bolin and Huxsoll 1991a; Bolin 1992).

Sy et al. (2005) reported that Julienne-style cut carrots showed a slight whitening after treatment with 1.4 mg L^{-1} of gaseous chlorine dioxide (ClO_2) for 6.4 to 10.5 min at 79% to 84% relative humidity. There are two possibilities for carrot whitening, the so-called white blushing or the carotene bleaching. It cannot be assured that the whitening reported by Sy et al. (2005) was either white blushing or carotene bleaching. Sy et al. (2005) also found that higher ClO_2 concentrations (2.7 and 4.1 mg L^{-1}) caused more whitening; once again these ClO_2 levels were also associated with relative humidity that is lower than 90% for even longer exposure times.

Emmambux and Minnaar (2003) reported that a polymeric packaging film maintaining a high relative humidity with a good moisture barrier should be considered to prevent white blush formation, which is the most important shelf life determinant for minimally processed carrots. Amanatidou et al. (2000) also reported that dipping in citric acid solution prevented the whitening of the carrot. They found that carrots dipped in citric acid, coated with Na alginate, and packaged under 50% O_2 and 30% CO_2 or 1% O_2 and 10% CO_2 atmospheres did not have any change in color until 12 days of storage. However, they observed a significant increase in whiteness index on day 12 comparing to day 0 under high O_2 MAP (80–90% O_2). Although they stated that air and low O_2 application (1%) caused surface darkening due to the oxidation of phenolics, they did not observe any darkening under air and low O_2 application during the 21 days of storage.

Loss of water causes loss of sheen and gloss at the cut surface (Gorny et al. 2000), and lignin formation (Howard and Griffin 1993) a whitening or dehydrated surface develops in cut carrot (Barry Ryan and O'Beirne 1998), shredded green papaya (Techavuthiporn et al. 2003), and sliced tomato (Artes et al. 1999). Cutting induces the development of translucency or water-soaking in fleshy tissues (Bai et al. 2001; Portela and Cantwell 2001).

Yellowing or Degreening

Studies on chlorophyll loss in fresh-cut product have predominately produced evidence for breakdown induced by oxygen radical oxidation of the chlorophyll molecule (Brown et al. 1991; Toivonen et al. 2008). Chlorophyll breakdown in parsley leaves has in one case been shown to involve pheophytin accumulation (Yamauchi and Watada 1991) and in another case to involve two breakdown intermediates, pheophytin and an unknown believed to be C13^2-hydroxychlorophyll a (Amir-Shapira et al. 1987). In broccoli, there are numerous reports strongly supporting the hypothesis that peroxidase- and/or chlorophyll oxidase- and/or lipoxygenase-mediated chlorophyll breakdown are important in whole product storage and packaged fresh-cut product (Zhang et al. 1994; Funamoto et al. 2002, 2003; Costa et al. 2005, 2006). Spinach leaf chlorophyll loss has been associated with peroxidase-mediated breakdown as well (Yamauchi and Watada 1991). In a final example, excised cabbage discs show chlorophyll losses strongly associated with lipoxygenase activity and concomitant fatty acid degradation (Cheour et al. 1992; Toivonen et al. 2008). It is reported that the treatment of broccoli florets with 1-MCP resulted in delayed loss of green color and onset of yellowing (Ku and Wills 1999). In a study conducted by Gomez and Artes (2005), the effects of two polymeric films on the external color of stored celery sticks were evaluated. Results showed that both MAP treatments kept the green color giving a product acceptable for commercial purposes. However, air-stored sticks showed an increased yellowing. Low-density polyethylene (LDPE)-bagged sticks had an intermediate hue value, while oriented polypropylene (OPP)-bagged sticks had the best, keeping a hue closer to that at harvest.

8.5 How to Maintain Quality of Fresh-Cut Vegetables

8.5.1 Low Temperatures

In general, fresh-cut products have a shorter shelf life, which is mainly due to mechanical stresses (Watada 1997). At the cut surface, cells and membranes are damaged leading to alterations in tissue metabolism. Although these alterations are different, many authors have observed an increase in CO_2 and ethylene evolution, water loss, and alterations in flavor and aroma and in volatile profiles and increase in the activity of enzymes related to enzymatic browning (Rolle and Chism 1987; Artes et al. 1998; Saltveit 2003).

Quality loss is a function of both time and temperature. If products are neglected, even for short periods at high temperatures during loading and off-loading, the shelf life is reduced. Refrigeration removes excess heat and facilitates temperature control for fresh-cut produce during storage and transportation. Maintaining the "cold chain" is the key to delivering wholesome fresh-cut products to the end user (James and Ngarmsak 2010).

Temperature control is the most common and important technology to minimize the effects of cutting in fruit and vegetables (Brecht 1995; Cantwell 1996; Watada et al. 1996). Whole and sliced carrots kept at 0 and 10 °C demonstrate the importance of low temperatures for the reduction of respiration and consequently metabolism. Whole carrots produced 1.0 and 1.4 μg kg^{-1} s^{-1} of CO_2 at 0 and 10 °C, respectively, which represents an increase of 40.54% in respiration at 10 °C, while carrot slices produced 1.7 and 3.0 μg kg^{-1} s^{-1} of CO_2 at 0 and 10 °C, respectively, which represents an increase of 66.67% in respiration at 10 °C. Sliced carrots showed an increase of 62.2 and 92.3% in respiration rate compared to whole carrots, at 0 and 10 °C, respectively (Cantwell 1992). Furthermore, Vitti et al. (2004) have observed that respiration rate of fresh-cut beets stored at 15 °C was two times higher than that of at 5 °C.

Most of the metabolic reactions that happen in fresh-cut products and result in changes in quality are enzyme catalyzed and as such very much dependent on temperature (Wiley 1994). Low temperature reduces respiration, inhibits microbial growth, and retards metabolic activity, ripening, and senescence (Wiley 1994; Wang 1999; Able et al. 2005). The maintenance of low temperature is also imperative to preserve the microbiological safety of these products, and because of that, the recommended temperature for fresh-cut products is ≤5 °C. Temperatures between 0 and 3 °C can extend the shelf life of minimally processed vegetables from 5 to 18 days, since the quality degradation is retarded by the lower temperature causing a reduction in the respiratory rate (Scott 1989).

Significant differences in respiration rate between shredded and sliced radish cut were obtained at 1 and 5 °C 1 h after processing. Also, shredded radish showed higher respiration than whole radish throughout the 4 h period (Saavedra del Aguila et al. 2006). The response of tissue to processing wounds usually increases as the severity of the injury increases (Brecht 1995; Saltveit 2003). This can explain the differences observed in the respiration rate between whole and shredded radish and between shredded and sliced radish. During cold storage, the respiration rate of whole radish remained stabile, while oscillations in fresh-cut radishes were observed. Respiration was generally higher in shredded radish (Saavedra del Aguila et al. 2006).

Regarding the storage temperature, similar results were obtained by Escalona et al. (2005), who reported an increase of respiration on fresh-cut butterhead lettuce at higher temperature.

There was no detectable ethylene production up to 9 h after processing the radish. After 9 h, ethylene production was higher in shredded and sliced radish compared to whole radish. No ethylene was detected 12 h after processing (Saavedra del Aguila et al. 2006). Ethylene production is another response of vegetables to wounding (Abeles et al. 1992; Sakr et al. 1997). Thus, the increase in ethylene production may be attributed to the biosynthesis of wound ethylene (Abeles et al. 1992). In radish, the ethylene production response to injury appears to be retarded if compared to other fresh-cut underground storage organs. For example, in shredded beets, ethylene (0.22 ng kg^{-1} s^{-1}) was detected after 1 h of processing (Vitti et al. 2004). In relation to storage temperature, it was verified that ethylene production was higher

with increase of temperature. Fresh-cut radish produced C_2H_4 at 0.03–0.08 ng kg^{-1} s^{-1} at 1 °C, 0.10–0.13 ng kg^{-1} s^{-1} at 5 °C and 0.17–0.22 ng kg^{-1} s^{-1} at 10 °C (Saavedra del Aguila et al. 2006).

8.5.2 Atmosphere Composition

Kader and Ben-Yehoushua (2000) reported that high O_2 concentration atmospheres may either stimulate, have no effect, or reduce the respiration rate and ethylene production, depending on the commodity, ripeness stage, storage time, temperature, and concentration of CO_2 and ethylene in the atmosphere. Shredded cabbage showed a higher respiration activity compared to intact cabbage leaves due to stress metabolism. Wounded tissue generally increases metabolic activity; Lakakul et al. (1999) reported a 2–3 times higher respiration rate for apple slices compared to intact apple. Mechanical damage, i.e., bruising, cutting, and insect attack, provokes increased respiration and ethylene production in many commodities (Santana-Lladó and Marrero-Domínguez 1998). Fresh-cut cabbage samples stored in an atmosphere containing 70% O_2 + 30% N_2 showed higher respiration activity than other atmospheres. Gorny (1997) found that high levels of O_2 in packages of fresh-cut spinach were beneficial in maintaining product quality. Allende et al. (2004) found a high O_2 atmosphere beneficial regarding the sensory quality of fresh-cut spinach.

Usually, low O_2 levels combined with moderate to high CO_2 levels are applied to extend the shelf life of fresh-cut commodities, and the optimal storage conditions depend on the metabolic characteristics of the specific product (Kader et al. 1989; Hertog et al. 1998). Nevertheless, some problems in using MAP regarding quality and microbial safety remain to be solved. Frequently O_2 is depleted to below the anaerobic compensation point of the commodities, resulting in fermentation within packages and production of off-odors and rapid deterioration (Zagory and Kader 1988). To maintain quality of fresh-cut green peppers in MAP, levels of about 3% O_2 combined with 5–10% CO_2 at temperatures between 0 and 5 °C are recommended (Gorny 1997). The respiration rates of fresh-cut bell peppers under diverse high and low O_2 levels, with or without 20% CO_2, at 2, 7, and 14 °C, were also studied by Conesa et al. (2007). In this study, fresh-cut peppers exposed to 0, 0.5, 1, 3, and 9% O_2 (all CO_2-free), and to 0% O_2 + 20% CO_2, had a lower respiration rate than peppers in the range of 20–100% O_2 with or without CO_2. Under high O_2, 20% CO_2 increased the respiration rate by about 20–40% compared to that in free CO_2 atmospheres. High O_2 had little or no effect in stimulating both CO_2 production and O_2 consumption compared to normal air. High CO_2 in the range 20–100% O_2 increased the respiratory activity of pepper dices, probably because physiological injury occurred at 14 °C. However, 20% CO_2 combined with superatmospheric O_2 neither induced a poor visual appearance nor off-odors (Conesa et al. 2007).

8.5.3 Modified Atmosphere Packaging (MAP)

Browning and other discolorations, softening, surface dehydration, water loss, translucency, off-flavor and off-odor development, and microbial spoilage are some of the most frequent causes of quality loss in fresh-cut products. Nowadays, the use of innovative MA and edible coatings stands out among other techniques in the struggle for maintaining freshness and safety of fresh-cut fruits and vegetables. A few studies have demonstrated the effectiveness of these techniques when applied to different fresh-cut commodities. However, treatment and storage conditions for fresh-cut fruits are still being largely explored to better keep their fresh-like quality attributes (Rojas-Grau et al. 2009).

MAP is a technology, whereby the composition of the atmosphere surrounding the product is different from the composition of air (O'Beirne 1990; Kim et al. 2004). MAP is effective in prolonging the shelf life of horticultural commodities by decreasing O_2 and increasing CO_2 concentrations in the package atmosphere (Jacxsens et al. 1999; Makino 2001). In general, major factors affect the equilibrium gas concentrations of packaged produce including packaged product weight and its respiration rate, package film oxygen/carbon dioxide transmission rates (OTRs) and the respiring surface area (Bell 1996), and storage temperature. However, for packaged fresh-cut vegetables in the retail market, package surface area and product fill weight are often predetermined to certain degree to achieve a market appeal, and the respiration rate is also influenced by numerous factors, including storage temperature, cut size, vegetables types, etc. Therefore, selecting package films with suitable OTRs plays an important role in developing MA packages for improved quality and shelf life of fresh-cut produce (Exama et al. 1993; Kim et al. 2004). Kim et al. (2004) conducted a research to develop a MAP system for fresh-cut salad savoy and to evaluate the effect of film OTR on package atmospheres, and consequently product quality changes during storage. The results showed that packages with 16.6 and 21.4 OTR films attained the desired O_2 (1.4–3.8%) and CO_2 levels (3.6–6.3%) on day 10 and throughout the storage period; products stored in these packages maintained freshness with high overall quality scores. Gimenez et al. (2003) determined the impact of the preservation technologies on sensory quality and on the growth of indicator microorganisms in minimally processed artichoke packaged with five different films (two PVC and three P-Plus). The respiratory activity showed by artichoke, even at storage temperatures of 4 °C, means that the different permeability of the films used caused differences in the composition of the atmosphere inside the packages after reaching an equilibrium. The impact of this factor on the development of microorganisms was the cause of the differences in the shelf life of the batches, on applying the legally established microbiological limits. The different atmospheres obtained, together with the differences in the permeability to the water vapor, also had a significant influence on the visual quality. The sensory and microbiological quality of fresh peeled white asparagus packaged in two different types of P-Plus films and stored at two different temperatures (5 and 10 °C) for up to 14 days was studied by Simón and Gonzalez-Fandos (2011). Quality was maintained better at 5 °C than at

10 °C; the main limiting factor was microbial spoilage. The atmosphere generated with film A (around 7% CO_2 + 15% O_2) inhibited spoilage and maintained the acidity of asparagus better than the atmosphere generated by film B (around 2% CO_2 + 20% O_2). The shelf life of asparagus packaged in film A and stored at 5 °C was 14 days. In previous studies, with peeled white asparagus (Simón et al. 2004) packed in micro-perforated polypropylene film (P-Plus), modified atmospheres with high O_2 levels without anaerobiosis and with adequate CO_2 levels were reached. The atmospheres generated in these types of films depend on their permeability to gases according to the number of micro-perforations. It is important to know the most appropriate atmosphere in order to select film permeability.

MAP with gas flush of 2–5% CO_2 + 2–5% O_2 was traditionally used for keeping fresh vegetables, but higher gas concentrations could be required for fresh-cut produce (Mohamed et al. 1996). Ayhan et al. (2008) found that storing minimally processed carrots in a high O_2 (80% O_2 + 10% CO_2) atmosphere retained better quality properties of the carrots compared to the low (O_2 5%) O_2 atmosphere. Plestenjak et al. (2008) studied different storage conditions on the storability of packaged shredded cabbage. The cabbage cultivar "Fieldrocket" was cut and packaged in glass jars and in polyethylene (PE) or polypropylene (PP) film. Several initial atmospheres were established within the packaged cut cabbage: 100% N_2, 5% O_2 + 95% N_2, 10% O_2 + 90% N_2, normal atmosphere (NA), 70% O_2 + 30% N_2, and 100% O_2. Samples were stored at two different temperatures of 0 and 10 °C for 7 days. Variation in CO_2 and O_2 concentrations was higher at 10 °C compared to 0 °C, and the highest at the atmosphere consisting of 70% O_2/30% N_2. A decrease of O_2 below 3–5% and an increase of CO_2 above 2–5% in the packed product resulted in the appearance of anaerobic metabolism. An initial atmosphere consisting of 100% O_2 and a storage temperature of 0 °C resulted in delayed anaerobic metabolism compared to other atmospheric conditions and storage temperature of 10 °C.

Furthermore, MAP has been used to extend the postharvest shelf life of vegetables by reducing respiration rate and delaying senescence. However, it causes anaerobiosis, and the fruit fails to ripen properly. Respiration of the product becomes anaerobic when O_2 levels decline. Therefore, restriction of O_2 leads to accumulation of ethyl alcohol or anaerobic metabolism that leads to off-flavors or off-odor (Sonti 2003). Off-odor in packages of fresh-cut products is often an indicator of anaerobic respiration as well as decay under low O_2 and elevated CO_2 levels. There are various reports on off-odor development of fresh-cut products such as fresh-cut broccoli, cauliflower, lettuce, etc. (Cameron et al. 1995; Smyth et al. 1998).

8.5.4 Edible Coatings and Films

The disadvantages of the techniques being used to preserve fresh-cuts and increasing environmental concerns (Arvanitoyannis and Gorris 1999; Sonti 2003) have created an urgency for the invention of alternative packaging techniques such edible coatings (Sonti 2003).

Studies have shown that ripening can be retarded, color changes can be delayed, water loss and decay can be reduced, and appearance can be improved by using a simple and environmentally friendly technology, edible coating (Baldwin 2001). The concept of edible films as protective films has been used since the 1800s (Sonti 2003). The first edible coating used was wax in China (Park 1999). Extensive research in this area has paved the way for different effective edible films and coatings. The use of edible films and coatings is extended for a wide range of food products including fresh-cut fruits and vegetables because they extend product shelf life, control degradative oxidation and respiration reactions, add to texture and sensory characteristics, and are environmentally friendly (Sonti 2003). Krochta (2001) indicated that the present commercial edible coatings are solvent based (ethanol) and the food industry should replace these solvent-based coatings with water-based coatings to ensure worker and environmental safety.

Coatings are applied and formed directly on the surface of the food product, whereas films are structures, which are applied after being formed separately (Guilbert et al. 1996; Sonti 2003). Because they may be consumed, the material used for the preparation of edible films and coatings should be regarded as GRAS (Krochta and Mulder-Johnston 1997) approved by FDA and must conform to the regulations that apply to the food product concerned (Guilbert et al. 1996). The purpose of edible films or coatings is to inhibit migration of moisture, O_2, CO_2, or any other solute materials, serve as a carrier for food additives like antioxidants or antimicrobials, and reduce the decay without affecting the quality of the food.

Specific requirements for edible films and coatings are (Arvanitoyannis and Gorris 1999; Sonti 2003):

1. The coating should be water-resistant so as to remain intact and to cover all parts of a product adequately when applied.
2. It should not deplete O_2 or build up excessive CO_2. A minimum of 1–3% O_2 is required around a commodity to avoid a shift from aerobic to anaerobic respiration.
3. It should reduce water vapor permeability.
4. It should improve appearance, maintain structural integrity, improve mechanical handling properties, carry active agents (antioxidants, antimicrobials etc.), and retain volatile flavor compounds.

In coating materials, mixtures of lipids, proteins, carbohydrates, plasticizers, surfactants, and additives are used dispersed in different kinds of solvents such as water or alcohols. Combining these materials in different ways provides the coating systems with a wide range of functional property values: barrier, appearance, mechanical, etc. The coating's effectiveness to limit respiration and dehydration of the processed products depends on the gas and water vapor barrier properties of the film. It has been determined that the permeability of hydrophilic films can be affected by factors such as the hydrophilic–hydrophobic nature both of the polymeric matrix and of the added components and by the matrix's morphology, thickness, and homogeneity (Erbil and Muftugil 1986).

Edible coatings are thin layers of edible material applied to the product surface in addition to or as a replacement for natural protective waxy coatings and provide a barrier to moisture, O_2, and solute movement for the food (McHugh and Senesi 2000; Sonti 2003). They are applied directly on the food surface by dipping, spraying, or brushing to create a modified atmosphere (McHugh and Senesi 2000; Krochta and Mulder-Johnston 1997; Guilbert et al. 1996).

Edible polymer film is defined as a thin layer of edible material formed on a product surface as a coating or placed (preformed) on or between food components (Krochta and Mulder-Johnston 1997). Several types of edible films have been applied successfully for preservation of fresh products (Sonti 2003). Fruit-based films provide enhanced nutrition for food products, while increasing their marketing allure (McHugh and Senesi 2000). Edible and biodegradable films must meet a number of special functional requirements, for example, moisture barrier, solute or gas barrier, water/lipid solubility, color and appearance, mechanical and rheological characteristics, non-toxicity, etc. These properties depend on the type of material used and its formation and application (Guilbert et al. 1996).

Some of the polysaccharides that have been used in coating formulations are starch and pectin (Baldwin 2001), cellulose (Tien et al. 2001; Baldwin 2001), chitosan (Zhang and Quantick 1997, 1998; Ghaouth et al. 1991; Jiang and Li 2001; Baldwin 2001), and alginate (Tien et al. 2001; Baldwin 2001). Nature Seal® (NS), is a cellulose-based edible coating, has been used (in combination with antimicrobials, plasticizers, antioxidants, etc.) to coat fresh-cut potatoes. The coating has also been used to effectively reduce the discoloration of mini-peeled carrots without affecting microbial and chemical quality (Ghaouth et al. 1991; Howard and Dewi 1995) but had minor effects on levels of O_2, CO_2, and ethanol in package headspace. In peeled carrots, the use of edible coatings, such as sodium caseinate–stearic acid (Avena-Bustillos et al. 1993), carboxymethyl cellulose (Nature Seal® (NS)) and polyhydric alcohols and salt solutions (Cisneros-Zevallos et al. 1997), and hydroxypropyl methylcellulose (HPMC) containing surfactant mixtures of sorbitan monostearate (SPAN 60) and sucrose palmitate (sucrose ester P-1570) in aqueous and hydroalcoholic media (Villalobos-Carvajal et al. 2009), was observed to reduce surface whiteness. A greater carotene retention, lower weight loss, and longer shelf life were achieved in peeled carrots and sliced carrots when Nature Seal® (NS) and alginate–citric acid were applied to form the coating (Amanatidou et al. 2000). Peeled carrots covered with xanthan gum and alpha-tocopheryl acetate (vitamin E) improved the surface color for 3 weeks without affecting taste, texture, or aroma (Mei et al. 2002). Recently, Jagannath et al. (2006), using a coating based on curcuma/casein starch and small quantities of polyvinyl alcohol and polyethylene glycol for fresh carrots, found that the color, carotene content, texture, and antimicrobial properties were maintained for 10 days.

Chitosan also reduced decay and improved appearance of carrots (Li and Barth 1998; Sonti 2003). Coating tomatoes with chitosan reduced respiration rate, internal O_2 levels, and ethylene production (with a greater effect at 2% than 1% chitosan) and increased titratable acidity (El Ghaouth et al. 1992).

Del Nobile et al. (2009) have investigated the influence of both postharvest treatments and film permeability on the quality loss kinetic of minimally processed artichokes. In particular, fresh-cut artichoke heads were subjected to dipping in citric acid/calcium chloride water solution, and coating with citric acid loaded sodium alginate, respectively. Three different packaging materials were used: a polyester-based biodegradable film, an aluminum-based multilayer film, and a commercially available oriented polypropylene film. Artichokes' quality loss kinetic during storage was determined by monitoring produce appearance, weight loss, pH, and viable cell load of the main spoilage microorganisms. Results suggest that among the selected treatments, coating shows the best performance in terms of artichokes shelf life. As far as the packaging material is concerned, the biodegradable film tested in this work seems to be the most suitable packaging to preserve the quality of the coated fresh-cut produce.

Some of the proteins that are used in coating formulations for fresh-cut products are soy protein, whey protein, casein and corn–zein, maize, egg albumen, collagen, and wheat (Baldwin et al. 1995). Corn–zein coating is a good barrier to O_2. It delays color change and loss of firmness and weight and extends shelf life of tomatoes. Its water vapor permeability, however, is about 800 times higher than a typical shrink-wrapping film (Park et al. 1994). Casein, a milk protein, contains four protein types: alpha-casein, beta-casein, delta-casein, and gamma-casein. Research conducted showed that casein–lipid coatings provide protection for fresh-cut products from moisture loss and oxidative browning (Baldwin et al. 1995). Calcium caseinate and whey protein solutions efficiently delayed browning of potato slices by acting as O_2 barriers (Tien et al. 2001). They were effective gas barriers to internal CO_2 and O_2, inhibited color changes, and reduced decay when coated on bell peppers (Lerdthanangkul and Krochta 1996). Peeled carrots covered with xanthan gum and alpha-tocopheryl acetate (vitamin E) improved the surface color for 3 weeks without affecting taste, texture, or aroma (Mei et al. 2002). Recently, Jagannath et al. (2006), using a coating based on curcuma/casein starch and small quantities of polyvinyl alcohol and polyethylene glycol for fresh carrots, found that the color, carotene content, texture, and antimicrobial properties were maintained for 10 days.

Some of the lipids that have been used effectively in coating formulations are beeswax, mineral oil, vegetable oil, surfactants, acetylated monoglycerides, carnauba, and paraffin wax (Kester and Fennema 1986). The mineral oil-based coating was a desirable edible coating for commercial application for bell pepper fruit (Lerdthanangkul and Krochta 1996). A film formed by milk protein (casein) and lipid (acetylated monoglyceride) for lightly processed apples and potatoes was reported to provide protection from moisture loss and oxidative browning for up to 3 days (Baldwin et al. 1995).

Generally, the potential benefits of edible coatings and films for lightly processed produce are to stabilize the product and thereby extend product shelf life (Sonti 2003). More specifically, coatings have the potential to reduce moisture (Avena-Bustillos et al. 1994, 1997; Baldwin et al. 1995) and firmness loss, provide moisture and O_2 barrier properties (Li and Barth 1998; Avena-Bustillos et al. 1994), retard respiration rates (Banks 1984; Sonti 2003), hinder solute movement (Li and Barth 1998),

retard loss of chlorophyll (Banks 1984; Sonti 2003), and ethylene production (Banks 1984; Baldwin et al. 1995), reduce metabolism and oxidation rates (Li and Barth 1998), seal in flavor volatiles, carry additives that could reduce discoloration and microbial growth (Sonti 2003; Baldwin et al. 1995), and improve the appearance (Sonti 2003). Some edible coatings can be very helpful in attaining relative humidity close to 100% (Watada et al. 1996).

8.5.5 1-Methylcyclopropene (1-MCP)

Other factors affecting the quality of fresh-cut produce include the fact that harvested fruits continue to respire by utilizing the store available sugars and adjunct organic acids increasing CO_2 and ethylene production that leads to rapid senescence (Kalia and Gupta 2006). Furthermore, cutting increases the water activity as well as stress-induced ethylene production which accelerates the water loss. Finally, the sugar availability promptly invites enhanced microbial invasion and rapid growth (Kalia and Gupta 2006).

As reported, ethylene production in plant tissue is regulated by the levels of both 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and 1-aminocyclopropane -1-carboxylic acid oxidase (ACO) activities (Yang and Hoffman 1984). The presence of exogenous ethylene affected the activity of these enzymes as previously reported in broccoli florets (Tian et al. 1994; Kato et al. 2002), in particular increasing the ACO activity, which causes the stimulation of ethylene production (Makhlouf et al. 1989) and the increase of tissue sensitivity to ethylene (Tian et al. 1994). 1-MCP delayed the senescence of broccoli by inhibiting the activities of enzymes involved in ethylene biosynthesis (ACS and ACO) and suppressing their gene expression (Ma et al. 2009). As a consequence of 1-MCP treatment on ethylene production, the respiration rate and the deleterious effects on quality due to exogenous ethylene were also limited as previously reported for broccoli florets (Ku and Wills 1999; Fan and Mattheis 2000; Able et al. 2002).

The effect of 1-MCP on some Brassicaceae vegetables, such as broccoli, Chinese cabbage, Chinese mustard, choy sum, mibuna, mizuna, pak choi, tatsoi, and broccoli raab, has been investigated (Watkins 2006; Cefola et al. 2010). Able et al. (2002) found that the use of 1-MCP to extend shelf life would be of marginal benefit for broccoli florets and of little benefit for pak choi leaves, especially when compared with the benefits from reduction in temperature alone. Ma et al. (2009) and Yuan et al. (2009) have reported that 1-MCP can delay yellowing, respiration activity, and decay and extend the shelf life of broccoli. Gong and Mattheis (2003) reported that 1-MCP treatment is useful to inhibit degreening in broccoli florets exposed to exogenous ethylene.

The increase in respiration rate in the presence of exogenous ethylene is a well-known physiological change which occurs to most vegetables (Saltveit 1999; Kays and Paull 2004) including broccoli florets (Asoda et al. 2009; Ma et al. 2009). Although leaves and other vegetative tissues are generally considered as nonclimacteric,

some authors (Makhlouf et al. 1989; Ma et al. 2009) found for broccoli a typical climacteric pattern. Broccoli raab is a very similar vegetable to broccoli; however, at present, no climacteric behavior for this vegetable has been reported. In a study, Cefola et al. (2010) treated broccoli raab florets with 1 μL L^{-1} of 1-MCP for 24 h at 20 °C and then stored, together with the untreated control, at 5 °C for 14 days in a humidified air flow and air +100 μL L^{-1} of ethylene. Treatment with 1-MCP extended the shelf life, reducing postharvest deterioration, retarding chlorophyll degradation, and delaying visual quality loss, as was also the case with samples stored in the presence of exogenous ethylene. Untreated broccoli raab florets stored either in air or in air + ethylene showed a significant increase in ammonia during storage, suggesting stressful storage conditions. These results indicate that a 1-MCP treatment could be a good candidate for extending shelf life, maintaining visual quality, and reducing loss of quality in broccoli raab florets.

8.6 Chemical-Based Microbial Control for Fresh-Cut Vegetables

8.6.1 Chlorine Dioxide

It is well known that fresh-cut processors usually rely on wash water sanitizers to reduce microbial counts in order to maintain quality and extend shelf life of the end product. Water is a useful tool for reducing potential contamination, but it can also transfer pathogenic microorganisms. Washing with sanitizers is important in fresh-cut produce hygiene, particularly removing soil and debris, but especially in water disinfection to avoid cross contamination between clean and contaminated product. In fact, despite the general idea that sanitizers are used to reduce the microbial population on the produce, their main effect is maintaining the microbial quality of the water (Gil et al. 2009). Generally, the success of the washing depends on different factors such as target microorganisms, characteristics of produce surfaces, attachment of cells to produce surfaces, formation of resistant biofilms and internalization of microorganisms, type of washing, exposure time, dose, pH, temperature, etc. Additionally, the remaining microbial load could grow rapidly, reaching similar values to those of the unwashed products. Hence, maintenance of this reduction during storage is as important as initial microbial reductions after washing (Ragaert et al. 2007). Most new alternative techniques accentuate the problems with chlorine suggesting that the industry should move away from this traditional disinfection agent. However, the use of chlorine-based sanitizers is presented as belonging to the most effective and efficient sanitizers when adequate doses are used (Gil et al. 2009).

Currently, most fresh-cut products are washed in chlorinated water to reduce levels of microorganisms. Sodium hypochlorite (NaClO) is the most widely used sanitizer in the fresh-cut industry (Lee and Baek 2008). The efficiency of chlorine and chlorine-based derivatives, providing adequate water-disinfecting capabilities, has been well proven over the past 30 years (Suslow 1997, 2001; IPFA 2001; Sapers 2003, 2005; Gómez-López et al. 2008a).

The use of chlorinated water as a decontamination stage in the washing of fresh-cut produce is widespread throughout the fresh produce industry. Without chlorine, there probably would not be a market for fresh-cut salads and vegetables. Approximately 76% of respondents in an industry survey reported the use of hypochlorite (Seymour 1999), although it was apparent that many of the important aspects of chlorine chemistry, e.g., pH control, were not fully understood. As a consequence, many users of hypochlorite were not using it under optimum conditions and therefore not achieving maximum effectiveness.

In the last few years, the outbreaks associated with foodborne pathogen contamination in fresh-cut vegetables raised the concerns about the efficacy of chlorine treatment in assuring the safety of the products. Moreover, it is known that the reaction of chlorine with natural organic matter results in the formation of carcinogenic halogenated disinfection by-products (DBP), like trihalomethanes (THMs) and haloacetic acids (HAAs) (Hua and Reckhow 2007; Singer 1994). The use of chlorine is also associated with the production of high amounts of wastewater with very high levels of biological oxygen demand (BOD). Due to these environmental and health risks posed by the use of chlorine, its use in organic production is forbidden in Europe. In some European countries, the use of chlorine has been forbidden even for conventional products. Actually, there is a trend in eliminating chlorine from the disinfection process (Olmez and Kretzschmar 2009). Due to the abovementioned problems, both the organic and the conventional processing sectors are now seeking alternatives to chlorine which assure the safety of the products, maintain the quality, and enable a shelf life as long as chlorine, while also reducing the water consumption rates in processing. Chlorine dioxide, ozone, organic acids, peracetic (peroxyacetic) acid, hydrogen peroxide, etc. are some of the alternative sanitizing agents that gain interest in recent years (Olmez and Kretzschmar 2009).

8.6.1.1 Aqueous Solution of Chlorine Dioxide

The use of alternative sanitizing agents instead of chlorine has been adopted by few fresh-cut companies as a marketing strategy to attract consumers. Additionally, the safety and efficacy of chlorine might eventually be the reason of the implementation of restrictions by regulatory agencies. Therefore, there is an increasing need to investigate the efficacy of new commercial sanitizers and other alternative technologies.

One of the postulated alternatives to sodium hypochlorite is an aqueous solution of chlorine dioxide (ClO_2), which possesses higher oxidation capacity and forms fewer halogenated by-products (Richardson et al. 2000; Hua and Reckhow 2007; Gómez-López et al. 2009). However, little information has been published regarding the impact of aqueous chlorine dioxide on the natural microbiota after washing and after storage. There are other important aspects related to the quality of fresh-cut produce, such as sensory quality, that needs to be preserved during storage, as well as the content of bioactive compounds, as they may be implicated in enzymatic browning after cutting, which is highly relevant in lettuce cultivars (Gómez-López et al. 2008b). Most of the scientific literature on the impact of aqueous chlorine dioxide in fresh-cut fruit and vegetables has involved using chlorine dioxide generators

at a laboratory scale, which is not always consistent with regard to the applied doses. However, commercial chlorine dioxide generators produce a continuous supply of aqueous solutions and include a chlorine dioxide analyzer, an ambient sensor, a probe, and a temperature controller. The suitability of aqueous chlorine dioxide (3 mg L^{-1}) as an effective sanitizer of fresh-cut iceberg lettuce stored under active MAP at refrigerated conditions was tested by López-Gálvez et al. (2010) and compared with sodium hypochlorite (100 mg L^{-1}). In this study, a commercial chlorine dioxide generator was used as an essential tool for industrial process simulation. In general, the natural microbiota of fresh-cut lettuce after washing and storage was equally affected by the different washing solutions, with the exception of yeasts which showed the highest growth after 10 days of storage in samples washed with chlorine dioxide. None of the tested washings negatively affected sensory quality, which was acceptable after 10 days of storage. The results suggest that aqueous chlorine dioxide is as suitable as sodium hypochlorite for fresh-cut lettuce sanitation with the advantage of preventing the formation of trihalomethanes (THMs).

8.6.1.2 Gaseous Chlorine Dioxide

Because gas has greater penetration ability than liquid, ClO_2 gas may be more effective for surface sanitation than aqueous ClO_2 (Han et al. 2001b) or other aqueous sanitizers. Different factors can influence the lethality of a ClO_2 gas treatment. Han et al. (2001a) reported in a study about the inactivation of *Escherichia coli* O157:H7 on green peppers that the order of significance of the factors from the most important to the least was ClO_2 gas concentration, time, relative humidity, and temperature; moreover a synergistic effect was found between gas concentration and relative humidity. One portion of the grated carrots (*Daucus carota* L.) was treated with ClO_2 gas, and the other was untreated for comparisons (Gómez-López et al. 2007). Fresh-cut carrots were decontaminated in a cabinet at 91% relative humidity and 28 °C for up to 6 min, including 30 s of ClO_2 injection to the cabinet, and then stored under equilibrium modified atmosphere (4.5% O_2 + 8.9% CO_2 + 86.6% N_2) at 7 °C for shelf life studies. After ClO_2 treatment, the decontamination levels (log CFU/g) achieved were 1.88, 1.71, 2.60, and 0.66 for mesophilic aerobic bacteria, psychrotrophs, and yeasts, respectively. The shelf life extension was limited to 1 day due to the restricted effect of the ClO_2 treatment on yeast counts. Nevertheless, ClO_2 seems to be a promising alternative to prolong the shelf life of grated carrots (Gómez-López et al. 2007).

Studies on the efficacy of ClO_2 to inactivate microorganisms inoculated onto fruit and vegetable surfaces have focused on pathogens such as *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium* (Han et al. 2000, 2004; Singh et al. 2002). Comparatively few studies have been devoted to the effect of ClO_2 gas on the spoilage microflora or sensory properties of the treated fruit and vegetables. Singh et al. (2002) reported the decolorization of lettuce leaves after treatment, which may have been due to oxidation of chlorophyll. A treatment with 4.1 mg L^{-1} of ClO_2 did not markedly affect the sensory quality of fruits stored for up to 10 days at 8 °C.

In a companion article, Sy et al. (2005) reported reductions in populations of yeasts and molds of 1.68 log CFU/apple and 2.65 log CFU/peach, but no significant reduction was found for those microorganisms on tomato and onion. Sensory qualities of peaches or fresh-cut cabbage, carrot, and lettuce were impaired by treatment with ClO_2, but apples, tomatoes, and onions were not markedly affected.

8.6.1.3 Acidified Sodium Chlorite

Acidified sodium chlorite (ASC) is a highly effective antimicrobial that is produced by lowering the pH (2.5–3.2) of a solution of sodium chlorite ($NaClO_2$, sodium chlorite (SC)) with any GRAS acid (Warf 2001; Allende et al. 2009). The FDA has approved ASC (0.5–1.2 g L^{-1}) for spray or dip application on various food products, including fresh and fresh-cut produce (Code of Federal Regulations 2000). Warf (2001) hypothesized that the mode of action of ASC derives from the uncharged chlorous acid, which is formed by the acidification of chlorite. Chlorous acid gradually decomposes to form chlorate ions, chlorine dioxide, and chloride ions. These reactive intermediates are highly oxidative with broad-spectrum germicidal activity (FDA 2007).

Inatsu et al. (2005) demonstrated the same sanitation efficacy of different organic acid-activated acidified sodium chlorite solutions. Currently, ASC is commercially supplied as a kit containing citric acid (CA) and SC. These chemicals when combined produce active chlorine dioxide (ClO_2), which is more soluble than sodium hypochlorite (NaOCl) in water and has about 2.5 times greater oxidizing capacity than hypochlorous acid (HOCl) (Inatsu et al. 2005; Allende et al. 2009). A number of reports have described the strong efficacy of ASC in the US Food and Drug Administration (FDA)-approved application concentration range of 0.5–1.2 g L^{-1} on inactivation of pathogens, including *E. coli* O157:H7 and *Salmonella* spp. (Gonzalez et al. 2004; Park and Beuchat 1999; Ruiz-Cruz et al. 2007). However, a negative impact on organoleptic quality of red meat and shredded carrots occurred when ASC was used within the approved concentration range (Bosilevac et al. 2004). ASC at 1 g L^{-1} has been found to effectively reduce aerobic bacterial growth in shredded carrots (Gonzalez et al. 2004; Ruiz-Cruz et al. 2006, 2007). Conner (2001) and Caldwell et al. (2003) affirmed that ASC applied to inoculated fresh fruits and vegetables at 1.2 g L^{-1} for 1 min killed at least 99.9% of *Salmonella* serotypes, *E. coli* O157:H7, and *L. monocytogenes* on carrots, strawberries, tomatoes, cucumbers, lettuce, cantaloupe, and apples. Gonzalez et al. (2004), Inatsu et al. (2005), and Ruiz-Cruz et al. (2007) found a strong *E. coli* O157:H7 reduction, even under process water conditions, when using 0.5 and 1 g L^{-1} ASC on shredded carrots and Chinese cabbage. Allende et al. (2009) treated cut cilantro with sodium hypochlorite (SH) at 0.2 g L^{-1} free chlorine and acidified sodium chlorite (ASC) at 0.1, 0.25, 0.5, and 1 g L^{-1}, along with the components of ASC, i.e., citric acid (CA) at 6 g L^{-1} and sodium chlorite (SC) at 1 g L^{-1}. In this study, it was found that SH inactivated, at maximum, 1–1.3 log CFU g^{-1} of background or pathogenic microflora present on cut cilantro. However, reductions of more than 3 log CFU g^{-1} were observed after

washing with 1 g L^{-1} of ASC. Moreover, when lower concentrations of ASC were used (0.25 and 0.5 g L^{-1}), microbial populations were reduced by about 2 log CFU g^{-1}. SC was as effective as ASC at 1 g L^{-1} in reducing aerobic mesophilic bacteria and *E. coli* O157:H7 populations, although it was not as effective as ASC in reducing yeast and mold populations.

Ruiz-Cruz et al. (2010) tested the effect of four sanitizers: sodium hypochlorite (NaOCl), peroxyacetic acid (PA), acidified sodium chlorite (ASC), and carvacrol on microbiological, sensorial, and nutritional quality (total phenols, vitamin C and antioxidant capacity) of fresh-cut jalapeño peppers stored at 5 °C during 27 days. All sanitizers (except carvacrol) maintained microbiological and overall quality of jalapeño peppers during 27 days. ASC (500 and 250 mg L^{-1}) maintained the best microbiological and sensorial properties at the end of the storage period. Carvacrol, active ingredient of oregano essential oil, maintained shelf life for only 17 days. ASC was the most effective sanitizer even though it was used at concentrations lower that those currently approved by the FDA.

8.6.2 Peroxyacetic Acid

Peroxyacetic acid is a combination of peracetic acid (CH_3CO_3H) and hydrogen peroxide (H_2O_2), usually commercialized as a liquid. Its breakdown products, acetic acid, O_2, CO_2, and water, are not particularly harmful for the ecosystem (Hilgren and Salverda 2000; Artes et al. 2009). It is applied for surface cleaning in concentrations ranging from 85 to 300 ppm, and the US Food and Drug Administration (FDA 1997) has set a minimum of 85 ppm peracetic acid for cleaning hard surfaces where food is handled. Stampi et al. (2001) indicated that for cleaning the surface of foods, 50 ppm is commonly enough, while, by comparison, concentrations used in the environmental and medical areas range from 1200 to 2600 ppm. Because of peroxyacetic acid tolerance to several factors such as temperature, pH, hardness, and soil contamination, its current main area of application is in fruit and vegetable processing (Artés et al. 2007, 2009). For the treatment of plant surfaces, recommended formulations combine 11% H_2O_2 and 15% CH_3CO_3H, at 80 ppm, followed by rinsing with tap water (Suslow 1997). However, population reductions for aerobic bacteria, coliforms, and yeasts and molds on fresh-cut celery, cabbage, and potatoes, treated with 80 ppm peroxyacetic acid, were less than 1.5 log units (Forney et al. 1991). Rodgers et al. (2004) reported that this was effective for controlling *E. coli* and *L. monocytogenes* in fresh-cut products. According to Kim et al. (2006a), *Enterobacter sakazakii* counts decreased 5 log units in lettuce with applications of peroxyacetic acid. Compared to 150 ppm NaClO, 68 ppm of peroxyacetic acid reduced psychrotrophic counts by 2 log units and mesophilic counts by 1 log unit in fresh-cut Galia melon, resulting in the fruit pieces having a shelf life of 10 days at 5 °C (Artes et al. 2009).

8.6.3 Calcium-Based Solutions

Temperature and Ca are necessary to delay flesh softening, since at high temperatures, PME activation occurs, generating free pectic acids which contain newly available carboxyl groups. Endogenous and exogenous ion Ca^{2+} bind to these carboxyl groups resulting in cellular wall stabilization and maintenance of flesh firmness (Ni et al. 2005). In relation to this point, there are several opinions in the literature. One is that high temperature, Ca content, and high PME activity are highly correlated (Manganaris et al. 2007). On the other hand, some researchers report that temperature is the main factor involved in firmness maintenance, while Ca salts have a marginal effect (Martin-Diana et al. 2006b). Beirão et al. (2008) reported that the firming effect found on kiwifruit slices dipped in $CaCl_2$ solution (1, 2, and 3%, w/v), combined with mild heating (25 min, 45 °C), is due to the activation of PME by high temperature, while the presence of Ca reduces or inhibits enzyme activation. Luna-Guzmán et al. (1999) treated melon cylinders using 2.5% $CaCl_2$ and Ca lactate at 60 °C, and Rico et al. (2007b) treated shredded carrot with 1% $CaCl_2$ at 50 °C, obtaining between 6 and 16% more firmness than the water control.

In the study conducted by Silveira et al. (2011), after preparing trapezoidal-shaped sections of "Galia" melons, the pieces were dipped for 1 min at 60 °C in Ca chloride, citrate, lactate, ascorbate, tartrate, silicate, propionate, or acetate using a Ca concentration equivalent to 0.4% (0.15 g g^{-1}) pure Ca chloride, combined with 50 mg L^{-1} H_2O_2 for controlling microbial growth. Dipping in sterile distilled water (without Ca salt) at 60 °C for 1 min was used as a control treatment. At the end of shelf life, Ca ascorbate, chloride, and lactate provided melon pieces with a lower respiration rate, increased tissue total Ca content, and maintained a good firmness. Similarly, Lamikanra and Watson (2004) and Luna-Guzmán and Barret (2000) found a reduction in respiratory rates of fresh-cut "cantaloupe" melon treated with $CaCl_2$ and lactate (2.5% at 25 and 60 °C for 1 min), an effect linked to ripening and senescence delay as a result of the combined additive effect of hot water dipping and Ca salts. This delay of senescence was discussed by Lester and Grusak (1999), who postulated that Ca applications can delay or slow down changes related to these processes having a direct effect in maintaining the functionality of the membranes. According to other authors, Ca increases membrane rigidity, thereby blocking gas exchange (Saftner et al. 1998).

Fresh-cut "honeydew" melon dipped for 30 s in different Ca salts (40 mM supplied as propionate and $CaCl_2$ and chelated with amino acids) reduced the microbial load by about 1 log unit (Saftner et al. 2003). Aguayo et al. (2008) also reported that $CaCl_2$ and lactate (0.18 g Ca 100 mL^{-1}) reduced microbial counts by 2 log units, while Ca propionate, at the same concentration, showed a greater reduction (4 log units). In addition, after preparing trapezoidal-shaped sections of "Galia" melons, the pieces were dipped for 1 min at 60 °C in Ca chloride, citrate, lactate, ascorbate, tartrate, silicate, propionate, or acetate, and these Ca salts reduced microbial growth (Silveira et al. 2011).

8.6.4 Ozone

Ozone has been used mostly in water applications for many years. After it gained GRAS status in 1997, its use in foods has been approved in Europe and in the United States. Ozone decomposes spontaneously during water treatment via a series of complex reaction mechanisms that involve the generation of hydroxyl free radicals (˙OH) (Hoigne and Bader 1983; Glaze 1987). The ˙OH radicals are the principal reactive oxidizing agents in water and are highly active in the inactivation of bacteria and virus (Vorontsov et al. 1997; Kim et al. 2003; Selma et al. 2006, 2007). A detailed assessment of the kinetics of microbial inactivation by ozone (with particular emphasis to aqueous systems) has been reported by Khadre et al. (2001). The antimicrobial action of ozone is due to the strong oxidizing activity of either molecular ozone itself or its decomposition products (e.g., the hydroxyl radical) rapidly reacting with intracellular enzymes, nucleic material, and components of their cell envelope, spore coats, or viral capsids (Khadre et al. 2001). Ozone is the fifth in thermodynamic oxidation potential behind elemental fluorine, chlorine trifluoride, atomic oxygen, and the hydroxyl free radical (Graham 1997).

It is also effective against many bacteria, molds, and yeast even at low concentrations (1–5 ppm) and for short exposure times (1–5 min). It has an antimicrobial activity higher than that of chlorine (Khadre et al. 2001) and acts more quickly than permissible levels of chlorine which makes it more suitable for washing procedures with short contact times. Due to its instability, ozone cannot produce persistent disinfection residuals (Kim et al. 2003; Singer 1994). Thus, it does not pose a residue problem and does not create any by-products enabling the reuse and recycling of process water. Since its activity is not pH dependent, there is no need to adjust the pH of the wash water.

Reductions between 1.5 and 2.5 log cycles were obtained in the natural microbial flora by the application of 1.5–3.0 ppm ozonated water (Kim et al. 2006b). These values are comparable with the reductions obtained by 100 ppm chlorine treatment. No further increase in the efficacy of ozone treatment was observed above 3 ppm ozone (Koseki and Isobe 2006). Kim et al. (2006b) observed about 2 log unit CFU g^{-1} reductions in *Listeria monocytogenes* counts on lettuce by applying 3 ppm ozonated water for 3 min. On the other hand, Yuk et al. (2006) reported that ozone did not affect the *L. monocytogenes* counts in shredded iceberg lettuce even applied at 5 ppm for 5 min and only reduced the counts of *Escherichia coli* O157:H7 about 1.1 log cycles. In contrary, Rodgers et al. (2004) showed that both *E. coli* O157:H7 and *L. monocytogenes* counts decreased more than 5 log cycles when inoculated whole green-leaf lettuce leaves were immersed in a 3 ppm ozonated water solution for 5 min or shredded lettuce leaves were misted with ozonated water for 3 min. Singh et al. (2002) observed only about 0.9 log cycle reduction in *E. coli* O157:H7 populations on shredded romaine lettuce after a washing treatment with 5.2 ppm of ozone for 5 min, but no significant reduction was achieved as a result of 1-min exposure neither at 5.2 ppm nor at 9.7 ppm ozone levels.

On the other hand, effectiveness of ozone treatment depends on the type of vegetable, the targeted microorganism, the initial inoculum level, the physiological states of the bacterial cells, and the ozone delivery methods that are the main factors that lead to differences in the efficacy of the treatment. Due the fact that ozone is a strong oxidative agent, it may cause physiological injury to the produce above certain levels. Kim et al. (2006b) found that above 5 ppm, ozonated water damaged the surface texture of the lettuce leaves. Koseki and Isobe (2006) observed a rapid onset of browning on iceberg lettuce treated with 10 ppm ozonated water. In the processing industry, it is important to keep the applied ozone levels as low as possible. It is known that the corrosion potential of stainless steel increases above 1 ppm ozone concentration. Most materials are compatible with ozone at moderate concentrations, that is, between 1 and 3 ppm (Brown et al. 1992; Pascual et al. 2007).

8.6.5 Electrolyzed Water

Electrolyzed water is produced by the electrolysis of ordinary tap water containing dissolved sodium chloride.

Electrolyzed water is a concept, which has been utilized experimentally in agriculture (Al-Haq et al. 2002), livestock management (Stevenson et al. 2004), medical sterilization (Vorobjeva et al. 2004), food sanitation (Park et al. 2002; Bari et al. 2003; Sharma and Demirci 2003; Okull and Laborde 2004; Ongeng et al. 2006), and also areas that rely on antimicrobial methodologies (Fabrizio and Cutter 2003). Electrolyzed water is conventionally generated by electrolysis of aqueous sodium chloride to produce an electrolyzed basic aqueous solution containing dilute sodium hydroxide at the cathode and an electrolyzed acidic solution at the anode (Kim et al. 2000; Ongeng et al. 2006).

Basically, the electrolyzed water apparatus consists of a disinfection cell (electrolysis cell) and a power supply. The electrolysis cell is made of specially coated, permanent electrodes. Direct current in the (safe) low voltage across the electrodes causes the formation of oxidizing agents principally derived from O_2, as well as free chlorine when chloride ions are present in the solution, both resulting in immediate disinfection (Ongeng et al. 2006). Through transmission of the reactive energy of the oxidants, the disinfection effect is upheld in the water causing a residual activity as well. These free oxidants are formed from the water itself, thus without adding chemicals, and eradicate bacteria, viruses, algae, and other microorganisms (Ryckeboer 2005; Ongeng et al. 2006).

The effect of electrolyzed water on total microbial count was evaluated on several fresh-cut vegetables. When fresh-cut carrots, bell peppers, spinach, Japanese radish, and potatoes were treated with electrolyzed water by dipping, rinsing, or dipping/blowing, microbes on all cuts were reduced by 0.6–2.6 logs CFU g^{-1}. Rinsing and dipping/blowing were more effective than dipping. Electrolyzed water containing 50 ppm available chlorine had a stronger bactericidal effect than that

containing 15 or 30 ppm chlorine for fresh-cut carrots, spinach, or cucumber. Electrolyzed water did not affect tissue pH, surface color, or general appearance of fresh-cut vegetables (Izumi 1999).

Rahman et al. (2011) have studied about the synergistic effect of alkaline electrolyzed water (AIEW) and citric acid with mild heat against background and pathogenic microorganisms on shredded carrots. The combined 1% citric acid and AlEW treatment at 50 °C showed a reduction of the total bacterial count and the yeast and fungi of around 3.7 log CFU g^{-1}, as well as effective reduction of *L. monocytogenes* (3.97 log CFU g^{-1}) and *E. coli* O157:H7 (4 log CFU g^{-1}). Combinations of alkaline electrolyzed water and citric acid better maintained the sensory and microbial quality of the fresh-cut carrots and enhanced the overall shelf life of the produce.

Additionally, a novel produce-washing procedure using a combination of alkaline electrolyzed water (AlEW), acidic electrolyzed water (AcEW), and mild heat induced a significant bactericidal effect when compared with treatment at ambient temperature (Koseki and Isobe 2007). Recently, combinations of alkaline electrolyzed water and citric acid showed a strong synergistic antimicrobial effect that reduced background flora and foodborne pathogens on fresh-cut produce and cereal grains (Park et al. 2004, 2009; Rahman et al. 2010).

8.6.6 Natural Preservatives for Treating Fresh-Cut Vegetables

8.6.6.1 Whey Permeate

Whey is a by-product of the cheese industry. Approximately, 9 kg of whey is produced for every kilogram of cheese manufactured. The high chemical oxygen demand (COD) (50 kg O_2/ton permeate) of whey makes its disposal a significant pollution problem. In order to minimize environmental impacts, different uses for this waste material have been devised by the dairy industry. The use of whey as a fermentation feedstock has long been of industrial interest for the production of lactic acid, acetic acid, propionic acid, ethanol, and single-cell protein (Bogaert 1997; Tyagi and Kluepfel 1998; Nykanen et al. 1998). However, these applications still do not utilize all the whey produced, and new uses for this by-product are continually being sought.

Whey permeate (WP) is obtained as a by-product when whey proteins are concentrated by ultrafiltration to produce whey protein concentrate. The use of WP for food preservation has been examined by Nykanen et al. (1998). These authors analyzed the effect of WP washing solutions on total counts and sensory characteristics in rainbow trout. They found that WP treatment gave a reduction in total counts and had no negative effect on sensory attributes. In a study conducted by Martin-Diana et al. (2006c), WP at different concentrations (0.5, 1.5, and 3%) was used as natural sanitizing agent in the washing treatment of fresh-cut lettuce and carrots. These treatments were compared with a chlorine 120 ppm widely used in the industry. WP at 3% resulted in equivalent or better microbial load reduction than chlorine. Sensory analysis panel considered all the samples of fresh-cut lettuce acceptable. However, in the sensory

results, the sliced carrots treated with 3% WP and chlorine scored lower acceptability due to higher surface whiteness, although these samples had lower microbial loads. Three percent WP controlled the browning-related enzymes better than 0.5% and 1.5% WP and chlorine and consequently the browning.

8.7 Alternatives to Chemical Microbial Control for Fresh-Cut Vegetables: Physical Treatments

8.7.1 Heat Treatment

Heat treatment (hot water, heat shock, hot air, precut heat, short-time blanching (steaming), etc.) has been used successfully in fresh-cut vegetables for different purposes. For example, in minimally processed celery, treatment by immersion in hot water allowed a better retention of the original color and the total chlorophyll content (Vina et al. 2007). Also, hot water dips controlled sprouting and rooting of garlic (Cantwell et al. 2003). Moreover, a 3 log decrease in microbial load was observed in response to heat treatment (Alegria et al. 2009, 2010).

In fresh-cut carrot, heat as an alternative decontamination treatment demonstrated high efficacy when compared to chlorinated water treatments (Alegria et al. 2009; Klaiber et al. 2005). Partial inhibition of quality-related enzymes in minimally processed vegetables, such as polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL) (Tomás-Barberán and Espín 2001; Saltveit 2000), and particularly peroxidase (POD), is also one of the beneficial effects of heat treatments. The partial inhibition of POD induced by heat was related to color maintenance during storage of minimally processed carrot (Howard et al. 1994). Also, reduction of respiratory activity (Rico et al. 2008; Serrano et al. 2004) is another favorable effect induced by heat treatments with significant impact over the fresh-like quality and, consequently, extension of its shelf life. Klaiber et al. (2005) found that during storage (9 days, 4 °C), heat-treated shredded carrot (50 °C/120 s) observed a continuous microbial development (up to 5 log 10 CFU g^{-1}) with similar counts in the chlorinated samples (200 ppm/120 s).

Heat shock is a method which usually implies a washing step at a temperature ranging 45–70 °C for a few minutes, usually less than 5 min (Rico et al. 2007b). Also this treatment appears to be very useful as a quality preservation agent for fresh-cut produce by preventing quality deterioration and helping to maintain texture and color qualities longer (Lamikanra and Watson 2007).

Heat shock treatments, alone or combined with other agents (calcium, chlorine), have also been used to prevent browning reactions and maintain texture in various vegetables and fruits (Hisaminato et al. 2001; Loaiza-Velarde et al. 1997). Heat treatment results in tissue firming in potatoes (Bartolome and Hoff 1972) and tomatoes (Floros et al. 1992). Firming effects obtained from heat treatments alone or combined with calcium treatments have been attributed to the action of heat-activated

pectin methylesterase (PME) and/or to increased calcium diffusion into tissues at higher temperatures (Garcia et al. 1996). In a study conducted by Martin-Diana et al. (2006a), fresh-cut lettuce was treated with 120 ppm chlorine and with 15 g L^{-1} calcium lactate at room temperature (18–20 °C) and at 50 °C (heat shock). Texturometer analysis showed that samples washed with calcium lactate had significantly ($p > 0.05$) higher crispness values than samples washed with chlorine. However, the use of 50 °C treatment (heat shock) gave better textural properties at the end of storage and significantly retarded the softening process, being in agreement with the sensorial results. The combination of calcium lactate and 50 °C washing temperature maintained objective and sensorial textural properties of fresh-cut lettuce better than the calcium lactate or chlorine washing treatments at room temperature.

Previously, it had been shown that hot air (Costa et al. 2005; Funamoto et al. 2002) can delay senescence in intact broccoli heads stored at 20 or 15 °C. However, senescence physiology could be completely different in fresh-cut broccoli due to the intense damage caused by cutting. A postharvest treatment with hot air (48 °C during 3 h) was applied to fresh-cut broccoli to investigate its effect on quality and senescence during storage at 0 °C (Lemoine et al. 2009). The treatment delayed yellowing as evidenced by lower decrease of hue values during storage. After 21 days of storage, treated broccoli had chlorophyll content approximately 40% higher than controls. Finally, after 3 weeks of storage, treated samples had higher levels of total sugars and total and soluble proteins. The results suggest that a short postharvest heat treatment may reduce senescence and tissue damage and contribute to maintain a better quality of the product during storage at 0 °C.

The effect of a precut heat treatment (100 °C/45 s) as an alternative decontamination treatment to chlorinated water (200 ppm active chlorine/1 min, 5 °C) was evaluated in minimally processed carrot (shredded). The use of heat in precut carrot proved to be more efficient than chlorinated water concerning microbial control (threshold concentration of 7 log 10 CFU g^{-1}), providing an acceptable fresh-like quality product during 10 days of storage (5 °C), which corresponds to a 3-day shelf life extension compared to control samples. Heat-treated shredded carrot showed lower respiratory and peroxidase (POD) activities than chlorinated samples suggesting that the use of heat provides a metabolic activity lowering effect besides the microbial effect which could be important to shelf life extension of the fresh-cut product (Alegria et al. 2010).

Previous analysis showed that short-time blanching (steaming) can be used as an alternative to chlorine (100 mg kg^{-1}) in sanitizing fresh-cut lettuce (Martin-Diana et al. 2006a). The use of steam produced a shocking effect on lettuce metabolism, reducing respiration of the product and causing partial inactivation of browning-related enzymes, thereby preventing browning apparition. Microbial results (mesophilic load) showed no differences between chlorine and steam and an improvement over water washing alone (Martin-Diana et al. 2006a).

Optimization of short-time blanching (steaming) was investigated by Rico et al. (2008), using response surface methodology by analyzing quality and microbial and nutritional markers over the shelf life of packaged fresh-cut lettuce. As a result, steamer treatment time (5–10 s) and storage (1–10 days) were used as independent

factors in order to optimize the process. Steamer treatment of 10 s could be considered the optimum time for maintaining the shelf life (mainly texture and browning) of fresh-cut lettuce for 7–10 days in optimum conditions (Rico et al. 2008). Time of exposure to steam is a critical factor in the case of the lettuce due to the high surface/volume ratio of the leaves. For this reason, operating conditions should be designed to reduce microbial load and extend shelf life with minimal adverse effects. Over-blanching may result in undesirable loss of quality (loss of texture, color, nutrients, etc.).

8.7.2 Ionizing Radiation

Ionizing radiation is highly effective for inactivation of foodborne pathogens and parasites in various vegetables (Bidawid et al. 2000). Additionally, several researchers have shown that ionizing irradiation is a suitable method to control foodborne pathogens on fresh fruits and in fruit juices, fresh-cut vegetables or salads, sprouts, and seeds (Buchanan et al. 1998; Chervin and Boisseau 1994; Hagenmaier and Baker 1997, 1998). Farkas et al. (1997) reported that ionizing irradiation at 1 kGy reduced loads of bacteria, improved microbiological shelf life, and extended sensory quality of precut peppers and carrots. The application of ionizing irradiation to control spoilage microorganism increases shelf life of irradiated strawberries, lettuce, sweet onions, and carrots (Thayer and Rajkowski 1999). Gamma rays applied to fresh-cut carrots stored in microporous bags resulted in limited respiration increase and ethylene production and considered to increase shelf life of the fresh-cut carrots (Chervin et al. 1992). Chaudry et al. (2004) noted that minimally processed carrots may be treated with a 2 kGy dose of gamma radiation to keep the appearance and flavor quality acceptable and extend the shelf life up to 14 days at refrigerated temperature. Zhang et al. (2006) evaluated the effects of irradiation on microorganisms and physiological quality of fresh-cut lettuce during storage at 4 °C. They found that total bacterial counts on fresh-cut lettuce irradiated with 1.0 kGy were reduced by the order of 2.35 log CFU g^{-1}, and the total coliform group was lowered to less than 30 MPN (most probable number)/100 g. The polyphenol oxidase activity of fresh-cut lettuce was significantly inhibited by irradiation. In addition, the loss of vitamin C of fresh-cut lettuce irradiated with 1.0 kGy was significantly lower than that of nonirradiated.

8.7.3 Ultraviolet-C (UV-C) Treatment

Nonionizing, artificial ultraviolet-C (UV-C) radiation is extensively used in a broad range of antimicrobial applications including disinfection of water, air, food preparation surfaces, and food containers (Wang et al. 2005) and surface disinfection of vegetable commodities (Erkan et al. 2001; Marquenie et al. 2002;

Gonzalez-Aguilar et al. 2007; Pombo et al. 2009). Treatment with ultraviolet energy could offer several advantages to fresh-cut fruit processors as it does not leave any residue and does not have legal restrictions and it is easy to use and lethal to a wide broad of microorganisms and does not require high economic investment and expensive safety equipment to be implemented (Rivera-Pastrana et al. 2007). UV is a nonionizing radiation with wavelengths from 100 to 400 nm, which is usually classified into three types: UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (100–280 nm). UV-C irradiation has its maximum at 254 nm and is, of the three, the one with the highest germicidal action and the most used for surface decontamination and control of microorganism growth in whole and fresh-cut products (Vicente et al. 2005). UV acts as an antimicrobial agent directly due to DNA damage (Gonzalez-Aguilar et al. 2010) and indirectly due to induction of a stress that simulates the production of phenylalanine ammonia-lyase (PAL), an enzyme that plays a key role in the synthesis of phytoalexins, phenolic compounds that improve the resistance of fruits to microorganisms (Charles et al. 2008, 2009).

It has been shown that UV-C can inactivate pathogenic microorganisms in vitro as well as inoculated onto vegetable surfaces. For example, *Salmonella typhi*, *Shigella sonnei*, and *Staphylococcus aureus* cultures (Chang et al. 1985) and *Salmonella* and *E. coli* O157:H7 inoculated onto agar surfaces (Yaun et al. 2003) and lettuce leaves (Yaun et al. 2004) have been inactivated by UV-C.

Mesophilic bacterial populations and yeast and fungal populations were higher in untreated slices of zucchini squash than in those exposed to 4.93 or 9.86 kJ m^{-2} at the end of storage for 22 and 14 days at 5 and 10 °C, respectively (Erkan et al. 2001). Minimally processed red oak leaf lettuce irradiated on one side with 0.41–8.14 kJ m^{-2} UV-C doses reduced the psychrotrophic population by about 0.5–2 log CFU g^{-1} from the beginning of the storage at 5 °C to almost the end of the study (9–10 days), resulting in a shelf life prolongation of 2 days or even longer when the highest fluences were used. Initial reductions in coliforms and yeast populations were kept during storage, but LAB populations were not affected by the treatments and grew to counts higher than those of controls during storage, although without reaching critical levels (Allende and Artés 2003). Treatment of minimally processed red oak leaf lettuce with up to 7.11 kJ m^{-2} UV-C on each side of the produce did not reduce total aerobic bacteria, facultative aerobic bacteria, and yeasts. Moreover, it resulted in about 1 log CFU g^{-1} reduction in lactic acid bacteria, but this group was not determinant for the shelf life of this minimally processed vegetable. The treatment slowed down the growth of total aerobic and facultative aerobic bacteria and yeasts, resulting in a shelf life extension of at least 2 days at 5 °C based on microbial growth (Allende et al. 2006b). Artes-Hernandez et al. (2010) evaluated the effects of four prepackaging UV-C illumination doses (1.6, 2.8, 4.8, and 7.2 kJ m^{-2}) on quality changes of watermelon cubes stored up to 11 days at 5 °C. Higher UV-C doses induced slightly higher CO_2 production throughout the storage period, while no changes in C_2H_4 production were monitored. UV-C decreased microbial counts just after illumination: after 11 days at 5 °C, mesophilic, psychrophilic, and enterobacteria populations were significantly lower in UV-C-treated watermelon. It has been observed that moderate UV-C doses (4.06 and 8.14 kJ m^{-2}) were effective in delaying senescence and deterioration of fresh-cut lettuce, although the surface of the product became shinier and firmer (Allende and

Artés 2003). As a main conclusion, UV-C radiation can be considered a promising tool for keeping overall quality of fresh-cut fruits. Combination of UV-C pretreatment with high CO_2 MAP deserves attention since it can extend reduction of microbial growth in several fresh-cut commodities (Artes-Hernandez et al. 2009).

8.7.4 Pulsed Light

Pulsed light (PL) is an emerging nonthermal technology for the rapid inactivation of pathogenic and spoilage microorganisms in foods. The significant microbial reductions in very short treatment times, the limited energy cost, the lack of residual compounds, and its great flexibility are some of the major benefits of the technique. PL involves the use of intense pulses of short duration and a broad spectrum to ensure microbial inactivation on the surface of either foods or packaging materials (Elmnasser et al. 2007). The main mechanism of microbial inactivation by PL is the photochemical effect, which includes structural changes in DNA of bacteria, viruses, and other pathogens, thus preventing cells from replicating (Takeshita et al. 2003). PL has the capacity to inactivate microorganisms in vitro (MacGregor et al. 1997; Rowan et al. 1999; Anderson et al. 2000; Roberts and Hope 2003), whereas its potential on foods is still under investigation. Experiments with carrot slices suggested that PL may reduce the loads of *Saccharomyces cerevisiae* inoculated on that product by about 3–4 log cycles (Kaack and Lyager 2007). According to Hoornstra et al. (2002), 2 log reductions in aerobic counts extended the shelf life of fresh-cut carrots by almost 4 additional days at 7 °C. However, the presence of off-odors seems to limit the shelf life of PL-treated shredded white cabbage stored under MAP at 7 °C for up to 9 days, whereas overall visual quality of PL-treated shredded lettuce was limited to 3 days (Gómez-López et al. 2005).

Oms-Oliu et al. (2010) investigated the impact of pulsed light treatments on microbial quality, enzymatic browning, texture, and antioxidant properties of fresh-cut mushrooms. The reduction of the native microflora of sliced mushrooms ranged from 0.6 to 2.2 log after 15 days of refrigerated storage by flashing at 4.8, 12, and 28 J cm^{-2}. Pulsed light treatments allowed extension of the microbiological shelf life of fresh-cut mushrooms by 2–3 days in the application of pulsed light at doses of 4.8 J cm^{-2}. Their results suggest that the application of pulsed light at doses of 4.8 J cm^{-2} could extend the shelf life of fresh-cut mushrooms without dramatically affecting texture and antioxidant properties.

8.8 Combination of Chemical and Physical Treatments for Microbial Control

Many combinations of physical and chemical treatments have been tested to determine if they showed enhanced antimicrobial action in recent years. Effective combined use of AlEW and AcEW with mild heat was reported by Koseki et al. (2004).

Conversely, AcEW combined with ozone was applied using sequential washes (Wang et al. 2004). Combination of UV-C pretreatment with high CO_2 deserves attention since it can extend reduction of microbial growth in several fresh-cut commodities (Artés-Hernández et al. 2009). However, there is scarce information about the influence of UV-C on microbial development and overall quality changes in fresh-cut watermelon and particularly on changes of some bioactive compounds such as lycopene, ascorbic acid, total antioxidant activity, and total polyphenol content throughout the shelf life. Previous reports indicate that 4.1 kJ m^{-2} was as efficient as 6.9 kJ m^{-2} for keeping microbial counts 1 log unit lower after 8 days at 3 °C when compared with chlorine and hydrogen peroxide (Fonseca and Rushing 2006). The effects of four prepackaging UV-C illumination doses (1.6, 2.8, 4.8, and 7.2 kJ m^{-2}) on quality changes of watermelon cubes stored up to 11 days at 5 °C were studied. UV-C decreased microbial counts just after illumination. After 11 days at 5 °C, mesophilic, psychrophilic, and enterobacteria populations were significantly lower in UV-C-treated watermelon. However, in some cases, high doses of UV-C may produce damage (Pan et al. 2004).

An alternative option is the advanced oxidation processes where two or more oxidants are used simultaneously. The most common process used to generate ˙OH is through the use of combined catalytic oxidants such as ozone–ultraviolet (O_3–UV), hydrogen peroxide–ultraviolet (H_2O_2–UV), and hydrogen peroxide–ozone (H_2O_2–O_3) (Gottschalk et al. 2000). Although these processes can produce ˙OH, the O_3–UV combination provides the maximum yield of ˙OH per oxidant (Gottschalk et al. 2000). For this reason, the O_3–UV process has been attracting increasing research interest (Teo et al. 2002; Beltran et al. 2005; Selma et al. 2006, 2007). However, little information is currently available about the use of O_3–UV treatment for water disinfection in the food industry.

Selma et al. (2008) investigated the disinfection efficacy of ozone (O_3) and UV-C illumination (UV) and their combination (O_3–UV) for reducing microbial flora of fresh-cut onion, escarole, carrot, and spinach wash waters collected from the industry. Furthermore, they analyzed the influence of water physicochemical parameters on the decontamination efficacy and the effect of these technologies on physicochemical quality of wash water. The results showed that O_3, UV, and O_3–UV were effective disinfection treatments on vegetable wash water, with a maximum microbial reduction of 6.6 log CFU mL^{-1} after 60-min treatment with O_3–UV. However, maximum total microbial reductions achieved by UV and O_3 treatments after 60 min were 4.0 and 5.9 log CFU mL^{-1}, lower than by O_3–UV treatment. Furthermore, turbidity of wash water was reduced significantly by O_3 and O_3–UV treatments, while UV treatment did not affect the physicochemical quality of the water. Conclusions derived from this study illustrate that O_3 and O_3–UV are alternatives to other sanitizers used in the fresh-cut washing processes.

The combined use of several disinfectant agents has been widely reported in the last few years (Beltran et al. 2005; Ukuku et al. 2005; Uyttendaele et al. 2004). Combinations of lactic acid, chlorinated water, thyme essential oil solution, sodium lactate, citric acid, hydrogen peroxide, ozone, and peroxyacetic acid have previously been tested, and combinations of chemical disinfectants have generally been found to maintain better sensory and microbial quality of the product. Bari et al. (2005)

investigated the efficacy of nisin and pediocin treatments in combination with EDTA, citric acid, sodium lactate, potassium sorbate, and phytic acid for reducing *L. monocytogenes* on fresh-cut produce. Yuk et al. (2007) showed that ozone treatment produced reductions of *E. coli* O157:H7 and *L. monocytogenes* that were less than 1.0 and 0.5 log, respectively, on enoki mushroom. The efficacy was improved using combined application of 3 ppm ozone and 1% citric acid, which resulted in reductions of 2.26 and 1.32 log, respectively.

Combination of thermoultrasound (temperature, 50, 55, 60 °C; time, 10, 15, 20 min with the frequency of 40 KHz) and calcium propionate (concentration: 1, 2, 3%, w/v) treatment was applied to decontaminate *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) from fresh-cut celery (Kwak et al. 2011). As a result, the optimum treatment conditions were 60 °C thermoultrasound with 2% calcium propionate for 15 min (*E. coli* O157:H7) and 59 °C thermoultrasound with 2% calcium propionate for 17 min (*S. typhimurium*). The combined treatment of thermoultrasound and calcium propionate contributes to the effective inactivation (more than 5 log reduction) of *E. coli* O157:H7 and *S. typhimurium* on fresh-cut celery.

8.9 Direction of Future Research

1. Evaluating new genotypes of vegetables for use as fresh-cut
2. Developing new products especially for children and old people
3. Extending shelf life of fresh-cut vegetables
4. Evaluating cost–benefit ratio of fresh-cut produce
5. Developing new tools for monitoring product quality and microbial contamination during the handling along with meeting the traceability requirements
6. Identifying the cultivar-specific optimal harvest stage for different vegetables to be used for fresh-cut products to provide good flavor to the consumer and adequate shelf life
7. Evaluating effect of various procedures such as modified atmospheres, atmosphere modification, and ethylene on microbial contamination, flavor, texture, and appearance of fresh-cut vegetables
8. Developing new technologies for reducing microbial contamination and labor costs by automation of as many of the processing steps as possible without significant losses in yield or quality of the fresh-cut vegetables
9. Developing new value-added fresh-cut vegetables that appeal to various consumers

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Chapter 9
Fresh-Cut Fruits

Elif Çandır

Abbreviations

1-MCP	1-Methylcyclopropene
4-HR	4-Hexylresorcinol
AsA	Ascorbic Acid
Ca	Calcium
CA	Controlled Atmosphere
CI	Chilling Injury
CitA	Citric Acid
CMC	Carboxymethyl Cellulose
CO_2	Carbon Dioxide
DBD	Double Barrier Discharge
GSH	Glutathione
HW	Hot Water
iso-AsA	Isoascorbic Acid
MA	Modified Atmosphere
MAP	Modified Atmosphere Packaging
N_2	Nitrogen
N_2O	Nitrous Oxide
Na	Sodium
NAC	N-Acetyl-Cysteine
O_2	Oxygen
PE	Polyethylene
PET	Polyethylene Terephthalate
PP	Polypropylene

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PPO	Polyphenol Oxidase
PVC	Polyvinyl Chloride
RH	Relative Humidity
SSC	Soluble Solid Content
US	United States
UV-C	Ultraviolet-C

9.1 Introduction

Fresh-cut fruit products for both retail and food service applications have increasingly appeared in the market place recently, but fresh-cut vegetables, bagged salad in particular, still dominate the production of minimally processed foods. Fresh-cut fruit market share accounts about 29% and 10% of total value added or fresh-cut fruit and vegetable market in the United States (US) and Europe, respectively, while bagged salads hold about 49% and 50% of total US and European fresh-cut market volume (Rabobank 2011; Cook 2015). A number of consumer market research reports have predicted that demand for fresh-cut fruit products will continually increase, with food services establishments and school lunch programs being major customers (Rojas-Graü et al. 2011). According to Mayen and Marshall (2005), the emerging fresh-cut fruit sector will likely overshadow salad sales in the future because fresh-cut fruits are more attractive to young consumers and aging baby boomers and in general are more likely to be consumed as snack products. From the retail side, fresh-cut fruit on average has higher margins than bagged salads, which will result in ample space for display in the stores. US sales of fresh-cut fruits, fresh-cut vegetables and bagged salads in 2014 increased by 12%, 10.9% and 7.8%, respectively, compared to 2013 (Cook 2015). Mixed fruit, apples, pineapple and watermelon account for the largest dollar (>13%) and unit share (>12%) of fresh-cut fruit sales in US grocery retail, followed by cantaloupe, mango, mixed melon, berries and honeydew with about ≤5% of dollar and unit share (Cook 2015). Some fresh-cut fruits currently available in European and US supermarkets are given in Fig. 9.1. According to FAO (2010) report, fresh-cut fruits, in particular, have gained popularity in urban centers of Asian countries. Fresh-cut fruit market is growing very fast and makes up about 11% and 5% of total sales in Japan and Korea, respectively (Kim 2007). Fresh-cut vegetables had a larger market share than fresh-cut fruits in Thailand (Sa-nguanpuag et al. 2007).

Fresh-cut fruits undergo abrasion, peeling, slicing or chopping before placing in modified atmosphere (MA) packages. Most fruit are very susceptible to bruising and mechanical injury. Fresh-cut processing removes the fruit's natural cuticle or skin barrier to gas diffusion and microbial invasion. Large and usually damaged surface area from peeling and cutting often provokes increased respiration, ethylene production and enhanced susceptibility to water loss and a greater possibility of enzymatic and microbial deterioration (Watada et al. 1996; Beaulieu and Gorny 2004).

- Pineapple chunks, slices, pieces
- Sliced melon selection
- Mango chunks and pieces
- Pomegranate arils
- Apple segments
- Red grape
- Peeled citrus fruits segments
- Peach slices

- Apple slices + grapes
- Melon + grape
- Grape + kiwi + pineapple
- Fruit selection
- Mango + lime wedge
- Grape + melon
- Melon selection

- Classic salad
- Luxury fruit salad
- Tropical fruit salad
- Fruit salad
- Fresh fruit salad
- Rainbow fruit salad
- Mixed fruit salad
- Fruit cocktail

Fig. 9.1 Commonly marketed fresh-cut fruits (Garner 2006)

Respiration rates of sliced peaches, bananas and kiwifruits average about 65% higher than rates of the corresponding intact fruits at 0–10 °C (Watada et al. 1996). Increased ethylene production upon cutting and other mechanical damages were reported in muskmelon fruit slices and green banana slices (Watada et al. 1990). The effects of slicing on rate of respiration and ethylene production differ between climacteric and non-climacteric fruit. In a study by Rosen and Kader (1989), slicing increased respiration of strawberries by 50% at 2.5 °C, but had no effect on ethylene production at the low temperature, which is typical of non-climacteric fruit. With partially ripe pears, a climacteric fruit, slicing increased respiration by 30% and decreased ethylene production at 2.5 °C. Stage of ripeness at the time of processing may alter the physiological responses to cutting. Wounding induces ethylene production, particularly with climacteric fruit at the pre-climacteric stage, but not with those at the post-climacteric stage as reported with pears (Rosen and Kader 1989; Gorny et al. 2000) and cantaloupe (Luna-Guzmán et al. 1999) due to loss of tissue capacity to produce ethylene (Luna-Guzmán et al. 1999). Besides stages of ripeness, the piece size also affects the physiological response of the fresh-cut fruits. Cantaloupe cut into very small pieces had a large increase in ethylene production at different stages of ripeness, whereas large pieces were not different in their physiology from the intact fruit (Cantwell and Suslow 2002). These physiological changes accompanied by increases in rates of other biochemical reactions responsible for changes in color (cut surface browning or color loss), flavor, texture and nutritional quality (sugar, acid, vitamin content), as well as surface desiccation, decay result in shorter shelf life (Cantwell and Suslow 2002; Gorny et al. 2000; Beaulieu and Gorny 2004).

Potential post-cutting life at 2–5 °C varies from 2 to 9 days for strawberry slices, melon chunks, mango cubes, citrus segments, kiwi, peach and pear slices, grape berries and 10–14 days for apple wedges, pineapple chunks and pomegranate arils (Cantwell 2011). The limitation for the commercial marketing of fresh-cut fruit products is relatively short post-cutting life due to mostly excessive tissue softening

Table 9.1 Storage characteristics of fresh-cut fruits (Gorny 1997; Cantwell and Suslow 2002)

Fresh-cut fruits	Common quality defects (other than microbial growth)	Beneficial atmosphere at 0–5 °C	
		% O_2	% CO_2
Apple sliced	Browning	<1	–
Kiwifruit sliced	Juice leakage, texture loss	2–4	5–10
Cantaloupe cubed	Leakage, softening, glassiness (translucency)	3–5	5–15
Honeydew cubed	Leakage; softening; glassiness (translucency)	2–3	5–15
Orange sliced; sectioned	Juice leakage, off flavors	14–21	7–10
Peach sliced	Browning	1–2	5–12
Pear sliced	Browning	0.5	<10
Persimmon sliced	Glassiness (translucency), darkening	2	12
Pineapple cubed	Leakage; discoloration	3	10
Pomegranate arils	Color loss, juice leakage	21	15–20
Strawberry sliced; topped	Loss of texture, juice, color	1–2	5–10
Watermelon cubed	Leakage, softening	3–5	5–15

and cut surface browning (Table 9.1). Major factors affecting fresh-cut fruit quality are cultivar, pre-harvest cultural practices (Romig 1995), quality of the intact fruit and its maintenance between harvest and preparation of the fresh-cut products, the stage of ripeness (Gorny et al. 1998a) physiological status of the intact fruits, post-harvest handling and storage, processing technique, sanitation (Hurst 1995), packaging and temperature management during shipping and marketing (Beaulieu and Gorny 2004; Cantwell 2011). Processing of fresh-cut fruit faces challenges including being high cost and labor-intensive production, availability of fruit (domestic and offshore source, storage condition of whole fruit), managing stage of ripeness, perishability of cut fruit (softening, browning, microbial) and flavor quality (Cantwell 2011). In order to deliver fresh-like fruits with extended shelf life and at the same time maintain food safety, nutritional and sensory quality, it is essential to maintain sanitation during fresh-cut preparation, use proper semipermeable packaging films and store fresh-cut fruits at as low a temperature as possible. Low oxygen (O_2) and/ or elevated carbon dioxide (CO_2) environments generated by modified atmosphere packaging (MAP) of fresh-cut produce can extend produce shelf life by (1) slowing browning reactions at cut surfaces, (2) reducing the rates of product transpiration (water loss) and respiration, and (3) reducing ethylene biosynthesis and action (Gorny 1997). Storage characteristics and recommended atmospheres of fresh-cut fruits were presented in Table 9.1. A range of physical and chemical treatments and appropriate MAP types to overcome the difficulties in preserving their nutritional, sensory and fresh-like quality of fresh-cut fruits during prolonged periods while extending the microbial shelf life will be reviewed in detail in Sect. 9.2.

9.2 Commercially Important Fresh-Cut Fruits

9.2.1 Apple

Fresh-cut apple slices are desired as a convenient snack for general consumers, as a component in school lunch programs and in food service. Their quality was well maintained for at least 2–3 weeks at 5 °C (Toivonen et al. 2003; Saftner et al. 2005). The secondary browning caused by microbial attack (Toivonen et al. 2003) and cut-edge browning caused by enzymatic-browning reactions (Rupasinghe et al. 2005) were major limiting factors on shelf life of fresh-cut apples. Secondary browning occurs sometime after cutting, usually towards the expected limit of shelf life and is localized in nature, while cut-edge browning occurs within hours of cutting and tends to be diffused across most of the cut edge (Toivonen 2006).

The shelf life and quality of fresh-cut apples depend mainly on cultivar (Saftner et al. 2005), harvest time (Toivonen 2008) ripeness stage at cutting (Soliva-Fortuny et al. 2002a; Rojas-Graü et al. 2007a), storage condition and duration before slicing (Toivonen 2006), and processing and packaging technologies (Soliva-Fortuny et al. 2002a). Granny Smith and Fuji are often used commercially for production of apple slices (Saftner et al. 2005). Saftner et al. (2005) compared GoldRush and Pink Lady to Granny Smith and Fuji and reported that the acceptability of flavor, texture and overall eating quality of GoldRush slices was as good as that for Pink Lady and Fuji, whereas Granny Smith slices generally rated lower for overall eating quality than the other cultivars. Apple cultivars show differences in their propensity for cut-edge browning (Fig. 9.2a). Luo and Barbosa-Canovas (1997) investigated enzymatic browning rate fresh-cut apples of new cultivars (Braeburn, Criterion, Fuji, Gala and Jonagold) and compared with traditional cultivars (Golden Delicious and McIntosh). Subjected to dip treatment of ascorbic acid (AsA) and 4-hexylresorcinol (4-HR), Jonagold had the slowest browning rate, while Braeburn had the fastest rate among the new cultivars. Of the two traditional cultivars, McIntosh had the faster browning rate. Golden Delicious apple was found to be more suitable variety for minimal processing due its low browning potential, compared to Granny Smith and Scarlet

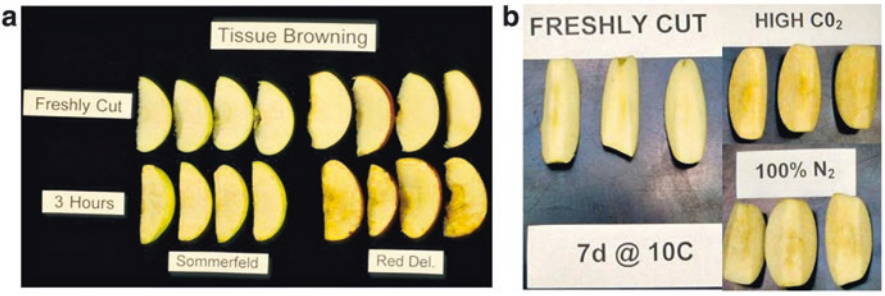

Fig. 9.2 (**a**) Genotypic differences in browning potential of apples (Cantwell 2011), (**b**) effects of 100% N_2 atmosphere on sliced apple browning (Gorny 2008)

Spur apple slices (Chiabrando and Giacalone 2012). Soliva-Fortuny et al. (2002a) showed a significant effect of ripeness stage at processing on the quality of apple slices. They reported that mature-green Golden delicious apple because of its lower respiration and ethanol formation was the most suitable to fresh-cut processing for extending post-cutting shelf life. Lightness of partially ripe apple slices after a dip in AsA and calcium (Ca) chloride solution and stored under the 100% nitrogen (N_2) atmosphere was slightly more efficiently preserved lightness than mature-green apples. On the other hand, ripe tissues of fresh-cut apples experienced greater changes in color and softening throughout time. Partially ripe Fuji apples were found to be the most suitable to prepare the fresh-cut due to their lower ethanol production and maintenance of their original color and firmness (Rojas-Graü et al. 2007a). Granny Smith apples harvested 2 weeks earlier than the optimal maturity had significant levels of cut-edge browning despite the post-cutting anti-browning dip and therefore should be avoided for use in fresh slicing (Toivonen 2008).

Controlled atmosphere (CA) storage suppressed the physiological activity of apples which become susceptible to accelerated deterioration upon cutting. Holding fruit for 2 weeks in air storage allowed recovery of physiological activity, which resulted in greater resistance to deterioration in response to fresh-cut processing. Fresh-cut slices from Spartan and Delicious apples held in post-CA air storage for 2 or 4 weeks showed the least changes in cut surface color as compared with those made from apples immediately on removal from CA (Toivonen et al. 2010). Tardelli et al. (2013) reported that post-CA air storage for 1 or 2 weeks prior to slicing reduced browning and had an overall positive effect on quality of fresh-cut apple slices. The reduction in browning occurred due to the suppression of both phenolic and o-quinones accumulation in treated slices.

1-Methylcyclopropene (1-MCP) treatment of freshly harvested whole apples before longer-term storage improved firmness and lightness retention of fresh-cut apples depending on cultivars (Perera et al. 2003; Bai et al. 2004; Calderon-Lopez et al. 2005), but did not affect the deterioration rate of apples slices (Calderon-Lopez et al. 2005). Firmness and acidity retention were consistently improved in Gala apple slices from apples treated with 1-MCP just before processing and dipped in the anti-browning solution containing Ca ascorbate, Ca propionate and N-acetyl-cysteine (NAC) during 19 days storage at 5.5 °C, although decay development was promoted on the cut surface by 1-MCP treatment (Bai et al. 2004). 1-MCP was found ineffective if 1-MCP treated apples air-stored for 3 months before cutting to prevent browning in Pacific Rose apple slices kept at 0 °C (Perera et al. 2003). The post-cutting treatment of Golden Delicious apple slices with 1-MCP had no significant effects on quality during storage of 10 days at 4 °C (Jiang and Joyce 2002).

Secondary browning is associated with the growth of soft-rotting microorganisms such as *Pseudomonas* spp. and *Enterobacteriacae*, yeasts and molds on the slices (Toivonen et al. 2003). Secondary browning occurs as a consequence of cross-contamination between apple slices in post-cut dip solutions (Toivonen and Delaquis 2006). Apple slices release a high level of organic load into dip tanks, along with microbial inoculum. The consequence is that sanitation chemicals are very quickly depleted in the sanitation wash and also inoculum accumulates in the

dip treatment tank (Toivonen et al. 2003). Spray application of anti-browning solution containing 7% of Ca ascorbate resulted in significantly higher reduction in the incidence and severity of microbiologically associated secondary browning as compared with dip application, but application of anti-browning solution at cutting does not control it to any great extent (Toivonen and Delaquis 2006). Addition of vanillin into the anti-browning solution resulted in a 50% reduction of the incidence of secondary browning (Toivonen and Delaquis 2006). Pre-cutting treatments of ethanol vapor (5 ml kg^{-1} for 24 h at 25 °C) and heat (4 days at 38 °C and >98% RH) were found successful to disinfest apples and control secondary browning (Bai et al. 2004). Secondary browning was also reduced for 2 weeks by post-CA air storage (Toivonen et al. 2010).

Cut surface browning occurred within hours of cutting limited shelf life of the cut apple to less than 3 days if not used any dip treatments (Rocha et al. 1998). A range of treatments have been applied to control cut-edge browning and extend the shelf life of fresh-cut apples (Table 9.2). Gil et al. (1998) reported that Fuji apple slices treated with AsA and held in an atmosphere of 100% N_2 at 10 °C had no significant browning and maintained visual quality for up to 15 days (Fig. 9.2a). However, AsA had temporary effect in controlling of enzymatic browning in fresh-cut apples (Rojas-Graü et al. 2008a). AsA, Ca chloride and/or citric acid (CitA) were shown to inhibit color deterioration and loss of firmness in apple cubes kept at 4 °C in air for 5–10 days (Rocha et al. 1998; Chiabrando and Giacalone 2012) and under 0% O_2 in plastic bags for at least 3 weeks (Soliva-Fortuny et al. 2005). Luo and Barbosa-Canovas (1996) found that browning inhibition using a mixture of AsA and 4-HR in vacuum packaged apple slices for up to 8 weeks during storage at 0.5 °C was comparable to that achieved by sulfites. Addition of sodium (Na) hexametaphosphate to an acidic browning inhibitor formulation containing AsA and CitA suppressed browning of Granny Smith and Fuji wedges for at least 3 weeks at 4 °C (Pilizota and Sapers 2004).

Treatments containing natural products such as 4-HR, isoascorbic acid (iso-AsA), NAC and Ca proprionate effectively inhibit browning and maintain quality of fresh-cut Delicious apple slices for up to 5 weeks at 5 °C with no apparent microbial growth (Buta et al. 1999). Oxalic acid, oxalacetic acid, ascorbic acid-2-phosphate, cysteine, glutathione (GSH), NAC, kojic acid and 4-HR showed the highest inhibitory activity on browning of apple slices (Son et al. 2001). Rojas-Graü et al. (2006) determined that 1-min dip with 4-HR concentrations lower than 0.5%, NAC concentrations higher than 0.75% and the NAC and GSH combination in concentrations higher than 0.60% was the most effective treatments in preventing browning in Fuji apple slices stored for 14 days at 4 °C. In comparison to ascorbic acid, 4-HR and GSH, a post-cutting dip in NAC was more effective treatment to prevent cut surface browning by inhibited polyphenol oxidase (PPO) activity and preserve the initial appearance of Fuji apple slices in air for 14 days or under low O_2 and elevated CO_2 for more than 1 month at 4 °C (Rojas-Graü et al. 2007a; Rojas-Graü et al. 2008a). Use of malic acid in combination with anti-browning dip solution containing NAC, GSH and Ca lactate improved controlling apple browning, softening and reducing the populations of pathogenic microorganisms in Fuji apple pieces during

Table 9.2 Effective anti-browning dip treatments for fresh-cut apples slices

Cultivar	Treatment	Storage condition	References
Fuji	AsA (2%)	0% O_2 at 10 °C 15 days at 10 °C	Gil et al. (1998)
Johnagored	AsA (0.75%), Ca chloride (0.75%); 5 min	10 days; 4 °C	Rocha et al. (1998)
Delicious	4-HR (0.001 M), iso-AsA (0.5 M), Ca propionate (0.05 M), NAC (0.025 M); 30 s	5 weeks; 5 °C	Buta et al. (1999)
Granny Smith Fuji	Na hexametaphosphate (1%), AsA (2%), CitA (1%) at pH 2.9; 2 min	3 weeks; 4 °C	Pilizota and Sapers (2004)
Golden Delicious	AsA(10 g l^{-1}), Ca chloride (5 g l^{-1}); 1 min	0% O_2 3–4 weeks; 4 °C	Soliva-Fortuny et al. (2005)
Empire Crispin	Ca ascorbate (6%); 3 min	21 days; 4 °C	Rupasinghe et al. (2005)
Fuji	NAC (1%); 1 min	Air for 14 days; 4 °C or 2.5% O_2 + 7% CO_2 > 30 days; 4 °C	Rojas-Graü et al. (2007a), (2008a)
Fuji	Malic acid (2.5%), NAC (1%), GSH (1%), Ca lactate (1%); 1 min	30 days; 5 °C	Raybaudi-Massilia et al. (2009a)
Bramley	Ca ascorbate (6%); 2 min	5 days ;2–4 °C	Rößle et al. (2009)
Braeburn	Ca ascorbate (6%); 2 min	Air or MA; 21–28 days; 4 °C	Aguayo et al. (2010)
Golden Delicious Scarlet Spur Granny Smith	Ca chloride (1%), CitA (1%), AsA (1%) ; 2 min	5 days at 4 °C	Chiabrando and Giacalone (2012)
Granny Smith	Na chloride (0.1 mol l^{-1}), Na fluoride (0.1 mol l^{-1}); 5 min	3 days at 4 °C	Li et al. (2015)

30 days storage at 5 °C (Raybaudi-Massilia et al. 2009a). Ca ascorbate (6%) was found to be effective in maintaining cut-surface firmness and inhibiting enzymatic browning for 21 days at 4 °C in Empire and Crispin (Rupasinghe et al. 2005), 5 days at 2–4 °C in Bramley (Rößle et al. 2009) and 21–28 days at 4 °C in Braeburn (Aguayo et al. 2010) apple slices stored in air or under MA conditions. Ca ascorbate treatment at higher concentrations than 6% and for longer dip times than 2 min resulted in significant residue levels in the wedges (Rößle et al. 2009).

Another approach used to avoid browning in apples has been the use of reducing agents in combination with application of edible coatings, hot water (HW), ultraviolet-C (UV-C), MA or CA and low temperature storage. A carboxymethyl cellulose (CMC)–based edible coating with the additives (antioxidants, acidulants and preservatives) has been demonstrated to control browning and microbial populations and prolong the shelf life of fresh-cut apple when stored in overwrapped trays by about 1 week at 4 °C (Baldwin et al. 1996). CMC-based coatings with Ca chloride (0.5%) and AsA (2%) maintained quality and reduced surface browning of fresh-cut

apples during refrigerated storage of 12 days (Saba and Sogvar 2016). Whey protein concentrate-beeswax-based coatings with AsA (1%) or L-cysteine (0.5%) were reported to be the most effective treatments in reducing browning of Golden Delicious apple slices during storage for 13 days at 5 °C (Perez-Gago et al. 2006). Alginate and gellan and apple puree-alginate-based edible coating incorporated with Ca chloride and NAC inhibited the growth of microorganism and maintained firmness and color in fresh-cut Fuji apples kept at 4 °C for 21–23 days storage (Rojas-Graü et al. 2007b, 2008b). The edible coating containing glycerol (1%), xanthan gum (0.5%), Ca chloride (1%), AsA (1%) and CitA (0.25%) resulted in an increase of firmness and a reduction of mass loss, oxidative browning and growth of microorganisms in fresh-cut Gala apples during refrigerated storage for 12 days (Freitas et al. 2013). Nano-emulsion-based edible coatings with lemongrass essential oil (0.1%) significantly slowed down microbial growth and reduced browning while maintaining firmness in fresh-cut Fuji apples during 2 weeks (Salvia-Trujillo et al. 2015). Browning index, firmness and acidity remained more stable throughout the 14 days at 2–4 °C when applied an edible coating (prebiotics of oligofructose and inulin) to fresh-cut Braeburn apple wedges, compared to the control (Rößle et al. 2011). Chitosan-coating in combination with anti-browning agents (2% AsA and 0.5% Ca chloride) effectively retarded enzymatic browning and tissue softening on Fuji apple slices during 8 days storage at 5 °C, but did not perform very well as water vapor barrier (Qi et al. 2011). Coatings with chitosan-based nanoparticles showed less browning and higher antimicrobial activity against microorganisms than conventional chitosan coating in Gala apple slices (Pilon et al. 2015).

The combination of HW treatment at 48 °C for 2 min followed by Ca ascorbate dip maintained quality, in particular, the sensory taste and aroma of fresh-cut Mahana Red apple packaged in bags and extended its shelf life up to 21 days at 4 °C (Aguayo et al. 2015). Dip solutions containing Ca ascorbate as the principal ingredient are currently used in fresh-cut apple production to inhibit enzymatic-browning reactions and to maintain quality and prolong shelf life. Ca ascorbate is a relatively expensive chemical and hence the washing solution is usually reused to reduce production costs. The reuse of Ca ascorbate solution, however, results in an accumulation of nutrients, microorganisms and even human pathogens, if present, which renders the washing solution a source of contamination. Acidic electrolyzed water followed by Ca ascorbate (Wang et al. 2007) or Na chlorite (Luo et al. 2011) was suggested as a promising treatment for the dual control of browning and bacteria growth in fresh-cut apples. Na halide salts has been shown effective in inhibiting cut-edge browning occurred within hours of cutting due to decreasing PPO activity for Granny Smith apple slices and suggested as a replacement for other anti-browning additives (Li et al. 2015).

UV-C light treatment (1.2 kJ m^{-2}) was demonstrated to be an effective treatment in apple slice surface decontamination (Manzocco et al. 2011a). These effects were attributed not only to the direct inactivation of spoilage microorganisms and enzymes by UV-C light but also to the formation of a thin, dried film on the surface of the product. This edible protective film inhibited microbial growth and prevented dehydration during storage but was too thin to be perceived by consumers. The use

of a gellan-gum-based coating incorporating apple fiber followed by the application of a pulsed light (1.2 kJ m^{-2} of UV-C light) significantly reduced softening and browning of Golden Delicious apple pieces without dramatically affecting its fresh-like quality attributes through 14 days storage (Moreira et al. 2015). UV-C treatment with or without CitA significantly decreased the occurrence of browning reactions and reduced the weight loss and microbial growth in fresh-cut Fuji apple slices during 15 days of storage at 5 °C (Chen et al. 2016).

Browning of fresh-cut Delicious apples was reduced during storage by high CO_2 levels of 15–30% CO_2 to only a limited extent, although elevated CO_2 reduced accumulation of fermentation products (Gunes et al. 2001). CA (2% O_2 + 4 to 12% CO_2) storage was found to be advantageous over air storage in terms of overall preservation of fresh-cut Jonagored apple for 3 days (Rocha and Morais 2000). CA-stored apple cubes were firmer, showed better color and higher fructose content and soluble solid content (SSC) than air-stored cubes. MAP with a 0% O_2 of initial atmosphere was very effective in preserving the initial color and firmness of Golden Delicious apple cubes dipped in AsA and Ca chloride for at least 3 weeks of refrigerated storage (Soliva-Fortuny et al. 2005). Low O_2 and elevated CO_2 (2.5% O_2 + 7% CO_2) atmosphere into polypropylene (PP) trays extended the shelf life of Fuji apple slices during refrigerated storage because of a significant inhibition of ethylene production (Rojas-Graü et al. 2007a). The initial appearance of Fuji apple slices immersed in anti-browning solutions (2% AsA, 2% CitA and 1% Ca chloride) for 15 min was retained and the browning index was prevented by nano-packaging synthesized by coating of nano-zinc oxide powder on polyvinyl chloride (PVC) film during storage for 12 days at 4 °C (Li et al. 2011).

9.2.2 Kiwifruit

Kiwifruit is commercially important as fresh-cut fruit (Antunes et al. 2010) and exhibit excessive tissue softening indicated as the major quality loss when peeled and/or sliced (O'Connor-Shaw et al. 1994; Agar et al. 1999) even at low temperatures storage (Gil et al. 2006). Kiwifruit slices do not store well because of their susceptibility to infections by gray mold, blue mold and *Phomopsis* spp. (Wang and Buta 2003). No visible cut surface browning was observed in stored kiwifruit slices (O'Connor-Shaw et al. 1994; Agar et al. 1999), but the cut surface darkening occurred was due to induction of a translucent water-soaked tissue and not to enzymatic browning due to low tannin content, low PPO and high AsA content of kiwifruit (Agar et al. 1999). Kiwifruit slices also developed bitter flavors (O'Connor-Shaw et al. 1994) and showed a significant loss in vitamin C (Agar et al. 1999; Gil et al. 2006) during refrigerated storage. Loss of vitamin C were around 8%, 12–13% and 21% after 6 days at 0, 5 and 10 °C, respectively, compared to initial values in kiwifruit slices (Agar et al. 1999; Gil et al. 2006).

Storage temperature and atmosphere, degree of tissue damage (Agar et al. 1999) and stage of ripeness at cutting (Tappi et al. 2013) were important factors for quality

retention of fresh-cut kiwifruit slices. The shelf life of slices held at 0–2 °C was longer compared to slices at 5 or 10 °C (Agar et al. 1999). The post-cutting life based on visual appearance was 2 days at 4 °C and shorter than 6 days at 5 °C for fresh-cut kiwifruit slices (O'Connor-Shaw et al. 1994; Gil et al. 2006). Light exposure during storage has been shown to promote a decrease in the content of vitamin C in kiwifruit slices (Gil et al. 2006). Physical damage or wounding caused by slicing and/or peeling resulted in increased CO_2 and ethylene production and caused higher mass loss of kiwifruit slices during storage (Agar et al. 1999). Raw kiwifruits with a high firmness and low SSC were used in the fresh-cut industry in order to perform the mechanical operations, but resulted in an inadequate level of ripening for consumption (Tappi et al. 2013). The intensity of the wounded response and effectiveness of pre-cutting treatments in kiwifruit slices were affected by initial maturity state. Kiwifruit slices at a lower stage of ripening (11–12 °Brix) exhibited a better retention of their firmness and visual appearance during storage at 10 °C, while more mature fruits (13–15 °Brix) were more sensible to peeling and cutting, showing a faster softening process compared to partially ripe fruits (Tappi et al. 2013). Mild heat treatment to whole fruit to delay quality loss in fresh-cut kiwifruit was effective only if the fruit was at an early maturity stage (firm ripe) (Beirão-da-Costa et al. 2006).

Several treatments to maintain quality and extend the shelf life of kiwifruit slices include application of volatile compounds, Ca chloride, edible coating, double barrier discharge (DBD) cold plasma after cutting, packaging with passive or active MAP, application of 1-MCP before or after cutting, application of mild heat treatments and UV-C radiation to whole kiwifruit before processing. Fresh-cut kiwifruit slices retained firmness and AsA content with a shelf life of 9–12 days if treated with 1% Ca chloride or 2% Ca lactate, in combination with chlorine water (100 $\mu l \cdot l^{-1}$) dip and stored at 0–2 °C and >90% relative humidity (RH) in an ethylene-free atmosphere of 2–4% O_2 and/or 5–10% CO_2 (Agar et al. 1999).

Alginate-based coating reduced respiration, dehydration and visible mold growth of fresh-cut kiwifruit both in passive and active MAP, thus extending its shelf life to 12–13 days (Mastromatteo et al. 2011). *Aloe vera* (5%) coating improved the quality of kiwifruit slices packaged under passive MA and stored at 4 °C for 12 days by retaining color, tissue firmness and sensory attributes and reducing the microbial proliferation (Benítez et al. 2013, 2015). MA containing 90% nitrous oxide (N_2O), 5% O_2 and 5% CO_2 was suggested as the best mixture of gases regarding color retention, firmness and SSC of sliced kiwifruit (Rocculi et al. 2005). Kiwifruit slices exposed to volatile compounds (11.2 or 22.4 ml l^{-1} methyl jasmonate) maintained higher levels of sugars and organic acids without fungal decay after 3 weeks at 10 °C (Wang and Buta 2003). Ramazzina et al. (2015) reported that DBD cold plasma treatment positively influenced the quality maintenance of kiwifruit slices by improving color retention and reducing the darkened area and not inducing any textural change during storage for 4 days at 10 °C (Fig. 9.3).

1-MCP treatment (1 $\mu l\ l^{-1}$ for 10 h at 20 °C) prior to cutting showed beneficial effects on reducing wound responses and delaying softening and color change in fresh-cut kiwifruit (Mao et al. 2007). 1-MCP application (1 $\mu l\ l^{-1}$ for 6 h at 10 °C)

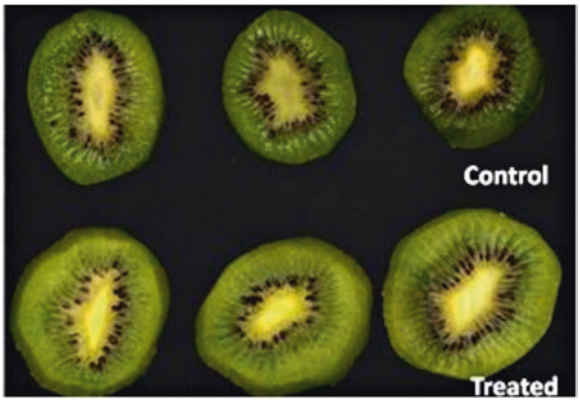

Fig. 9.3 Control and treated kiwifruit slices with DBD gas plasma after 4 days of storage (Ramazzina et al. 2015)

either before or after processing reduced ethylene production and softening, but did not affect the color of fresh-cut Hayward kiwifruit slices during storage for 7 days at 5 °C (Vilas-Boas and Kader 2007). A 2-min dip in Ca chloride (1%) synergistically increased the effect of 1-MCP on firmness retention (Vilas-Boas and Kader 2007). However, the application of 1-MCP to whole kiwifruit had no significant affect quality and nutritional properties through 8-day shelf life at 2 °C in fresh-cut kiwifruit prepared from fruit previously stored for 3 months after 1-MCP treatment (Antunes et al. 2010). Mild heat pre-treatments of intact firm ripe kiwifruit at temperatures below 45 °C during less than 25 min improve the quality, mainly the firmness, during storage for 9–10 days at 4 °C (Beirão-da-Costa et al. 2006, 2008). UV-C treatment (1 kJ m^{-2}) before cutting was found to be effective in sanitizing and preserving overall quality of fresh-cut kiwifruit slices since UV-C-treated kiwifruit slices showed improved firmness with lower existence of water-soaked tissues and microbial load below the legal or recommended limits during storage for 6 days at 4 °C(Beirão-da-Costa et al. 2014). The combination of ultrasound treatment (40 KHz) to whole fruit followed by nano-zinc oxide coating (1.2 g l^{-1}) of slices reduced production of ethylene and CO_2, water loss and softening of fresh-cut kiwifruit slices kept at 4 °C for 10 days (Meng et al. 2014).

9.2.3 Mango

Post-cutting life of fresh-cut mango was 8–10 days at 5 °C and limited by brown discoloration (Limbanyen et al. 1998; Beaulieu and Lea 2003; Poubol and Izumi 2005a; DeSouza et al. 2006), development of water-soaked appearance (Rattanapanone et al. 2001; Poubol and Izumi 2005a; DeSouza et al. 2006), loss of firmness (Limbanyen et al. 1998; DeSouza et al. 2006) and aroma (Beaulieu and Lea 2003), surface desiccation (Beaulieu and Lea 2003; DeSouza et al. 2006) and microbial growth (Rattanapanone et al. 2001). Beaulieu and Lea (2003) indicated

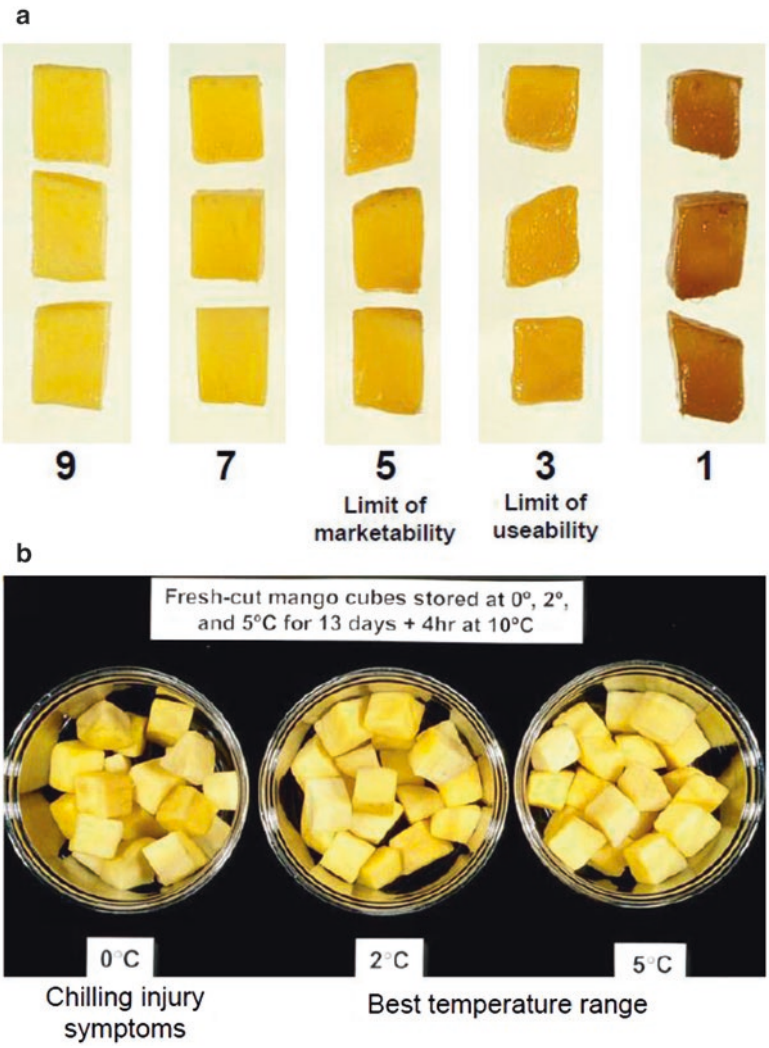

Fig. 9.4 (**a**) Overall visual quality evaluation, (**b**) effects of temperature on the quality of fresh-cut mango (Kader 2008)

that desiccation (dried cut surface) and edge or tissue damage (cut edge damage or brown and bruising-like appearance) were major limitations to fresh-cut mango quality. The 9-point rating scale was developed for overall visual quality evaluation of fresh-cut mango (Fig. 9.4a). Fresh-cut mangoes visually spoil before any significant nutrient losses occur since no significant losses in total carotenoids, vitamin C and total phenolics content were noted until day 9 at 5 °C (Gil et al. 2006). The optimum storage temperature range was found to be 2–5 °C for fresh-cut mango cubes (Fig. 9.4b). Storage at 0 °C for more than 10 days caused chilling injury (CI)

symptoms appeared as surface darkening (Kader 2008). Post-cutting life of fresh-cut mango at 5 °C was 8–10 days with no evidence of CI (Limbanyen et al. 1998). Izumi et al. (2003) recommended 5 °C as the best temperature for maintaining quality of partially ripe fresh-cut Carabao mango cubes for 4–6 days. Fresh-cut mango slices had longer shelf life when stored at 5 °C than at 12 °C despite the possibility of CI, because more rapid visual and textural deterioration and off-odor development occurred at the higher temperature were more objectionable than the relatively minor negative changes that occurred at the lower temperature (Dea et al. 2010a). The shelf life of fresh-cut mango slices prepared from different cultivars was 10 days at 3 °C for Tommy Atkins (DeSouza et al. 2005), 6 days at 5 °C or 4 days at 13 °C for Carabao (Poubol and Izumi 2005a), 2 days at 5 °C or 1 day at 13 °C for Nam Dokmai and 3–4 days at 12 °C or 5–6 days at 5 °C for Kent mangoes (Dea et al. 2010a).

Quality of fresh-cut mangoes depends upon cultivars, maturity and quality of the intact mangoes at harvest, maintaining quality until preparation, ripeness stage when cut, method of preparation (sharpness of the cutting tools, size and surface area of the cut pieces, washing and removal of surface moisture), subsequent handling procedures (packaging, speed of cooling, maintaining optimum ranges of temperature and relative humidity, expedited marketing and proper sanitation procedures) and time between harvest and consumption (Kader 2008). Kent and Keitt cultivars were the most preferred cultivars by nearly all the processors because of better availability of large sizes and consistency of good flavor when cut at the firm-ripe stage to facilitate processing and reduce mechanical damage during processing (Kader 2008). Some processors indicated that Ataulfo is requested by some of their customers because of its superior flavor when at optimal ripeness stage (dark-yellow to orange flesh color). The shelf life of fresh-cut Ataulfo mangoes was 21 days while that of fresh-cut Keitt and Kent mangoes was only 9 and 12 days, respectively, at 5 °C (González-Aguilar et al. 2008). This was attributed to better response of Ataulfo than Keitt and Kent mangoes to treatment with antioxidants. Haden and Tommy Atkins mangoes were not used or preferred for fresh-cut processing due to lack of large sizes and higher fiber content and inconsistency of flavor when ripe (Kader 2008). Kent mango was found to be more suitable than Tommy Atkins for fresh-cut processing in terms of less tissue browning and higher consumer liking (Ngamchuachit et al. 2014).

Tommy Atkins, Haden and Palmer mangoes with yellow flesh color (no green color remaining) were reported to have optimum maturity for fresh-cut in terms of maintenance of acceptable appearance, texture and taste while riper fruit developed flesh breakdown and more browning (Limbanyen et al. 1998). Allong et al. (2000) found that fresh-cut slices made from half-ripe (12.5–14% SSC) and firm-ripe (14.5–17% SSC) Julie and Graham mangoes had a shelf life of 8 days at 5 °C or 4 days at 10 °C. They concluded that half-ripe (13–16% SSC) mangoes are ideal for fresh-cut purposes in terms of maintenance of acceptable appearance, texture and taste during post-cutting life at 5 °C. Rattanapanone et al. (2001) recommended that Tommy Atkins and Kent mangoes should be at 13–27 N of firmness when cut to have an acceptable quality and reasonable shelf life as a fresh-cut product. Beaulieu and Lea (2003) compared volatile and quality changes in stored fresh-cut mango cubes prepared from firm-ripe (86–92 N of flesh firmness and 9–10% SSC) and

soft-ripe (27–29 N and 12.5–14% SSC) Keitt and Palmer mangoes. They found that soft-ripe cubes were unmarketable by day 7 at 4 °C and that firm-ripe cubes were not ripe enough to deliver an optimum product to consumers, even though their storage life was greater than soft-ripe cubes. DeSouza et al. (2005) reported that fresh-cut Tommy Atkins mangoes prepared from naturally ripened mango presented the best flavor and consumer preference with a commercial shelf life of 13 days at 3 °C, while the shelf life of those from mature-green mangoes that were ripened with ethylene for 12 h at 25–30 °C before cutting was limited to 11 days due to browning in the external surface. The initial ripeness stage of 35 N for Kent mango and 25 N for Tommy Atkins mango was reported as optimal ripeness stage for fresh-cut mango in terms of handling, visual quality and quality maintenance during storage and was also well received by consumers (Ngamchuachit et al. 2015). According to Kader (2008), most processors prefer to receive mangoes at the ready-to-cut (almost ready-to-eat) ripeness stage (flesh firmness of 13 to 27 N and 12–14% of SSC) since they do not have the facilities to ripen fruits. Therefore, mangoes must be ripened, at least partially (almost ready-to-eat), before cutting to assure better flavor quality in the fresh-cut products.

Proper sanitation and good temperature and humidity management may allow a shelf life of 5–7 days for fresh-cut mangoes prepared from firm-ripe mangoes (Kader 2008). Narciso and Plotto (2005) pointed out that the method of whole fruit sanitation plays a role in determining the cleanliness of the cut fruit. Use of peroxyacetic acid (100 $\mu l \cdot l^{-1}$) to sanitize whole Keitt mangoes followed by a 30 s dip of cut slices in peroxyacetic acid (50 $\mu l \cdot l^{-1}$) or acidified Na hypochlorite (200 $\mu l \cdot l^{-1}$) effectively reduced microbial growth and kept microbial counts low on cut fruit surfaces for 21 days. Ngarmsak et al. (2005) reported that washing whole Chok Anun mangoes in hot (50 °C) or cold (12 °C) chlorinated (100 $\mu l \cdot l^{-1}$) water for 5 min significantly reduced total microbial populations initially and after 7 days at 5 °C. HW quarantine treatment (dip in 46 °C water for 65–110 min) of whole mangoes did not significantly affect the quality of fresh-cut Kent mango slices stored at 5 °C (Dea et al. 2010b).

The respiration and ethylene production rates of mango cubes were only slightly higher than whole mangoes (Chantanawarangoon and Kader 2000). Therefore, wounding had a minor effect on physiology of fresh-cut mangoes, which is helpful in extending post-cutting life (Kader 2008). On the other hand wounding increases rates of water loss, softening and browning. Using very sharp tools to peel mangoes and cut their flesh limits cellular damage and reduces leakage of cellular contents and enzymatic browning mediated by the enzymes of PPO and phenol oxidase (Kader 2008). Allong et al. (2001) found that storage of fresh-cut Julie and Graham mangoes at 5 °C reduced the negative effects of wounding, including the level of microbial contamination. Gil et al. (2006) recommended complete removal of the mango skin (peel) with a very sharp knife or peeler to avoid brown discoloration of the remaining peel tissues, which appears faster than flesh tissue browning of fresh-cut mango products. High pressure processing (300 and 600 mPa for 1 min) led to decline typical flavor and increase off-flavor of fresh-cut mangoes during storage for 9 weeks at 3 °C, but color and other sensory attributes were affected very little

by high pressure processing (Boynton et al. 2002). High pressure also prevented increases in microbial load that were noted in the control.

The use of anti-browning, anti-softening chemical dips, edible coating and MAP extended the post-cutting life 9–12 days (Kader 2008). It is necessary to treat fresh-cut mangoes with antioxidants to prevent color darkening during storage (Plotto et al. 2004). Chantanawarangoon and Kader (2000) suggested to use the dip containing Ca chloride (1%) for maintaining firmness of fresh-cut mango cubes and extending their post-cutting life to about 9 days, compared with 5 days for untreated cubes. Chonhenchob et al. (2007) reported that the most effective chemical treatment was AsA (0.1 M) to reduce browning, softening and decay of fresh-cut Namdokami mangoes. Addition of AsA as an anti-browning agent to CitA and Ca chloride dip not only retarded quality loss of fresh-cut mango cubes but also promoted significant increases in antioxidant activity of fresh-cut Kent mangoes stored at 5 °C (Robles-Sánchez et al. 2009). The anti-browning dips with Ca ascorbate, CitA and NAC reduced firmness loss and maintained visual quality for fresh-cut Kent and Keitt mangoes during storage at 5 °C for 14–19 days (Plotto et al. 2010). Ngamchuachit et al. (2014) recommended a dip treatment at 10 °C in Ca chloride (0.136 M) for 2.5 min and 1 min for Tommy Atkins and Kent mango cubes, respectively, to retain firmness and consumer acceptance during 9 days of storage at 5 °C. The AsA, CitA and Ca chloride were found to be the best dip treatment for fresh-cut Tommy Atkins mangoes to maintain color and firmness during 12 days of storage at 4 °C (Siddiq et al. 2013).

Edible coatings including CMC, maltodextrin, chitosan, potato starch, whey protein and soybean oil emulsion, alginate and cassava starch have been studied to prevent surface desiccation and loss and flavor of fresh-cut mangoes. Plotto et al. (2004) found that coating with CMC (1%) and/or maltodextrin (0.5%) after a dip treatment with antioxidants solution (2% Ca ascorbate and 0.5% NAC) maintained the best visual quality of Tommy Atkins mango pieces for up to 21 days at 5 °C or 14 days at 10 °C, among the various coatings. CMC-based coating alone maintained visual quality similar to anti-browning dip treatments, but carrageenan or chitosan coatings decreased color values of Keitt and Kent slices kept at 5 °C (Plotto et al. 2010). Incorporating the anti-browning mixture into a CMC-based coating, or adding a carrageenan coating after the anti-browning dips, gave positive results in comparison to untreated control Keitt and Kent slices, but visual quality and firmness of cut slices was mostly maintained by the anti-browning dip. Tommy Atkins mango slices coated with cassava starch containing CitA had lower weight loss and maintained their mechanical properties and color parameters while those coated with Na alginate containing CitA presented lower browning during 15 days of storage at 5 °C (Chiumarelli et al. 2011). The combination of alginate coating and anti-browning agent (AsA and CitA) preserved the color of fresh-cut mangoes Kent stored at 4 °C and increased the antioxidant potential of cubes (Robles-Sánchez et al. 2013). Chitosan coating (0.25%) was not effective to maintain firmness and color, but inhibited microbial growth of fresh-cut Tommy Atkins mangoes during 9 days of storage at 6 °C (Djioua et al. 2010).

MA (10% O_2 + 10% CO_2) slowed browning and softening of fresh-cut mangoes as compared to air control (Limbanyen et al. 1998). Rattanapanone and Watada (2000)

concluded that fresh-cut Tommy Atkins mango cubes can be held in low O_2 atmospheres (0.5–4.0% O_2, balance N_2) at 5 °C. Marketability was limited by the development of watery condition and slight darkening in air and 4% O_2 atmosphere. Rattanapanone et al. (2001) reported that the marketable period of fresh-cut Tommy Atkins and Kent mango cubes was 3–5 days at 10 °C or 5–8 days at 5 °C and was extended by 1–2 days when cubes were held in 2–4% O_2 + 10% CO_2. They concluded that while CA was beneficial in maintaining quality of the cubes, temperature was more effective than CA. Temperature of 5 °C is recommended for holding mango cubes; however, CA with low O_2 (1–2% O_2, balance N_2) had an added beneficial effect in maintaining firmness surface lightness of fresh-cut cubes from partially ripe mango (Izumi et al. 2003). MA of 4% O_2 + 10% CO_2 resulted in longer shelf life (25 days at 5 °C) by inhibiting the growth of spoilage microorganisms, particularly molds and yeasts for the partially ripened Keitt mango cubes (11–12% of SSC) in comparison with vacuum packaging, 100% O_2 and air control (Martinez-Ferrer et al. 2002). Tommy Atkins mango slices packed in the polyethylene terephthalate (PET) clamshell trays had a shelf life of 14 days at 3 °C versus 11 days for the mango cubes in the other packages (Donadon and Durigan 2004). A 10% CO_2-enriched atmosphere enhanced texture and retarded the development of water-soaked Carabao cubes at 5 °C and 13 °C and reduced bacterial count on mango cubes held at 13 °C (Poubol and Izumi 2005a). Superatmosferic or high O_2 atmosphere (60%) accelerated browning of Carabao mango cubes kept at 13 °C (Poubol and Izumi 2005b). Low O_2 (2.5%) atmosphere was effective in controlling tissue darkening and the development of a glassy appearance (DeSouza et al. 2006). The combination of the dip treatment (1% Ca chloride, 1% AsA and 0.5% L-cysteine) with MA/CA (2% O_2 + 10% CO_2) was effective in maintaining the visual quality and reducing microbial growth on Haden, Keitt and Kent fresh-cut mango cubes for up to 12–17 days at 5 °C (Chantanawarangoon and Kader 2000; Kader 2008). The result of this study is presented in Fig. 9.5. González-Aguilar et al. (2000) found that combinations of anti-browning agents (0.001 M 4-HR, 0.05 M potassium sorbate and 0.5 M iso-AsA) and MAP reduced browning and deterioration of fresh-cut Kent mangoes stored at 10 °C for up to 14 days. Fresh-cut Tommy Atkins mango dipped in a solution of Ca chloride (3.5%) at 35 °C for 20 min and packaged under active MA (5% O_2 + 5% CO_2) maintained good quality for 5 days at 5 °C (Trindade et al. 2003). A combination a dip treatment with 3% Ca chloride and packaging under 2.5% O_2 atmosphere allowed Kensington mango slices to be held for at least 15 days at 3 °C (DeSouza et al. 2006). They reported that low O_2 was effective at controlling tissue darkening and the development of a glassy appearance and Ca chloride application significantly delayed tissue softening, while CO_2 (5–40%) and CitA application promoted tissue softening. González-Aguilar et al. (2008) reported that combinations of Ca chloride, antioxidants (AsA and CitA) and two commercial film coatings resulted in a reduction of browning and deterioration of fresh-cut Keitt, Kent and Ataulfo mangoes stored at 5 °C.

Applications of 1-MCP, heat or HW, ethanol and UV-C before or after slicing were investigated to maintain quality and extend shelf life of mango cubes. 1-MCP (25 $\mu l \cdot l^{-1}$) and heat treatments (38 °C and 98% RH for 12 or 24 h) of whole Kent

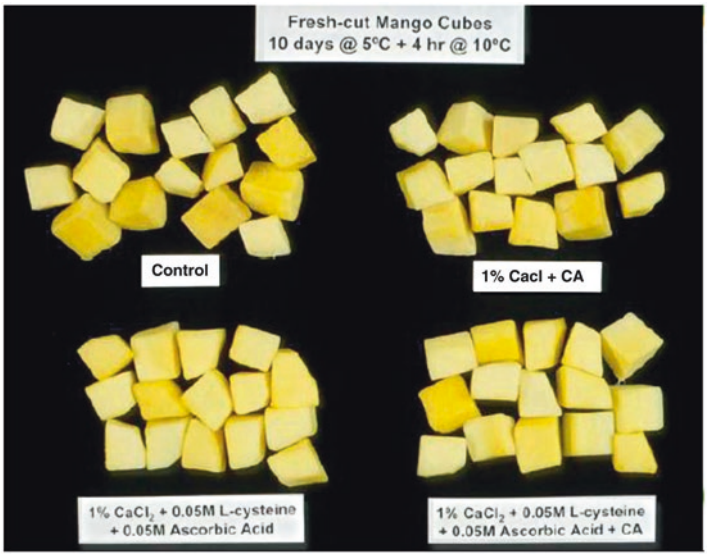

Fig. 9.5 Effects of chemical dips and CA (2% O_2 + 10% CO_2) on the quality of fresh-cut mango (Kader 2008)

mangoes decreased firmness of fresh-cut pieces, while the ethanol treatment (5 g kg^{-1}) maintained the best visual quality and firmness after 12 days at 7–8 °C, but resulted in off-flavor (Plotto et al. 2003). These ripening inhibition treatments did not influence shelf life of fresh-cut Tommy Atkins mangoes, but delayed spoilage of Kent mangoes by 2 days. Vilas-Boas and Kader (2007) found that softening and browning were delayed when 1-MCP (0.5 or 1.0 μl·l^{-1} for 6 h) was applied directly on fresh-cut Kent and Keitt mango slices. Respiration rate of mango slices was not affected by 1-MCP whereas ethylene production rate was affected only toward the end of 9 days at 5 °C. Treating whole mangoes before cutting was less effective than treating the cut product. Combination of 1-MCP treatment with Ca treatment and/or MAP results in synergistic effects on maintaining good appearance and textural quality. A HW dipping of whole fruit at 50 °C for 30 min maintained firmness and color of fresh-cut Keitt and Tommy Atkins mangoes for 6 to 9 days of storage at 6 °C (Djioua et al. 2009, 2010). Chitosan coating (0.25%) was less effective to maintain the firmness and the color, compared to HW dipping, but inhibited the microbial growth of fresh-cut Tommy Atkins during 9 days of storage at 6 °C (Djioua et al. 2010). González-Aguilar et al. (2007) reported that exposure of mango cubes to UV-C irradiation for 10 min appears to be a good technique to improve the total antioxidant capacity by increasing phenolics and flavonoid contents of fresh-cut Tommy Atkins mangoes stored for 15 days at 5 °C. However, this treatment reduced vitamin C and carotenoids contents. Pulsed light treatment with a total fluence of 8 J cm^{-2} maintained the firmness, the color and nutritional properties (phenol and total AsA contents) of fresh-cut Kent mangoes during storage for 7 days at 6 °C (Charles et al. 2013).

9.2.4 Melon

The main obstacles to marketing of fresh-cut melons are softening (Aguayo et al. 2008; Silveira et al. 2011) and development of translucency appearance (O'Connor-Shaw et al. 1994; Portela and Cantwell 2001; Bai et al. 2003; Aguayo et al. 2004), microbial growth (Aguayo et al. 2008), visible mold growth (O'Connor-Shaw et al. 1994), the loss of typical flavor (O'Connor-Shaw et al. 1994) or development of off-flavor (Bai et al. 2003). Translucent appearance (Portela and Cantwell 2001), a physiological disorder, also termed "glassiness" or "watercore" is characterized by the alteration of flesh texture to become dark and glassy (Aguayo et al. 2004). Translucent appearance has also been reported to be a symptom of senescence (Aguayo et al. 2003) produced by an advanced stage of maturity (Bai et al. 2001) and treatments that accelerate ripening (Aguayo et al. 2003) such as severity of wounding (Portela and Cantwell 2001; Aguayo et al. 2004), high temperatures of storage (Aguayo et al. 2004), prolonged storage time and unwashed cut melon (Ayhan et al. 1998). Biochemical parameters such as pH, titratable acidity, °Brix and organic and amino acids were not suggested to use as indicators of stored cut cantaloupe quality because they did not change significantly when fresh-cut melon was stored at 4 °C for a period of 2 weeks (Lamikanra et al. 2000). At this temperature, the microbial population also remains constant relative to amounts initially present at the time of processing for about 3–4 days (Lamikanra et al. 2000). However, rapid changes in the quality parameters such as texture, flavor and appearance occurred when the microbial growth increased on fresh-cut cantaloupe and honeydew melons (Ayhan et al. 1998).

Cantaloupe melon is used more than any other fruit in fresh-cut processing (Lamikanra et al. 2000) especially in the United States followed by honeydew melon, also a common component of fresh-cut fruit products (Portela and Cantwell 1998). In Europe, fresh-cut melons could be prepared from most commonly produced melon types Galia and European cantaloupe and Amarillo and Piel de Sapo (Aguayo et al. 2004). Fresh-cut melons may be processed as cylinders (Luna-Guzmán and Barrett 2000; Portela and Cantwell 2001), cubes (O'Connor-Shaw et al. 1996; Ayhan et al. 1998; Qi et al. 1999; Bai et al. 2001), slices (Lamikanra et al. 2000) or trapezoidal sections (Aguayo et al. 2004).

For fresh-cut melons, a 10-day shelf life is desirable in the distribution chain, but retail stores get an average shelf life of only 3 days (Luna-Guzmán et al. 1999). The post-cutting life of fresh-cut cantaloupe and honeydew melons without any treatment was found to be 4–9 days and 11–14 days, respectively, at 4–5 °C in a closed PP container (O'Connor-Shaw et al. 1994; Gil et al. 2006). Proper sanitation of melon before or after cutting prior to the MAP and the refrigerated storage can ensure a shelf life of 15 days for cantaloupe and honeydew pieces (Ayhan et al. 1998). Ayhan et al. (1998) recommended chlorine dip (200 $\mu l \cdot l^{-1}$ at 10–15 °C for 30 s) for cantaloupes and honeydews before and after cutting to reduce the microbial population. HW treatment (76 °C for 3 min) of whole fruit was found to be superior to chlorine dip (20 $\mu l \cdot l^{-1}$ at 10 °C for 20 min) in reducing microbial population of whole and fresh-cut cantaloupes kept at 4 °C for 13–20 days (Fan et al. 2008).

The combination of HW (75 °C for 1 min) and gaseous ozone (10,000 $\mu l \cdot l^{-1}$ for 30 min) applied to whole fruit was found effective in controlling microbial growth while maintaining full typical aroma, color and a very firm and turgid texture of fresh-cut cantaloupes after storage for 8 days at 5 °C (Selma et al. 2008a).

Maturity is an important quality attribute for the fresh-cut fruit because immature fruit lack good sensory quality and over-mature fruit has limited shelf life. For fresh-cut honeydew cubes, mature fruit (11% SSC) had acceptable eating quality at 10 °C, while immature honeydew cubes (8.8% SSC) failed to retain honeydew taste/aroma. Very mature honeydew cubes (13% SSC) had good taste/aroma, but deteriorated more rapidly than those with 11% SSC at 10 °C (Watada and Qi 1999). For fresh-cut cantaloupe cubes, a desirable sensorial attributes can be prepared with fruit when harvested at ≥½ slip, but not from ¼-slip fruit which was firmer, but had less intense fruity and sweet aromatic flavor than the other three maturities through 14 days storage of fresh-cut melons at 4 °C (Beaulieu et al. 2004). Green-mature stage of ripeness at processing was reported to be the most suitable to extend the shelf life of fresh-cut Piel de Sapo melon (Oms-Oliu et al. 2007). They observed the more advanced the fruit maturity at processing, the greater occurrence of translucency, higher the CO_2 production ethanol accumulation, microbiological counts in fresh-cut Piel de Sapo melon packaged under MA (2.5% O_2 + 7% CO_2) or non-MA throughout 35 days of storage at 4 °C.

Minimizing mechanical injury by using very sharp cutting blades during preparation of fresh-cut melon significantly reduced surface translucency and thus helped to maintain marketable visual quality for at least 6 days at 5 °C (Portela and Cantwell 2001). The most notable difference between the blunt and sharp-cut pieces was in color and appearance (Fig. 9.6a). Blunt-cut pieces had a darker orange color and a progressive appearance of translucency during storage after 10 days. The types of melon (Portela and Cantwell 1998; Aguayo et al. 2004), kind of cut (Aguayo et al. 2004) and storage temperature (Portela et al. 1997; Lamikanra et al. 2000; Aguayo et al. 2004; Bett-Garber et al. 2011) affected quality changes such as translucency, softening, off-flavor, microbial growth in fresh-cut melons. Initial firmness values of honeydew pieces were much higher than those of cantaloupe pieces, while loss of firmness was much greater in honeydew than cantaloupe pieces (Portela and Cantwell 1998). Processing as cylinders, high temperatures (10 °C) and some melon types (Cantaloupe, Galia and Piel de Sapo) increased translucency, while a cylindrical shape followed by trapezoidal sections, low temperatures (0 °C) and some melon types (for example, Cantaloupe, Piel de Sapo and Amarillo) decreased softness (Aguayo et al. 2004). Although whole cantaloupe suffered from CI below temperatures of 2–5 °C (Portela and Cantwell 2001), temperatures near 0 °C are considered ideal to minimize biochemical changes and to keep microbial growth as low as possible for fresh-cut cantaloupe (O'Connor-Shaw et al. 1994). Storage temperature was the most important factor for maintaining firmness of fresh-cut melons. After 9 days of storage, cantaloupe cylinders in air at 5 °C had firmness values similar to those in CA at 10 °C (Portela et al. 1997). Aguayo et al. (2004) stored fresh-cut melons at 0 °C and 5 °C and observed that softness, weight loss, translucency, wound stress and respiration rate were lower and sensorial quality was higher during

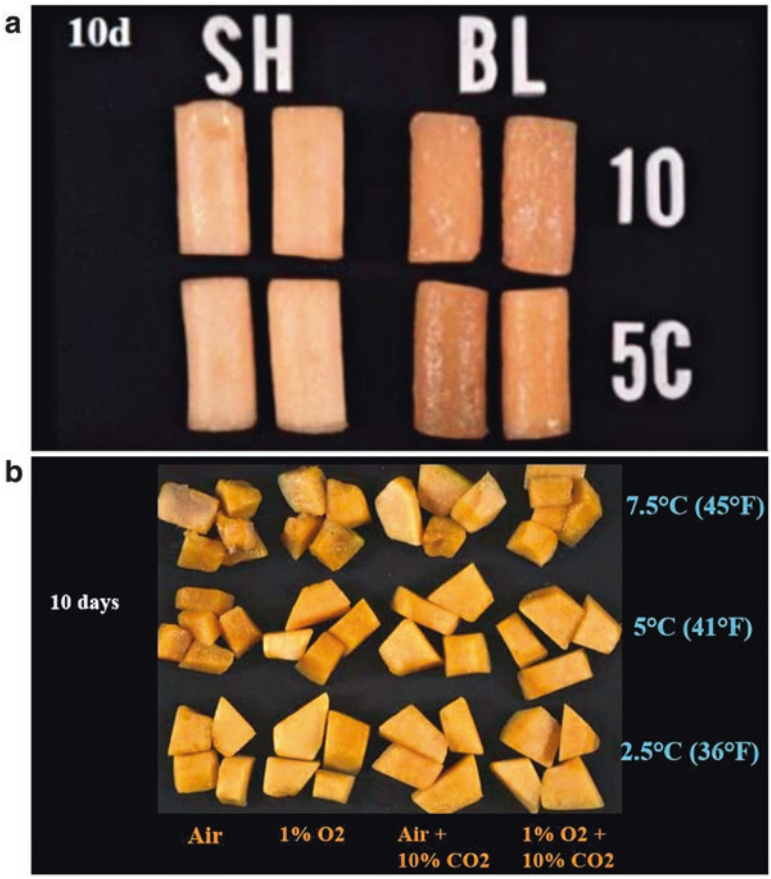

Fig. 9.6 (**a**) Translucent appearance of blunt and sharp-cut cantaloupe pieces, (**b**) relative importance of temperature and MA for fresh-cut melon (Cantwell 2011)

storage at 0 °C than at 5 °C. The fresh-cut cantaloupes maintained the fruity/melon aroma and flavor if held at a constant temperature of 4 °C or even 10 °C while developed off-flavor if held at 4 °C for 24–48 h then exposed to higher temperatures of 10 °C (Bett-Garber et al. 2011).

Fresh-cut cantaloupe shelf life may be extended by dipping in Ca salt solution alone or in combination with heat treatments, application of edible coating alone or incorporated with antimicrobial compounds, gaseous ozone, UV-C and packaging under MA. Fresh-cut melon is very susceptible to softening during storage, even under low temperature storage and MAP (Aguayo et al. 2008; Silveira et al. 2011). This softening process is related to Ca levels in fruit tissue (Silveira et al. 2011). Ca chloride (2.5%) dips for 1 min applied to melon cylinders taken from commercially ripe (3:4 to full slip) cantaloupe melons maintained or improved firmness, especially at higher dip temperatures (Luna-Guzmán et al. 1999). Luna-Guzmán and Barrett (2000) noticed

an undesirable bitterness flavor with cylinders of Cantaloupe dipped in Ca chloride (2.5%) at 25 and 60 °C, while cylinders of cantaloupe dipped for 1 min in Ca lactate (2.5%) maintained firmer tissue without bitterness (Luna-Guzmán and Barrett 2000). Saftner et al. (2003) doubled tissue Ca content and inhibited changes in firmness when fresh-cut honeydew pieces were dipped in Ca chloride (0.6%), Ca propionate (0.7%) or Ca amino chelate (1.5%). Dipping fresh-cut Amarillo melon in Ca salts (Ca chloride and Ca propionate and lactate) for 1 min at 60 °C, was very effective in reducing microbial growth and maintaining fruit firmness during 8 days storage at 5 °C, but Ca propionate led to whitish color to melon flesh and a slight off flavor (Aguayo et al. 2008). A dip treatment with 0.4% of Ca solution containing Ca chlorine, lactate and ascorbate (at 60 °C for 1 min) followed by hydrogen peroxide treatment (50 mg l^{-1} at 0 °C for 5 min) increased tissue total Ca content and maintained firmness, acceptable flavor and microbial quality in trapezoidal shaped sections of Galia melons kept at 5 °C for 10 days under a passive MA reaching 4.5% O_2 and 14.7% CO_2 (Silveira et al. 2011). Dip treatment with 1 mM of carvacrol or cinnamic acid delayed spoilage of fresh-cut honeydew melon for 3 days at 8 °C and 5 days at 4 °C without adverse sensory consequences (Roller and Seedhar 2002).

Edible coatings containing pectin or alginate prevented desiccation and best maintained fruit firmness of fresh-cut Piel de Sapo melon throughout storage, but the high economic cost of low methoxyl pectins for industrial applications may limit to its commercial use (Oms-Oliu et al. 2008a). Alginate-based edible coating maintained microbiological (up to 9.6 days) and physico-chemical (>14 days) quality of fresh-cut "Piel de Sapo" melon at 5 °C (Raybaudi-Massilia et al. 2009b). Edible coating incorporated with palmarosa essential oil (0.3%) prolonged the microbiological shelf life to more than 21 days by inhibiting the native flora growth and reducing *Salmonella* spp. population and maintained the fruit quality parameters with a good acceptation by panelists (Raybaudi-Massilia et al. 2009b). Application of a multilayered edible coating composed of trans-cinnamaldehyde (2%), chitosan (2%) and pectin (1%) maintained physico-chemical and sensory quality attributes of fresh-cut cantaloupe for longer period (7–9 days) than uncoated controls (4 days) at 4 °C (Martiñon et al. 2014). Edible coating of chitosan (2%) combined with Ca chloride (1%) treatment synergistically extends the shelf life of fresh-cut honeydew melon by reducing weight loss and microbial growth and by maintaining firmness during 13 days storage at 7 °C (Chong et al. 2015). The chitosan-based coating (2%) with antimicrobial trans-cinnamaldehyde (500 mg l^{-1}) maintained quality of fresh-cut cantaloupe melons during storage at 4 °C by preserving total vitamin C, carotenoid, lightness and firmness (Carvalho et al. 2016).

Fresh-cut cantaloupe treated with gaseous ozone (5000–20,000 $\mu l \cdot l^{-1}$ for 30 min at 11 °C) maintained an acceptable visual quality, aroma and firmness and reduced microbial load during 7 days of storage at 5 °C (Selma et al. 2008b). Application of gaseous ozone treatment and hazelnut oil to trapezoidal sections of fresh-cut Kırkağaç melon was found to be as effective as Na hypochlorite in reducing microbial populations while preserving the quality during cold storage for 9 days and 6 days, respectively (Dilmaçünal et al. 2014).

Cutting fruit under UV-C radiation (1.18 mJ cm^{-2}) reduced microbial populations and improved firmness retention of fresh-cut cantaloupe melon kept at 10 °C, while post-cut application of UV-C improved shelf life by reducing microbial growth, but not as effective as cutting fruit under UV-C radiation in maintaining firmness (Lamikanra et al. 2005). Post-cut UV-C irradiation (120 s, 0.04 kJ s^{-1} m^{-2}) treatment extended the shelf life of fresh-cut Galia melon by reducing tissue softening and color changes for 10 days at 5 °C (Chisari et al. 2011). UV-C treatment (20 W m^{-2}) was found effective in achieving surface decontamination, improving sensory properties and decreasing melon leakage, probably due to the formation of a thin dried film on the product surface, thus extending shelf life of fresh-cut cantaloupe cubes to 14 days at 6 °C (Manzocco et al. 2011b).

MA or CA with 2% O_2 + 10% CO_2 was suggested for fresh-cut honeydew cubes to maintain overall visual quality and retard microbial growth, if held at a constant temperature of 5 °C to avoid anaerobic respiration (Qi et al. 1999). Importance of temperature and MA for fresh-cut melon is presented in Fig. 9.6b. CA storage of honeydew pieces (air +15% CO_2) maintained overall visual quality of honeydew pieces for 12 days at 5 °C by reducing development of macroscopic decay, translucency and off-odors (Portela and Cantwell 1998). Cantaloupe cylinders stored in air or CA softened at similar rates at 5 °C, but at 10 °C, pieces stored in 15% CO_2, 1.5% O_2 and combinations of 3% O_2 with 7.5 or 15% CO_2 maintained firmness longer than pieces stored in air (Portela et al. 1997). Diced cantaloupe prepared with a very sterile system kept at 4.5 °C under CA (6% O_2 + 6% CO_2, 3.5% O_2 + 9.5% CO_2 or 6% O_2 + 15% CO_2) had an acceptable quality up to 28 days (O'Connor-Shaw et al. 1996). Honeydew and cantaloupe melon chunks packaged under an MA of 5% O_2 and 95% N_2 developed off-flavors after 10 days at 2.2 °C (Ayhan et al. 1998). MAP conditions with final gas composition of 4% O_2 + 12–13% CO_2 at 5 °C led to a maximum 10-day shelf life of fresh-cut Amarillo melon by maintaining sensorial quality and microbial safety and reducing weight loss and translucency (Aguayo et al. 2003). They recommended the use of a CitA dip before MAP to decrease the microbial count, avoid discoloration, increase lightness and improve visual appearance of the melon pieces. Fresh-cut cantaloupe and honeydew cubes packaged with active MAP packages (initially flushed with 4% O_2 and 10% CO_2 for cantaloupe and 5% O_2 and 5% CO_2 for honeydew) showed better color retention by reduced translucency, respiration rate and microbial population and retained salable quality for 9 days and 11 days, respectively, at 5 °C (Bai et al. 2001, 2003). Cantaloupe and honeydew cubes packaged with perforated MAP packages had a shelf life limited to 5–7 days and 9–10 days, respectively, at 5 °C due to tissue translucency and/or off-odor development (Bai et al. 2001, 2003). The use of dips, containing AsA and Ca chloride, combined to MAP (2.5% O_2 + 7% CO_2) have been shown to preserve fresh-cut melon from softening, thus extending the shelf life of green-mature fresh-cut Piel de Sapo melon to 10 days at 5 °C (Oms-Oliu et al. 2007). Low O_2 and CO_2 enriched atmospheres (2.5% O_2 + 7% CO_2) stimulated anaerobic metabolism of fresh-cut Piel de Sapo melon after 10–14 days at 4 °C, while high O_2 levels (70% O_2) delayed the deteriorative changes related to softening, off-odors and off-flavors in

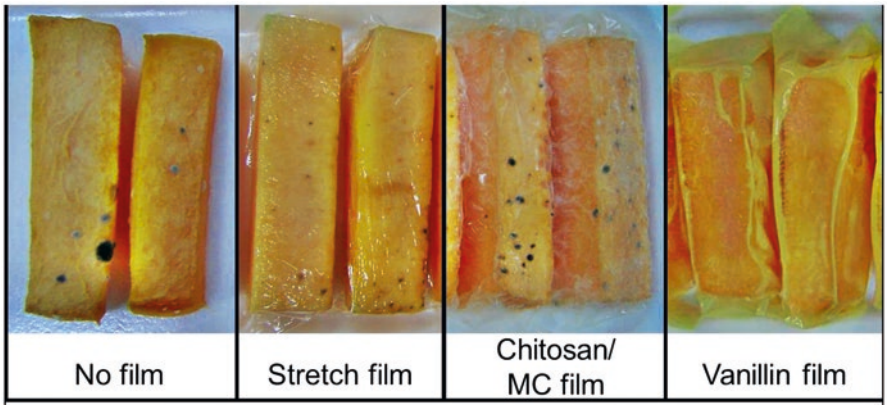

Fig. 9.7 Effects of antimicrobial biodegradable film on microbial and quality characteristics of fresh-cut melon (Sangsuwan et al. 2008)

those kept at 4 °C for 2 weeks (Oms-Oliu et al. 2008b). Application of cinnamic acid vapors (0.15 and 0.30 g l −1) through active MAP had a good antimicrobial effect, mainly against the mesophilic bacteria and allowed for better retention vitamin C and total phenolic content without affecting the odor acceptability in fresh-cut cantaloupe melon stored for 10 days at 5 °C (Silveira et al. 2015). However, Raybaudi-Massilia et al. (2009b) reported that aroma and taste characteristic of fresh-cut Piel de Sapo melon was significantly affected by cinnamon (0.7%) when incorporated to alginate-based edible coating, leading to a lower acceptance. Sangsuwan et al. (2008) showed that fresh-cut cantaloupe wedges wrapped with vanillin film had no or less mold growth, compared to those wrapped with stretch or chitosan/methylcellulose film and unwrapped fruit after 12 days at 10 °C (Fig. 9.7).

9.2.5 Peaches and Nectarines

The commercial success of fresh-cut peach and nectarine slices has been limited due to their short shelf life, because of cut surface browning and pit cavity breakdown (Gorny et al. 1999). Cultivar, fruit ripeness at cutting, storage temperature and atmosphere significantly affected the shelf life of peach and nectarine slices (Gorny et al. 1998a, 1999). Shelf life of fresh-cut peaches and of nectarines varied between 2 and 12 days at 0 °C. Among the peach cultivars, Cal Red, Red Cal and Elegant Lady slices had the longest shelf life of about 7 days, while Summer Lady and Ryan Sun slices had the shortest shelf life (<2 days) (Gorny et al. 1999). The clingstone non-melting peaches of Settembrina di Leonforte, Settembrina di Bivona (Allegra et al. 2015), Romea (González-Buesa et al. 2011) and nectarines of Early Top and Nectaprima (Nogales-Delgado et al. 2014) were found to be suitable for fresh-cut peach processing due to their lower degree of browning during 9–12 days at 4 or 5 °C in air or under MA. The 18–31 N of firmness was determined as the optimal

stage of ripeness for whole fruit to be processed into peach and nectarine slices held at 0 °C (Gorny et al. 1998a).

CA containing 2% O_2, 12% CO_2 in air and 2% O_2 + 12% CO_2 had no effect on quality attributes of sliced Fay Elberta peaches over 7 days of storage at 5 °C (Wright and Kader 1997a). Gorny et al. (1999) reported that CA of 0.25% O_2 and/or 10% or 20% CO_2 extended the shelf life at 10 °C of O'Henry or Elegant Lady peach slices by 1–2 days beyond the air control based on visual quality (Fig. 9.8).

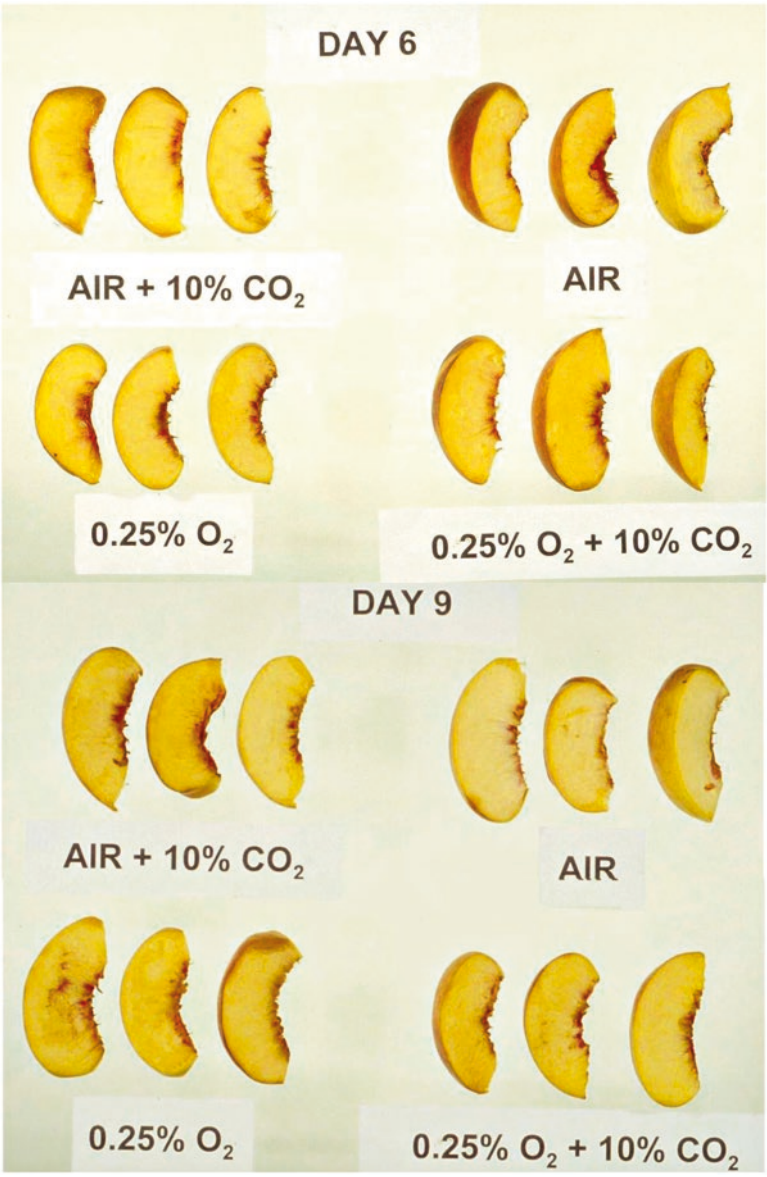

Fig. 9.8 Effect of CA on quality of their fresh-cut slices (Mitcham 2008)

Low O_2 (0.25%) with CO_2 levels of 10% and 20% to induced fermentative metabolism as indicated by ethanol and acetaldehyde production.

A post-cutting dip containing AsA (2%) and Ca lactate (1%) resulted in slight reduction of cut surface browning and tissue softening in Carnival peach slices (Gorny et al. 1999). A dip treatment of AsA (2%) and Ca lactate (1%) preserved phytochemical content, antioxidant capacity and microbiological safety of fresh-cut Big Top nectarines stored at 4 °C for up to 12 days under 10% O_2 and 10% CO_2 using microperforated MAP film (Cefola et al. 2014). In comparison to AsA and Ca lactate, L-cysteine (0.5%) better retained visual quality of fresh-cut peach wedges during storage at 5 °C for 8 days (Colantuono et al. 2015). Tara gum-based edible coating incorporated with CitA, AsA and Na chloride maintained firmness and color with lower mass loss and yeast-mold growth in Granada peach cubes during storage for 12 days at 4 °C (Pizato et al. 2013). Ayala-Zavala et al. (2013) demonstrated that pectin-cinnamon leaf oil coating preserved quality of fresh-cut Jefferson peach slices by increasing its antioxidant status, odor acceptability and decreasing bacterial growth during storage at 5 °C for up to 15 days. Coated (chitosan, pectin and Na caseinate) or non-coated Babygold nectarine sections maintained texture, color and microbiological quality for 7 days at 4 °C, coating treatment did not extent the shelf life (Ramirez et al. 2015).

Heat treatment (50 °C for 10 min) 4 h before cutting effectively controlled browning and retained firmness fresh-cut peaches during storage for days at 5 °C (Koukounaras et al. 2008). Mild heat treatment (40 °C for 70 min) before cutting in combination with active (3% O_2 and 5% CO_2) or passive MAP had no effects on lightness of fresh-cut peach slices during storage for 8 days at 4 °C, although significant firmness improvements were observed due to the activation of pectin methyl esterase (Steiner et al. 2006). High pressure processing in combination with vacuum packaging maintained quality by successfully inhibiting fermentation and browning of fresh-cut peaches for at least 21 days at 10 °C, with only a slight translucency caused by vacuum packaging (Denoya et al. 2015).

9.2.6 Pears

Fresh-cut pear slices have limited shelf life due to excessive tissue softening and cut surface browning (Gorny et al. 2000). The cut surface loss of sheen or gloss was reported in Bosc, Anjou, Red Anjou and Conference pear slices as a result of localized dehydration of ruptured cells at the cut surface (Gorny et al. 2000; Soliva-Fortuny et al. 2004). Pear slices also exhibited development of an abrasive surface texture due to protruding stone cell (Gorny et al. 2000).

Many factors affect the shelf life of fresh-cut pear slices including cultivar (Gorny et al. 2000; Arias et al. 2008), stage of ripeness at cutting (Sapers and Miller 1998; Gorny et al. 2000; Soliva-Fortuny et al. 2004), fruit size (Gorny et al. 2000) and storage regime before (Gorny et al. 2000) or after processing (Gorny et al. 2000). Bartlett pear is the best suited cultivar for fresh-cut processing because of lower

browning intensity and longer post-cutting shelf life, compared to Bosc, Anjou and Red Anjou pears (Gorny et al. 2000). Conference was found to be more suitable variety than Williams and Passacrassana for fresh-cut pear processing (Arias et al. 2008) due to its organoleptic attributes, low browning sensitivity and excellent physiological response to processing operations (Soliva-Fortuny et al. 2004). Anjou, Red Anjou (Gorny et al. 2000), Blanquilla (Arias et al. 2009) and Rocha (Abreu et al. 2011) pears were reported to be highly sensitive to browning when processed as fresh-cut products. Response of fresh-cut pears to anti-browning dip treatments depends on cultivar. Anjou and Bartlett pears responded better than Bosc to the Na erythorbate dip treatment (Sapers and Miller 1998). Fruit ripeness stage at cutting had a significant effect on shelf life and severity of browning of pear slices. The optimal pear fruit ripeness stage for fresh-cut processing based on flesh firmness is between 44 and 58 N. If softer fruit are used, a reduction in shelf life occurs due to increased cut surface browning. If mature-green fruit are used, eating quality may be compromised due to lack of juiciness and fruity aroma (Gorny et al. 2000). It is suggested to use partially ripened Bartlett, Bosc and Anjou to 27–45 N, Red Anjou to 65 N, (Gorny et al. 2000), Conference to 44 N (Soliva-Fortuny et al. 2004) and Anjou to 22–31 N (Chen et al. 2003) and Flor de Invierno to 43 N (Oms-Oliu et al. 2009a) for fresh-cut pear slices. Partially ripe and mature-green pear slices exhibited significantly less cut surface browning at 0 °C than ripe fruit. Slices made from ripe pears of different varieties held at 0 °C, 4 °C or 10 °C exhibited the most intense enzymatic browning (Gorny et al. 2000; Soliva-Fortuny et al. 2004). Browning of cut surfaces of fresh-cut Bartlett, Bosc and Anjou pears during storage was greater in less firm fruit than in more firm fruit in the presence of browning inhibitors due to higher susceptibility of less firm fruits to mechanical injury during handling or cutting (Sapers and Miller 1998). Amiot et al. (1995) found that, in pear fruits of different varieties picked at dates close to the commercial maturity state, the susceptibility of pears to browning and the phenolic content were not greatly different, although a significant decrease in the phenolic content occurred with delayed harvest times. Bai et al. (2009) reported that fresh-cut fruit salad produced from 1-month delayed-harvest pears had less browning potential than those from pear fruits at commercial maturity. Furthermore delayed-harvest pear fruits produced slices with better flavor because pear fruits at commercial maturity are firm and crisp, but its flavor is flat. Ripe Bartlett, Bosc and Anjou and Red Anjou pear slices had a marketable shelf life of about 2 days at 0 °C, while partially ripe slices had a shelf life based on visual quality of 8 days at 0 °C (Gorny et al. 2000). Conference pears processed at partially ripe maturity preserved their initial fresh-like quality during at least 14 days at 4 °C (Soliva-Fortuny et al. 2004). A shelf life of 10 days at 4 °C was suggested for partially ripe fresh-cut Flor de Invierno pears packaged under a 2.5% O_2 + 7% CO_2 atmosphere, while an advanced ripeness stage at processing could be a limiting factor on the shelf life due to softening and accelerated microbial spoilage (Oms-Oliu et al. 2009a). Pear slices from smaller size fruit have greater cut surface discoloration and deteriorate more rapidly than slices from larger fruit (Gorny et al. 2000). Pears may be stored for many months at low temperature with or without atmospheric modification before processing. Storage condition of

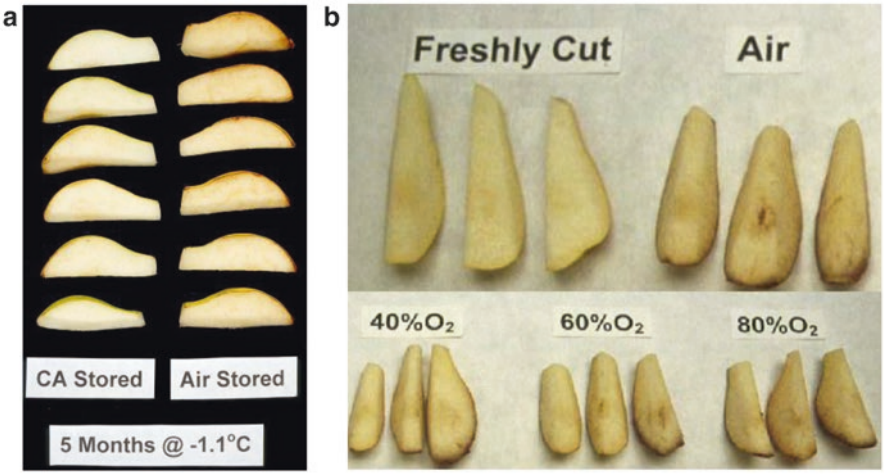

Fig. 9.9 (**a**) Effect of whole pears storage atmospheres on quality of their fresh-cut slices, (**b**) effects of high O_2 atmospheres on sliced pears (Gorny 2008; Mitcham 2008)

intact fruits affects the subsequent shelf life of the fresh-cut fruit slices (Fig. 9.9a). Recently harvested Bartlett pear fruit and whole pears held at −1 °C in a CA of 2% O_2 + 98% N_2 had a longer post-cutting shelf life than those held in air at −1 °C for the same duration (Gorny et al. 2000). Storage temperature significantly affected the rate and intensity of enzymatic browning of pear slice. Pear slices held at 10 °C exhibited more intense enzymatic browning than those held at 0 °C. Shelf life of pear slices at 10 °C was generally about half of that at 0 °C (Gorny et al. 2000).

Mechanical operations such as peeling and cutting during fresh-cut processing involve enzymatic browning of fruit tissue. Cut surface browning in sliced pears is caused by the action of PPO on phenolic compounds released during the process of cutting (Amiot et al. 1995). Effective anti-browning treatments for fresh-cut pear slices were given in Table 9.3. A combination of a Ca chloride dip and 0.5% O_2 + 12% CO_2 atmosphere reduced softening and browning rates of Bartlett pear slices (Rosen and Kader 1989). Chen et al. (2003) suggested a 30-s dipping in the solution contained AsA and potassium chloride at pH 2.3 as optimum anti-browning agent for Anjou wedges over a 14-day period at −1.1 to 1.7 °C. A post-cutting dip containing Ca chloride, Ca lactate and AsA or AsA and Ca chloride reduced cut surface browning and firmness loss in fresh-cut Bartlett pears (Gorny et al. 1998b).

The dip treatment consisting of AsA and Ca chloride of preserved the initial appearance of Conference pear cubes packaged in plastic bags for at least 14 days at 4 °C (Soliva-Fortuny et al. 2004). Alandes et al. (2009a) have shown that Ca lactate treatment resulted in improvement of the texture of fresh-cut Flor de Invierno pears stored for 3 weeks at 4 °C. However, Ca lactate alone had no anti-browning effect on cut pear (Dong et al. 2000; Gorny et al. 2002; Gomes et al. 2010a) since Ca lactate had the least inhibitory effect on PPO among the Ca salts (Gomes et al. 2014). Color and firmness are better preserved when Rocha pear slices were dipped

Table 9.3 Effective anti-browning treatments for fresh-cut pear slices

Cultivar	Treatment	Storage condition	References
Bartlett	Ca chloride (1%)	7 days; 2.5 °C 0.5% O_2+ 12% CO_2	Rosen and Kader (1989)
Bartlett	Ca chloride (1%), Ca lactate (1%) and AsA (2%)	Air or 0.5% O_2 + 5% CO_2, 9 days; 5 °C	Gorny et al. (1998b)
Anjou Bartlett	Na erythorbate (4%), Ca chloride (0.2%), 4-HR (100 µl·l^{-1}); 1 min	14 days ; 4 °C	Sapers and Miller (1998)
Anjou, Bartlett, Bosc	4-HR(0.01%), AsA (0.5%), Ca lactate (1%); 2 min	15–30 days; 2–3 °C	Dong et al. (2000)
Bartlett	AsA(2%), Ca lactate (1%), L-cysteine (0.5%); pH 7.0; 5 min	8 days; 0 °C	Gorny et al. (2002)
Anjou, Bartlett	4-HR, iso-AsA, NAC, potassium sorbate; 30 s	9 days ; 5 °C	Abbott and Buta (2002)
Anjou'	10% AsA, 2% potassium chloride ; pH 2.3; 30 s	14 days ; −1.1–1.7 °C	Chen et al. (2003)
Conference	AsA (10 g l^{-1}), Ca chloride (5 g l^{-1}); 1 min	14 days; 4 °C	Soliva-Fortuny et al. (2004)
Flor de Invierno	NAC (0.75%), GSH (0.7%); 2 min	28 days; 4 °C 21 days; 4 °C	Oms-Oliu et al. (2006)
Flor de Invierno	Alginate (2%), pectin (2%), gellan-based (0.5%) edible coatings with NAC (0.75%), GSH (0.75%) ; 2 min	14 days; 4 °C	Oms-Oliu et al. (2008c)
Williams, conference, passacrassana	AsA (2%) 4-HR(0.01%), Ca chloride (1%); 15 min	10% O_2 + 10% CO_2; 9 days; 4 °C	Arias et al. (2008)
Flor de Invierno	NAC (1%), GSH (1%), Ca lactate (0.8%), malic acid (2.5%); 1 min	4 weeks; 4 °C	Alandes et al. (2009b)

in a solution of Ca additives such as Ca chloride, Ca ascorbate, Ca lactate and Ca propionate at pH 7.0, but microbial growth is reduced at pH 3.0 (Gomes et al. 2010a). AsA in combination with Ca chloride is more effective in reducing browning of fresh-cut pear under neutral (pH 7.7) than under acidic (pH 3.3) conditions (Sapers and Miller 1998). It was suggested that the pH of anti-browning additives for fresh-cut pears should be corrected to 6 or higher to reduce the browning potential (Gomes et al. 2014). Reducing enzymatic browning of fresh-cut pear using AsA and Ca salt dipping is not completely not completely control post-cutting enzymatic browning of fresh-cut pear slices (Gorny et al. 2002). When cysteine is used as an inhibitor of enzymatic browning on sliced pears (Sapers and Miller 1998), pinkish-red colored compounds are formed and this off-color formation can be prevented by altering the treatment solution pH (Gorny et al. 2002). A post-cutting dip of AsA, Ca lactate and L-cysteine at pH 7.0 significantly extended shelf life of the pear slices by inhibiting loss of firmness and cut surface browning without any objectionable off-flavors (Gorny et al. 2002). The thiol-containing compounds such as NAC and GSH, natural chemicals with antioxidant properties, are suggested as browning

inhibitors to prevent darkening fresh-cut pears. The combined treatment with NAC/GSH, Ca lactate and malic acid preserved quality of fresh-cut Flor de Invierno pears with low microbial load for 4 weeks at 4 °C (Alandes et al. 2009b). Treatments containing a 4-HR and iso-AsA or AsA were effective in inhibiting the browning and maintaining firmness of Anjou, Bartlett and Bosc pears slices during storage at 5 °C for 14 days of marketing period (Buta and Abbott 2000). A dip treatment containing Na erythorbate, Ca chloride and 4-HR followed by appropriate MAP retarded browning of cut surfaces, cut edges of the skin and residual core tissue, with no apparent deterioration of flavor or texture of slightly under ripe Anjou and Bartlett fresh-cut pears for at least 14 days at 4 °C (Sapers and Miller 1998). A 2-min dip in 4-HR, AsA and Ca lactate provided 15–30 days shelf life at 2–3 °C for Anjou, Bartlett and Bosc pears without a flavor difference between 4-HR-treated pears and controls (Dong et al. 2000). A 30-s dip in a solution of 4-HR, iso-AsA, NAC and potassium sorbate prevented browning of fresh-cut Anjou and Bartlett pears for 9 days at 5 °C in air (Abbott and Buta 2002). Oms-Oliu et al. (2006) reported that NAC and GSH were effective to prevent browning of Flor de Invierno pear slices up to 28 days and 21 days at 4 °C, due to the inhibition of PPO, but AsA or 4-HR treatments did not completely prevent browning of pear wedges throughout the storage period. They also indicated these browning inhibitors slightly reduced firmness of fresh-cut pears. Effectiveness of anti-browning additives in PPO inhibition and browning depends on pH and cultivars. The inhibition PPO activity and browning were more effective with 4-HR than AsA in Conference pear (Arias et al. 2007), AsA + 4-HR + Ca chloride than AsA + CitA + Ca chloride in Williams, Conference and Passacrassana pear slices (Arias et al. 2008) and AsA + NAC + Ca ascorbate than Ca chloride + Ca lactate + Ca propionate +4-HR in Rocha pear (Gomes et al. 2014).

The use of polysaccharide-based edible coatings increased the water vapor resistance and reduced ethylene production of coated fresh-cut Flor de Invierno pears (Oms-Oliu et al. 2008c). The incorporation of NAC and GSH into gellan-, alginate- or pectin-based coating was effective in preventing fresh-cut pears from browning for 14 days at 4 °C and reduced microbial growth without affecting firmness of pear fruit wedges (Oms-Oliu et al. 2008c). Xiao et al. (2011) demonstrated beneficial effects of combination of Na chlorite dip treatment and carboxymethyl chitosan coatings in reducing cut-surface discoloration of fresh-cut Anjou pears during 10 days of storage at 4 °C. Sharma and Rao (2015) reported that Xanthan gum-based edible coating (2.5 g l^{-1}) with cinnamic acid (1 g l^{-1}) reduced the surface browning and enhance the shelf life of fresh-cut Nashpati and Babughosha pears for 4 days and 8 days, respectively, at 4 °C (Sharma and Rao 2015). Chitosan coating containing anti-browning agents (AsA and CitA) or cinnamon oil inhibited PPO activity, maintained overall visual quality and retarded the microbiological deterioration of fresh-cut pear slices for 15 days at 4 °C (Xu et al. 2013). Medeiros et al. (2012) applied nano-layered coatings of κ-carrageenan and lysozyme on intact and fresh-cut Rocha pears reporting the positive effect on gas barrier properties and antimicrobial action, also maintaining the color and reducing mass loss.

Passive or active MAP containing low O_2 and high CO_2 in conjunction with anti-browning dip treatments prevents browning, and thus extends shelf life of

fresh-cut pears. Anjou pears wedges treated with a browning inhibitor formulation containing Na erythorbate (4%), Ca chloride (0.2%), 4-HR (100 µl l^{-1}) and packaged with a laminated polyethylene (PE) film having oxygen transmission rate of 1395 cm^{-3} m^{-2} day^{-1} had a shelf life for at least 14 days at 4 °C (Sapers and Miller 1998). The beneficial MA have initial O_2 levels of 2.5% (Soliva-Fortuny et al. 2007; Oms-Oliu et al. 2008d), or even closer to 0% (Soliva-Fortuny et al. 2002b, 2007). The browning reaction rate is influenced by the amount of O_2 and CO_2 available in the surroundings of the fruit tissue. In fresh-cut Conference pears, color changes as well as PPO activity were strongly influenced by the package headspace gas composition as indicated by a linear increase in PPO activity in related to the availability of O_2 in the package headspace (Soliva-Fortuny et al. 2002c). Firmness of fresh-cut Conference pears was maintained up to several weeks at 4 °C with low O_2 atmospheres developed by an initial atmosphere of 100% N_2 combined with bags of low O_2 permeability of 15 cm^{-3} m^{-2} bar^{-1} day^{-1}(Soliva-Fortuny et al. 2002b). Low O_2 (0.25 or 0.5%) or elevated CO_2 (air + 5, 10 or 20% CO_2) or high O_2 (40, 60 or 80%) atmospheres alone did not effectively prevent cut surface browning or firmness loss in fresh-cut pear slices (Gorny et al. 2002). Pear slices kept in air, 40, 60 or 80% O_2 (balance N_2) softened at similar rates and exhibited similar severities of cut surface browning during storage at 10 °C (Fig. 9.9b). Gomes et al. (2012) reported that no significant improvements of quality attributes of fresh-cut Rocha pear can be obtained by low O_2 (0.25 or 1.8%) inside the MAP packages since low O_2 atmospheres did not offer any reduction in metabolic activity without the danger of inducing anaerobiosis, especially between 0 and 10 °C, the temperatures normally found during storage and marketing (Gomes et al. 2010b). A packaging atmosphere of 2.5% O_2 + 7% CO_2 with a dip in 10 g l^{-1} AsA and 5 g l^{-1} Ca chloride preserved an acceptable sensory quality of partially ripe Conference pear cubes kept at 4 °C for 3 weeks without significant changes in relation to untreated freshly prepared samples (Soliva-Fortuny et al. 2007). The application of a low O_2 and elevated CO_2 atmospheres (2.5% O_2 and 7% CO_2) inside MAP packages after a dip in NAC (0.75%) and GSH (0.75%) preserved a quality color and texture of fresh-cut Flor de Invierno pears and provided a commercial shelf life up to 28 days at 4 °C (Jandric et al. 2010). Pear slices are susceptible to CO_2 injury when atmospheric levels of CO_2 are at or above 10% (Gorny et al. 1998b). The CO_2 injury symptoms expressed are similar to those in whole pear fruit. Symptoms of CO_2 injury in pear slices include tissue browning, necrosis and softening, beginning near the core and radiating out toward the peel as the duration of exposure to CO_2 increased. Arias et al. (2008) found that a MA of 10–12% O_2 and 8–10% CO_2 developed with a microperforated film is the most appropriate to preserve Williams, Conference and Passacrassana pear slices for 12 days at 4 °C. Oms-Oliu et al. (2009a) recommended MA with 2.5% O_2 + 7% CO_2 for keeping quality and safety of fresh-cut Flor de Invierno pears processed in a partially ripe state for about 10 days although low O_2 atmospheres did not prevent effectively cut surface browning, which occurred during the few first hours after processing. Soliva-Fortuny et al. (2007) found that storage of Conference pear cubes under low O_2 atmosphere (initial 0% O_2 in PE bags) was detrimental to flavor perception and even harmful to the fruit tissue when combined

with high CO_2 concentrations (38.9–49.1%), which caused off-flavor production and massive production of fermentative metabolites beyond 3 weeks at 4 °C (Soliva-Fortuny et al. 2007). High O_2 atmospheres (70% of O_2) limited shelf life of Flor de Invierno fresh-cut pears to 14–21 days at 4 °C due to the browning appearance of the cut surfaces and off-odors (Oms-Oliu et al. 2008d), lower acidity, softer texture although microbiological stability was assured under high O_2 atmospheres throughout storage (Jandric et al. 2010).

Common post-harvest methods, such as the use of anti-browning compounds and/or MAP, may fail to preserve quality of pear slices long enough to be marketable. Mild heat pre-treatment of 35–45 °C for 40–150 min and 35 °C for less than 20 min before cutting were effective in avoiding the cut surface browning and maintaining a good overall acceptable quality of Rocha pear quarters for 7 days at 2 °C (Abreu et al. 2003) and for 8 days at 5 °C (Abreu et al. 2011), respectively. The treatment of intact pears with 1-MCP before cutting and peeling considerably improved textural properties and color of Blanquilla pear slices for 5 days at 4 °C by a decrease in respiratory activity and ethylene production, but the high phenolic concentrations in this variety promoted browning and makes fresh-cut pears unmarketable after 5 days which is not long enough for selling of Blanquilla fresh-cut pear in supermarkets, but it is long enough for food services and quick service restaurants (Arias et al. 2009). Lu et al. (2009) suggested in package application of gaseous 1-MCP instead of pre-slicing treatment with 1-MCP for Anjou pear slices within a MA package stored for 3 weeks at 5 °C to reduce secondary browning induced by enzymes of microbial origin and better maintain the measured juiciness of slices. The cut-surface browning and loss of firmness were significantly inhibited in the Huangguan pear wedges following pre-treatment of the whole fruit with 100% O_2 before cutting. A pure O_2 pre-treatment combined with a chitosan coating (2%) included rosemary extracts (0.03%) presented the lowest rate of browning, softening and sensory degradation in the pear wedges after 3 days at 20 °C (Xiao et al. 2010). The combination of UV-C irradiation (3.7 kJ m^{-2}; 7.5 min) with hydrogen peroxide (3%) for 5 min treatment resulted in less browning and kept optimal microbial stability of fresh-cut William pears for 6 days at 5 °C (Schenk et al. 2012).

9.2.7 Pineapples

Pineapple, a large size fruit and relatively hard to peel, is well suited to be prepared and sold as fresh cut (Benítez et al. 2014). Fresh-cut pineapple is already found in many supermarkets and food service chains (Marrero and Kader 2006). The shelf life of fresh-cut pineapple is heavily dependent on storage temperature (Marrero and Kader 2001) and limited mainly by brown discoloration (O'Connor-Shaw et al. (1994), juice leakage (Marrero and Kader 2006), microbial (Bierhals et al. 2011) and visible fungal growth (Chonhenchob et al. 2007). Other researchers have described a reduction in the shelf life of fresh-cut pineapple as indicated by a 10% decrease in vitamin C content and a 25% decrease in carotenes during the first

6 days of storage at 5 °C (Gil et al. 2006). The post-cutting life of fresh-cut pineapple pieces ranged from 4 days at 10 °C to over 2 weeks at 0 °C (Marrero and Kader 2001; Marrero and Kader 2006). No signs of CI were detected at any point in the fresh-cut pineapple pieces, even for those kept at 0 °C for 2 weeks while prolonged storage more than 2 weeks at 0 °C led to the appearance of the appearance of CI symptoms, off-flavors and odors and microbial spoilage (Marrero and Kader 2006). O'Connor-Shaw et al. (1994) reported that pineapple cubes stored in PP containers at 4 °C kept their sensory attributes for 7 days, but showed brown discoloration after 11 days and off-odors and softening were apparent after 14 days. They indicated that the main problem of fresh-cut pineapple was browning after 6 days of storage at 4 °C, not microbial decay. Fresh-cut pineapple can be stored for 2 weeks at 2 °C, 1 week at 10 °C and 2 days at 25 °C (Latifah et al. 2011). Light exposure promoted browning in pineapple pieces during storage at 5 °C (Gil et al. 2006). Torri et al. (2010) evaluated the shelf life of fresh-cut pineapple by using an electronic nose. They reported that the shelf life was about 5 days at 4 °C, 2 days at 7.6 °C and 1 day at 16 °C. Egidio et al. (2009) evaluated the shelf life of fresh-cut pineapple using infrared spectroscopy and microbiological analysis. They reported that the microbial shelf life was 8–10 days at 5.3 °C, 4–5 days at 8.6 °C and about 2 days at 15.8 °C. The shelf life of Gold fresh-cut pineapple was limited to 14 days at 5 °C by mesophilic bacterial growth (Montero-Calderón et al. 2008).

Fresh pineapples at the M2 or M3 stages of maturity are suitable for fresh-cut purposes (Fig. 9.10a). Cut ripe pineapple cubes have a longer post-cutting life than those cut green (Fig. 9.10b). When pulp pieces of the two cultivars were stored at 5 °C for 2 weeks, those of the Premium Select retained their luminosity better than SC3620, and the final difference in color among the two cultivars was even more prominent than at the beginning (Marrero and Kader 2006). Fresh-cut wedges of Premium Select pineapples have comparable shelf life as Champaka pineapples (Fig. 9.10c).

Several treatments including dip treatment with anti-browning agents, packaging with passive or active MAP, application of edible coating and UV-C have been studied to maintain quality and extend shelf life of fresh-cut pineapples. A combination of ASA (300 µl l^{-1}) and 4-HR (200 µl l^{-1}) effectively reduced browning and microbial spoilage of fresh-cut pineapple slices during refrigerated storage (Mohammed and Wickham 2005). Chemical treatments with Na chloride, Ca chloride and AsA can improve the taste, flesh firmness and overcome the browning problem in cut Josephine pineapple (Latifah et al. 2011). Pineapple slices packaged in polystyrene trays maintained in good condition for up 14 days at 10 °C following dip treatment for 2 min with anti-browning agents of 0.1 mol l^{-1}iso-AsA, 0.05 mol l^{-1} AsA or 0.05 mol l^{-1} NAC (González-Aguilar et al. 2004). They concluded that iso-AsA was the most effective treatment in reducing cut surface browning, decay and firmness loss of Cayenne Lisa fresh-cut pineapples after 14 days of storage at 10 °C, followed by AsA and NAC treatments. González-Aguilar et al. (2005) recommended the use of AsA and iso-AsA to maintain compositional quality parameters such as sugars, vitamin C and phenolic content of Cayenne Lisa fresh-cut pineapples kept for 14 days at 10 °C. Although the highest external quality (reduced browning and

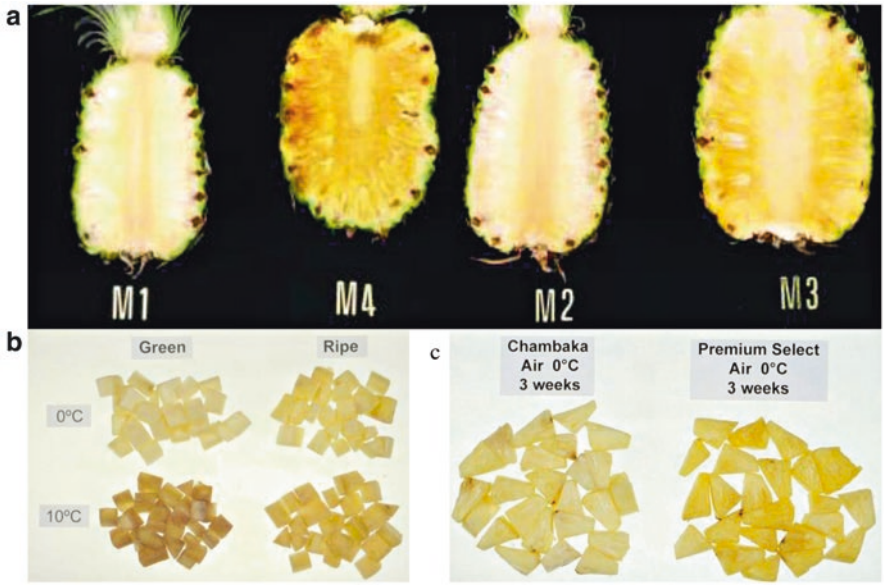

Fig. 9.10 (**a**) Effect of maturity on flesh color and translucency of pineapples (Mohammed 2004), (**b**) visual quality of cut ripe vs. cut green pineapple cubes, (**c**) effects of cultivars on visual quality pineapple cubes (Mitcham 2008)

better overall appearance) was achieved with the NAC treatment, NAC treatment was less effective in maintaining compositional quality (González-Aguilar et al. 2005). However, AsA-treated pineapple wedges showed a significant loss of the luminosity after 15 days of storage (Benítez et al. 2014). Immersing the pineapple slices in a solution (0.25% AsA and 10% sucrose) for 2 min and packaging with MA containing 4% O_2 + 10% CO_2 extended shelf life of fresh-cut pineapples to 7 days at 4 °C by reducing the respiration rate, ethylene production, textural, color and microbial deteriorations, as well as the overall sensory deterioration, while untreated slices exhibited wet surface and off-flavor after storage at 4 °C for 3 days (Liu et al. 2007). Ca lactate dip (0.5%) combined with the use of low permeable film maintained the quality of MD2 Del Monte fresh-cut pineapple for up to 15 days of storage at 4 °C by delaying the ripening process and reducing juice leakage and the loss of luminosity (Benítez et al. 2014).

MAP and refrigeration are the main tools used to slow undesirable quality changes and increase the shelf life of fresh-cut pineapples. Marrero and Kader (2001, 2006) reported on post-cutting life of fresh-cut Smooth Cayenne pineapple pieces from 4 day at 10 °C to over 2 weeks at 0 °C (10% CO_2 + 8% O_2), while González-Aguilar et al. (2004) reported 14 days at 10 °C for the same cultivar (2–5% CO_2 and 12–15% O_2). The shelf life of fresh-cut Phuket pineapples was limited by visible fungal growth and extended from 6 days to 13 days at 10 °C under MA of 6% O_2 and 14% CO_2 in PET containers (Chonhenchob et al. 2007). Fresh-cut Gold pineapple packed in PP trays filled with air or wrapped with 64 μm of PP film

under low O_2 (11.4%) showed higher L^* and b^* values than those under high O_2 (40%), however, a significant decreased color values during 14 days of refrigerated storage in all packaging conditions was directly attributed to the translucency appearance of fruit flesh (Montero-Calderón et al. 2008). The application of high pressure argon treatment extended the shelf life of samples to 18 days during storage with MAP at 4 °C as compared to the control for only 12 days (Wu et al. 2012). 1-MCP treatment and MAP with N_2O enriched atmosphere (86.13% N_2O, 10.13% O_2 and 5.07% CO_2) delayed softening by the inhibition of respiration and ethylene production of fresh-cut pineapple and N_2O enriched atmosphere alone extended the shelf life by 3–4 day due to delaying microbial growth (Rocculi et al. 2009). Neither refrigeration nor atmosphere modification reduced the incidence of juice leakage. No differences were found in the amounts of juice leakage of the fresh-cut pineapples among the different temperatures at 2, 5 and 10 °C during 20 days of storage (Chomsri et al. 2013). Many factors such as cultivar, the fresh-cut shape, Ca treatment and edible coating could also play an important role in the volume of juice leakage. Fresh-cut pineapple from Premium Select cultivar was less prone to leakage than those from SC3620 (Marrero and Kader 2006). Peeled pineapples had a juice leakage in the range of 0.24–3.78% (Chomsri et al. 2013), while pineapple wedges showed a juice leakage of 0.5–4.5% (Marrero and Kader 2006).

Cassava starch coated Pérola fresh-cut pineapple showed lower juice leakage, but had only 7 days of shelf life at 5 °C due to the microbial growth (Bierhals et al. 2011). In contrast, Viana et al. (2009) did not find significant differences in juice leakage between control and cassava-starch coated pineapple wedges after 8 days of storage. Chitosan coating reduced yeast count (Sangsuwan et al. 2008), but resulted in the highest juice of fresh-cut pineapples leakage during 15 days of storage at 4 °C (Benítez et al. 2014). Juice leakage was reduced in pineapple wedges coated with alginate throughout 20 days of storage at 5 °C (Montero-Calderón et al. 2008). However, alginate coating alone showed a significant loss of the luminosity of pineapple wedges after 15 days of storage at 4 °C (Benítez et al. 2014). An alginate-based edible coating formulation incorporated with 0.3% lemongrass extended the shelf life and maintained the firmness, color, sensory characteristics and microbial quality of fresh-cut pineapple for 16 days at 10 °C (Azarakhsh et al. 2014). Multilayered edible coating (Ca chloride, alginate + Ca chloride, pectin, Ca chloride) with a microencapsulated trans-cinnamaldehyde extended the shelf life of fresh-cut pineapple to 15 days at 4 °C by inhibiting microbial growth, but resulted in off-flavor (Mantilla et al. 2013).

UV-C radiation significantly inhibited the decrease in the firmness of fresh-cut Comte de Paris pineapple, but UV-C treated slices showed markedly decreased in the vitamin C content and induced browning throughout the storage period at 10 °C (Pan and Zu 2012). UV-C light (200 J m^{-2}) showed slower microbial growth without adverse effect on color and consumer preference of pineapple sticks packaged in conventional trays sealed with PET/PE film during storage at 6 °C up to 15 days (Manzocco et al. 2016). Both UV-C and medium heat (70 °C) treatments reduced microbial count and extended shelf life of fresh-cut Josephine pineapple to 15 days at 5 °C, but medium heat treatment resulted in deterioration of AsA content

(George et al. 2015). They suggested using UV-C treatment for better retention of quality, effective microbial inactivation and enhancement of health promoting compounds for the benefit of consumers.

9.2.8 Pomegranate Aril

Minimally processed (extracted arils, fresh-cut) ready to eat pomegranate arils may become a convenient alternative to increase consumption of fresh pomegranate fruit which has difficulty in peeling (Gil et al. 1996a; Sepulveda et al. 2000). The difficulties involving extracting the arils are major problems of the commercialization of fresh pomegranate arils, although manual and mechanical extraction techniques are currently used commercially to a limited extent for fresh pomegranate arils (Erkan 2011). Instead of using an expensive and labor-intensive manual extraction, fully automated systems to extract pomegranate arils with minimal seed damage, increased output and labor cost savings provide opportunities to create a new and innovative market for fresh arils (Erkan 2011).

Cultivars (Ghasemnezhad et al. 2015), harvest time (Lopez-Rubira et al. 2005), pre-extraction storage duration (Hess-Pierce and Kader 1997) and post-extraction treatments, packaging, storage temperature and atmosphere (Hess-Pierce and Kader 1997; Gil et al. 1996a, 1996b) are important factors affecting quality and shelf life of pomegranate arils. Ghasemnezhad et al. (2015) comparing the quality of minimally processed different Iranian pomegranate genotypes during storage at 4 °C for 14 days suggested that Torsh Syabe Lorestan was a promising genotype for minimal processing technology due to the lower microbial count on the arils and enzymes activity associated with browning. Pomegranate arils extracted from Mridula packaged in 50 micron PP bags had longer shelf life of about 15 days with least browning and microbial count with the highest acceptability score compared to Kandhari and Bhagwa which had a limited shelf life to 12 days at 5 °C (Bhatia et al. 2015). Lopez-Rubira et al. (2005) showed effect of harvest date on arils quality of Mollar of Elche pomegranates harvested at the normal commercial date and at the end of the harvest season. They suggested that minimally processed arils from pomegranates harvested in the middle of the season had lower respiration rate and longer shelf life based on microbial growth and visual quality. Pomegranate fruits that are stored at 7 °C for up to 3 months in air or up to 5 months in CA (5% O_2 and 15% CO_2) produced arils that retain good sensory and microbial quality for up to 14 days shelf life at 5 °C (Hess-Pierce and Kader 1997). It should be noted that the longer the storage duration of intact pomegranates before extracting the arils, the shorter the post-extraction life of the arils (Erkan 2011).

Mechanical damage to the arils must be minimized during their extraction from the fruit, washing, drying to remove surface moisture and packaging since damaged arils are more susceptible to decay-causing fungi (Erkan 2011). Pomegranate arils that are not damaged or microbiologically contaminated can be kept at 0 °C for up to 21 days, at 2 °C for up to 18 days, or at 5 °C for up to 14 days in marketable

condition (Kader 2006). Although intact pomegranate fruits are CI-sensitive, the arils are CI-tolerant and should be kept at 0–5 °C to maintain their quality and microbial safety (Kader 2006). Storage at higher temperatures of 4–8 °C resulted in shorter shelf life of pomegranate arils, compared to storage at 1 °C (Gil et al. 1996b; Lopez-Rubira et al. 2005; O'Grady et al. 2014). Shelf life of fresh-cut pomegranate arils is short because they are very susceptible to shriveling, microbial growth, loss of nutritional value and, mainly to the enzymatic browning (Peña-Estévez et al. 2015). Minimally processed pomegranate arils has shown that browning is produced by the oxidation of phenolic compounds during storage, indicating that the stabilization of anthocyanin pigments is essential to achieve high quality, because an attractive color is one of the most important sensory characteristics of pomegranate arils (Gil 1996a, b). Lopez-Rubira et al. (2005) showed that Mollar de Elche arils stored at 5 °C for 13 days had no significant change in anthocyanin and antioxidant activity. O'Grady et al. (2014) observed no incidence of decay and a lower decline in anthocyanin content of Arakta arils kept at 1 °C, compared to those kept 4 °C and 8 °C during 14 days of storage.

Passive or active MAP either alone or in combination with antioxidant solution has been used to extend the shelf life of minimally processed pomegranate arils. Hess-Pierce and Kader (1997) reported that the shelf life of Wonderful pomegranate arils could be extended to 16 days at 5 °C with 20% CO_2, without changes in the physical and chemical characteristics of the fruit. They also indicated that the arils with mechanical damage presented more susceptibility to the molds after 12 days. Gil et al. (1996b) reported that storage at 1 °C under passive MA using perforated PP and biaxially oriented PP (40 μm) maintained anthocyanin content of minimally processed Mollar de Elche pomegranate arils for 7 days. Mollar de Elche pomegranate arils dipped with chlorinated water (100 μl l^{-1}) and antioxidant solution (0.5% AsA and/or 0.5% CitA) and packaged with biaxially oriented PP film maintained good quality and appearance for 7 days of storage at 1 °C without fungal attacks or off-flavor development (Gil et al. 1996a) and no significant change in anthocyanin as well as antioxidant activity (Lopez-Rubira et al. 2005). The use of ethyl vinyl acetate–based semipermeable packages with or without application of antioxidant solution (5% AsA and 5% CitA) preserved visual and microbiological quality of Wonderful pomegranate arils for 14 days at 4 °C (Sepulveda et al. 2000). The shelf life of pomegranate arils of Hicaznar pomegranates dipped in a solution containing CitA (1%) and chlorinated (100 μ l l^{-1}) water was 18 days under air, N_2 and enriched O_2 atmospheres, 15 days under low O_2 atmosphere with the package type of PP tray with biaxially oriented PP film and 10 days under 100% N_2 in PET packages at 5 °C, but drip loss was observed as an important quality defect (Ayhan and Esturk 2009). The post-harvest life of passive MA-packaged Acco and Herskawitz pomegranate arils was limited to 7 days due to fungal growth and off-flavor (Caleb et al. 2013). Hussein et al. (2015) suggested using perforated MAP (3 or 6 perforations) to avoid excessive CO_2 accumulation or anoxic state and to prevent the microorganism growth and water vapor condensation (Fig. 9.11), thereby overall quality of minimally processed Acco pomegranate arils for 15 days of storage. They reported that non-perforated and perforated packages with 9 perforations resulted in highest microbial counts.

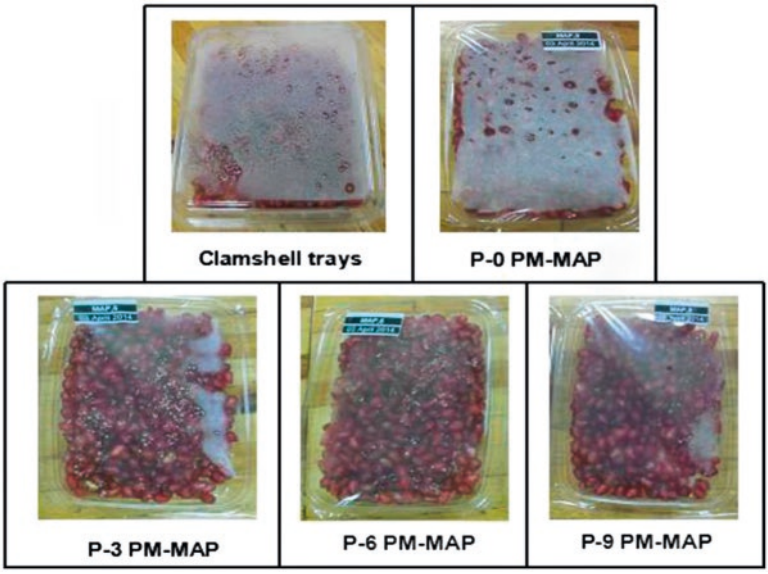

Fig. 9.11 Water vapor condensation on the surface of perforated MAP film and clamshell package after 15 day of storage for minimally processed Acco pomegranate arils stored at 5 °C. P-0, P-3, P-6, P-9 represent non-perforated, 3, 6 and 9 perforations, respectively (Hussein et al. 2015)

In addition to MAP and antioxidant dip treatments, application of edible coatings, UV-C, water or alcohol vapor has been shown to maintain quality and anthocyanin content of pomegranate arils. Honey treatments (10 or 20% diluted honey for 5 min) preserved fresh-like quality of minimally processed arils by delaying quality loss, microbial development and pigment changes for 10 days at 4 °C (Ergun and Ergun 2009). Chitosan (1%) coating extended the shelf life of pomegranate arils to 12–19 days at 4 °C by effectively reducing or delaying microbial growth and maintaining anthocyanin content (Çetin 2012; Ghasemnezhad et al. 2013). Öz and Ulukanlı (2012) suggested the use of combination of starch-based coating with *Nigella sativa* oil to maintain quality to reduce microbiological risks during 12 days of refrigerated storage of Silifkeaşısı arils from freshly harvested or long-stored whole fruit. UV-C treatment (6.5 kJ m^{-2} or 4.54 kJ m^{-2}) in air or under active MAP containing initial 90% of high O_2 increased total phenol content in Hicaznar and Mollar de Elche pomegranate arils during storage for 6 days at 2 °C and 14 days at 5 °C, respectively (Nunes et al. 2010; Maghoumi et al. 2013). Mollar de Elche pomegranate arils treated with UV-C either alone or in combination with high O_2 had lowest microbial growth with acceptable sensory properties after 14 days at 5 °C (Maghoumi et al. 2013). Water vapor treatments at 95 °C for 7 or 10 s applied to pomegranate arils led to an extended shelf life up to 18 days at 5 °C, compared to a conventional sanitizing treatment with chlorine (Peña-Estévez et al. 2015). Pomegranate arils exposed to vapors of 36% distillery ethanol and brandy maintained their anthocyanin content and microbiological and sensory quality with and extended shelf life for >5 days at 10 °C and >23 days at 4 °C (Kapetanakou et al. 2015).

9.2.9 Watermelon

Fresh-cut watermelon is marketed as halves, quarters and slices with rinds, or as rind-free chunks (Saftner et al. 2007). Quality degradation of fresh-cut watermelons is associated with decreased acceptability of texture, color and sweetness (Perkins-Veazie and Collins 2004). The shelf life is limited by water soaking, juice leakage (Cartaxo et al. 1997; Gil et al. 2006), off-odor development (Fonseca et al. 2004) and increased microbial growth and spoilage (Mao et al. 2006). The excellent initial quality of the watermelon prevented juice leakage of fresh-cut watermelons during 9 days of storage at 5 °C (Gil et al. 2006). Fresh-cut watermelon cubes showed increased juice leakage and darkening of product vibrated in non-compartmentalized packages (Fonseca et al. 1999). Removing the rind accelerated senescence and off-flavor production, while the presence of rind improved the overall storage stability of fresh-cut watermelon slices kept at 4 °C for 9 days (Petrou et al. 2013). The recommended temperature for storing whole watermelons is 14 °C to avoid CI, whereas fresh-cut watermelons are not as CI sensitive as the corresponding whole fruit and can be kept at 5 °C for 9 days with marketable visual quality (Gil et al. 2006). Fresh-cut watermelon held for 7 or more days at 2 °C had a slight loss of SSC, color saturation and lycopene, most likely caused by senescence (Perkins-Veazie and Collins 2004). Fresh-cut watermelon cubes had a 5% of loss in vitamin C after 6 days at 5 °C, but showed no losses in carotenoids and phenolics (Gil et al. 2006). Initial antioxidant potential of fresh-cut watermelon was best maintained at storage temperature of 5 °C for 14 days (Oms-Oliu et al. 2009b). Exogenous ethylene will induce placental tissue softening and water soaking, electrolyte leakage, rind softening and enhanced phospholipid degradation in watermelons (Karakurt and Huber 2002; Mao et al. 2004, 2006). These changes increased microbial population on fresh-cut watermelon after 6 days at 5 °C (Zhou et al. 2006). Figure 9.12 presents fresh-cut watermelon following exposure to ethylene (1 µl l^{-1}) and air for 3 days at 10 °C. Treatment of watermelons prior to ethylene exposure with 1-MCP (0.5 or 1.0 µl l^{-1} for 18 h) prevented ethylene-mediated quality deterioration and

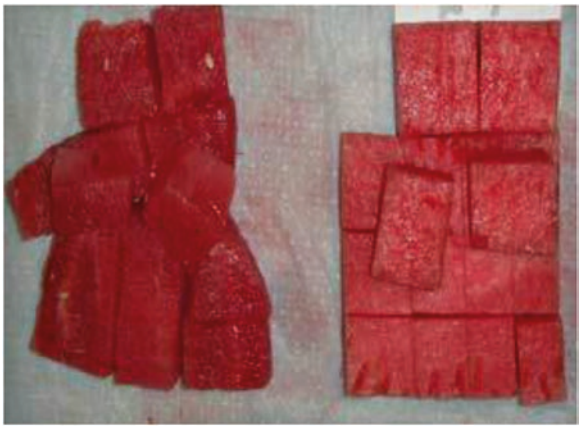

Fig. 9.12 Effects of ethylene exposure to fresh-cut watermelon (Brecht 2012)

microbial growth in fresh-cut watermelon slices stored under MA conditions at 5 °C (Zhou et al. 2006; Saftner et al. 2007).

Unwrapped watermelon slices lost 47% of their firmness after 4 days at 5 °C (Abbey et al. 1988). Undesirable flavor was the limiting factor in sliced wrapped watermelon stored 7 days at 5 °C even though aroma was still acceptable and microbial populations were not problematic until after 8 days (Abbey et al. 1988). Fresh-cut watermelon had an extended shelf life up to 9 days or longer at 1–3 °C under MA with >14% O_2 (Fonseca et al. 2004). CA (3% O_2 and 15–20% CO_2) inhibited bacterial development on fresh-cut watermelon kept at 3 °C for 15 days, but had negative effects on visual quality of the cubes (Cartaxo et al. 1997). The results from this study indicate that cutting and storing watermelon resulted in slight loss of color saturation and lycopene. The losses did not appear until after 7 days of storage and therefore were not directly due to the cutting process itself. Watermelon pieces stored in the PE containers developed MA conditions 10% O_2 and 10% CO_2 after 10 days at 2 °C which caused slight degradation of lycopene and color (Perkins-Veazie and Collins 2004). Alginate coating (1%) maintained the quality and sensory acceptance of fresh-cut watermelon while extending its shelf life up to 15 days at 4 °C (Sipahi et al. 2013). Exposing packaged watermelons cubes to UV-C light at 4.1 kJ m^{-2} reduced microbial populations without affecting juice leakage, color and overall visual quality while dipping cubes in chlorine (40 μl l^{-1}) and ozone (0.4 μl l^{-1}) was not effective in reducing microbial populations (Fonseca and Rushing 2006). The application of pulsed light (180–1100 nm, 12 J cm^{-2}) in combination with malic acid (2%) dips effectively controlled food borne pathogens on the surface of fresh-cut watermelons for at least 2 weeks of storage (Ramos-Villarroel et al. 2015). Low UV-C treated cubes (1.6 and 2.8 kJ m^{-2}) under MA with 3–6% O_2 and 13–17% CO_2 had lower microbial counts and higher initial lycopene content with an extended shelf life up to 11 days at 5 °C (Artés-Hernández et al. 2010).

9.2.10 Other Fruits

Fresh-cut citrus has received much attention because of its large potential market, but has relatively short shelf life due to excessive juice leakage from the cut segments (Artés-Hernández et al. 2007), loss of flavor (Rocha et al. 1995) and microbial spoilage even under refrigeration (Pao et al. 1996; Pao and Petracek 1997). Peeled and/or cut oranges and grapefruits generally have a microbiological shelf life limited to 10–15 days at 4–5 °C (Rocha et al. 1995; Pao et al. 1996). With respect to sensory quality, the shelf life of fresh-cut orange was only 5 days at 4 °C due to flavor changes (Rocha et al. 1995). Artés-Hernández et al. (2007) studied the effects of different cut types (wedges, slices, 1/2 and 1/4 slices) and storage temperatures (0, 2, 5 and 10 °C) on post-cutting life of Lisbon lemons. All cut types remained marketable for up to 7 days at all tested temperatures, but only the wedges, slices, and 1/2 slices stored at 0, 2 and 5 °C preserved their sensory attributes for up to 10 days. UV-C treatment (1.5 kJ m^{-2}) retained the quality attributes and AsA content

and improved health-related phenolic contents of fresh-cut Satsuma mandarin during 12 days of storage at 4 °C (Shen et al. 2013). Manually separated segments and enzymatically peeled whole oranges remained in a commercially viable condition for 1 week at 4 °C (Pretel et al. 1998). After 1 week, a loss of aroma and taste began to be perceived due to an increase in ethanol levels in especially manually separated segments packaged with the high-barrier plastic film. The losses of flavor quality of the chilled citrus segments could be reduced by the use of MAP. Pretel et al. (1998) observed slight microbial activity in fresh-cut orange segments without significant changes in quality parameters during 11 days of storage at 4 °C under passive MA using PP films, but orange segments were commercially viable for 1 week in terms of sensorial quality. Rapisarda et al. (2006) observed low microbial counts on Tarocco orange slices packaged with films of different permeability during 12 days of storage at 4 °C. Fresh-cut Tarocco slices packaged with the highest O_2 permeable film were the most appreciated regarding sensory evaluation. Grapefruit segments remained viable for 10 days under O_2 enriched atmosphere (70% O_2) and less than 10 days under active (20% O_2) and passive MA applications (Karacay and Ayhan 2010a). Orange segments remained in a commercially viable condition for 10 days under MAP containing of low O_2 (20% O_2, 10% CO_2, 70% N_2) or high O_2 (80% O_2, 10% CO_2, 10% N_2) due to sensory quality (Karacay and Ayhan 2010b). The relatively low respiration rate and the high acidity of citrus fruit make a stable product suitable for the fresh-cut market (Rocha et al. 1995). However, commercialization of fresh-cut oranges is limited mostly by technical difficulties in peeling as a result of the peculiarity of citrus peel (presence of albedo) and pulp (vesicle structure) (Pinnavaia et al. 2006). The use of mechanical peelers with blades is not as efficient as for other fruit such as kiwifruits and apples, because it is not possible to completely remove the peel from the citrus segments without damaging the segment surface, losing edible material and generating juice leakage (Pinnavaia et al. 2006). The methods have developed a process using enzyme infiltration under vacuum (Ismail et al. 2005) and water infusion (Pao et al. 1996) to facilitate citrus peeling. The enzyme, pectinase or cellulase, digested the albedo, facilitating peel removal (Ismail et al. 2005), but juice leakage, loss of texture, and off flavors caused by enzyme activity during storage were reported (Ismail et al. 2005). Although water infusion resulted in easy peeling of oranges with significantly less juice leakage and firmness loss during storage (Pao et al. 1996), the residual albedo tissue, affected appearance negatively, was remained (Pinnavaia et al. 2006). Microbial stability is also a concern for citrus processed through infusion of water or enzyme solutions because this could be a source of contamination (Pinnavaia et al. 2006). CitA-treated sliced (post enzyme treatment or by infusion) oranges compared to enzyme-infused oranges may extend shelf life by reducing fruit softening and juice leakage and microbial load depending on cultivars (Pao and Petracek 1997; Pinnavaia et al. 2006). The enzyme treatment did not affect peeling times of white or red grapefruit, oranges or tangelos. Pressure and vacuum infusion methods produced similar results. Grapefruit and oranges infused with water had significantly less juice leakage and were firmer than fruit infused with enzyme. Microbial levels and respiration and ethylene production rates during storage were the same for enzyme- and water-treated fruit (Pao et al. 1996).

Few studies have been published related to minimally processed or ready-to-eat (fresh-cut) table grapes. The quality losses of minimally processed table grapes include collapse, browning and decay on the stem end, splitting and decay on the berry surface (Ergun et al. 2008). The injury during minimal processing, e.g. removal of cap stems, causes grape berries susceptible to microbial growth, decay and quality losses (Kou et al. 2007). Dark colored table grapes seem to be more suitable for minimal processing than white colored table grapes (Ergun et al. 2008). Browning on the stem end was mostly observed in white colored table grapes whereas collapse on the stem end, decay on either stem end or berry surface and splitting did not follow a pattern, implying that the severity of these defects depends on the cultivars and types (Ergun et al. 2008). The cultivars with a very large berry size showed less quality losses (Ergun et al. 2008). In a study with chlorinated water, ethanol and HW treatments have been shown to reduce spoilage microorganisms in minimally processed table grapes kept at 5 °C for a period of about 30 days, with ethanol (50%) having greater effects than HW or chlorinated water treatment (Del Nobile et al. 2008). HW treatment (45 °C for 8 min) maintained a significantly lower decay rate for minimally processed grapes during 14 days of storage without any negative impact on grape color, texture and flavor (Kou et al. 2007). Sabir and Sabir (2013) reported that stem-retained berries that received HW treatment had higher visual quality score and lower decay rates at the end of 3-week storage at 1 °C, compared to stem-excised berries. They recommended cap stem retaining in fresh-cut processing for table grapes to maintain the quality of grapes. The use of passive MAP together with ethanol exhibited the best results in maintenance of overall quality parameters of minimally processed table grapes during storage at 0 °C for 4 weeks since ethanol sanitized the berry surface while MAP retarded tissue senescence by restricting respiration rate of the berries (Sabir et al. 2010). Passive MAP using oriented PP at the different thickness (20, 40 and 80 μm) assured a shelf life more than 70 days, while active MAP was not found to be effective in extending shelf life of ready-to-eat table grapes at 5 °C (Costa et al. 2011). Del Nobile et al. (2009) obtained the best results using high barrier films for preserving the quality of minimally processed table grapes for 35 days at 5 °C.

Few studies have been carried out for fresh-cut persimmon fruit, a new alternative fresh-cut produce (Ergun and Ergun 2010). Non-astringent and astringent persimmon cultivars can be prepared as fresh-cut wedges or slices. With astringent persimmon cultivars, e.g. Rojo Brillante, removal of astringency before cutting into slices improves commercialization and transport, maintaining a firm consistency and allows their commercialization as a fresh-cut commodity (Sanchís et al. 2015a). However, the shelf life of fresh-cut persimmon is limited due to enzymatic browning and softening (Sanchís et al. 2015a). Wright and Kader (1997a, 1997b) reported black areas on cut surfaces of sliced Fuyu persimmon fruit as causing a decrease in shelf life. They partially overcome this problem by applying 12% CO_2 which resulted in the maintenance of good visual quality for up to 8 days at 5 °C. Air- and 2% O_2-stored persimmon slices developed areas of faint black pigmentation on the cut surfaces (Wright and Kader 1997a, 1997b). The thin cut edge and the blossom end of the persimmon slices tended to soften and develop a slight water- soaked

appearance. For sliced persimmons, the limit of shelf life was reached before major losses of carotenoid and AsA content occurred (Wright and Kader 1997a, 1997b). Low O_2 (5%) atmosphere combined with application of 1% CitA reduced the enzymatic and non-enzymatic browning of fresh-cut Rojo Brillante persimmons and maintain shelf life up to 9 days of storage at 5 °C (Sanchís et al. 2013). Treatment with 1-MCP was effective in preventing the softening and darkening of fresh-cut Fuyu persimmons stored for 7 days at 5 °C only when applied on intact fruit before processing (Vilas-Boas and Kader 2007). Ethylene production was enhanced, but the respiration rate was not affected in fresh-cut persimmons treated with 1-MCP before processing (Vilas-Boas and Kader 2007). The application of 1-MCP (600 μl l^{-1}) at harvest allowed to fresh-cut processing of Rojo Brillante persimmon after 45 days of storage at 1 °C by reducing firmness loss significantly (Sanchís et al. 2015a). Antioxidant dip treatments (1.12% AsA and 0.21% CitA) were found to be the most effective treatments to control enzymatic browning of fresh-cut persimmons, reaching the limit of marketability in 5–7 days, whereas, 4-HR and Ca chloride did not reach 1 day of storage at 5 °C (Ghidelli et al. 2013). The persimmon slices dipped in the antioxidant solution (1% CitA and 1% Ca chloride) were evaluated above the limit of marketability after 9 days of storage at 5 °C (Sanchís et al. 2015a). The limit of marketability of fresh-cut 'Rojo Brillante' persimmons was reached after 6 and 8 days with AsA and CitA, respectively (Sanchís et al. 2015b). For commercial purposes, the persimmons harvested at the beginning of the season could be processed as a fresh-cut fruit, even after 3 days of storage at 15 °C if treated with 0.01 kg l^{-1} AsA or 0.01 kg l^{-1} CitA. However, processing fruits from late season immediately after harvest and being treated with AsA are recommended (Sanchís et al. 2015b). Apple pectin-based edible coatings incorporated with antioxidant solution (10 g kg^{-1} CitA and 10 g kg^{-1} Ca chloride) and antimicrobial agents (2 g kg^{-1} potassium sorbate or 4 g kg^{-1} Na benzoate) significantly controlled enzymatic browning and reduced the total aerobic mesophilic bacteria of fresh-cut 'Rojo Brillante' persimmon during storage at 5 °C, which accomplished a commercial shelf life of 7 days (Sanchís et al. 2016). Ergun and Ergun (2010) studied effects of honey treatments (10–20% diluted honey solution) on the shelf life of diced ripe Hachiya persimmon fruit during storage at 4 °C. They reported that honey treatments prevented off-aroma development, suppressed softness and exuding juice of the fresh-cut persimmon cubes and delayed jelling, an indication of over ripening and/or CI.

Fresh-cut strawberry can be found in the retail market and fruit salad bars of food service. Fresh-cut strawberry, which is sliced, halved or hulled, is packed in sealed plastic containers or wrapped with plastic film in a fruit mix. The shelf life fresh-cut strawberry was limited to less than 9 days at 5 °C (Aguayo et al. 2006; Gil et al. 2006) due to discoloration (Wright and Kader 1997b), softening (Rosen and Kader 1989) and microbial growth (Aguayo et al. 2006). The visual quality of the strawberries stored at 5 °C under various atmospheres decreased over 7 days due to dry surface and mealy texture and the limit of shelf life was reached before major losses of AsA content of sliced strawberries occurred (Wright and Kader 1997b). Firmness decreased by 30–40% after storage of fresh-cut strawberry at 2.5–5 °C (Rosen and Kader 1989; Gil et al. 2006). Sliced strawberries held for 8 days at 5 °C under air

+12% CO_2 and the 2% O_2 + 12% CO_2 lightened and appeared bleached by day 7 while those stored under air or 2% O_2 darkened (Wright and Kader 1997b). Rosen and Kader (1989) found that CA of 2% O_2 + 12% CO_2 at 2.5 °C was effective in minimizing firmness loss of sliced strawberries, depending on cultivars. CA with 1% O_2 + 10% CO_2 suppressed fungal and bacterial growth in hulled strawberries (Qi and Watada 1997). Ca chloride dips retarded flesh softening in fresh-cut strawberry (Morris et al. 1985; Rosen and Kader 1989). The combined treatment of 1-MCP (1 µl l^{-1} for 24 h at 5 °C) before and after cutting with Ca chloride dip (1% for 2 min) and CA storage (3% O_2 + 10% CO_2) slowed down softening, deterioration rates and microbial growth of strawberry wedges and extended shelf life to 9 days at 5 °C (Aguayo et al. 2006). Odriozola-Serrano et al. (2010) proposed low O_2 atmosphere (2.5% O_2 + 7% CO_2) for MAP of fresh-cut strawberries to prevent oxidation of the main antioxidant compounds during storage for 21 days at 4 °C.

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Chapter 10
Minimally Processed Herbs, Spices, Medicinal and Aromatic Plants

Manolya E. Oner

10.1 Introduction

Minimally processed food products have been preferred by health conscious consumers due to its convenience, microbial safety, and nutritional quality. There have been several studies on fresh-cut fruits and vegetables based on requirements for extending microbiological, sensory, and nutritional shelf life by analyzing each step of the production chain. Minimally processed herbs, spices, medicinal and aromatic plants (Figs. 10.1, 10.2, and 10.3) are also receiving attention of researchers because they were often found contaminated due to improper preparation conditions (USFDA 2001, 2003; Arthur et al. 2007). Spices are used as either powder or kernel in foods, but herbs can be consumed in different forms such as fresh, dried, whole, chopped, or ground.

There had been several outbreaks related to fresh herbs including cilantro contaminated with *Shigella boydii* in Chicago in 1999 (Agle et al. 2005), *Salmonella* Thompson infection of cilantro in California in 1999 (Campbell et al. 2001), and *Salmonella* Senftenberg identified in prepacked fresh basils imported from Israel (Pezzoli et al. 2008). Moreover, an outbreak of *Salmonella* Agona phage type 40 affecting more than 400 people was associated with the consumption of contaminated curry leaves in England (Foster 2013). Preservation techniques capable of maintaining the safety and quality of commodities long enough to make distribution feasible and achievable are required and referred as minimal processing.

In fresh herbs, visual quality is one of the most important quality attributes as an indication of freshness. Greenness is correlated with chlorophyll content in the tissue of herbs (Fan and Thayer 2001); therefore yellowing of fresh herbs occurs due

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Fig. 10.1 Historical Spice Bazaar in Gaziantep, Turkey

Fig. 10.2 Different varieties of dried herbs and spices in Spice Bazaar in Gaziantep, Turkey

Fig. 10.3 Common fresh herbs consumed as dried or raw

to chlorophyll degradation (Yamauchi and Watada 1993). Exposure of light or other specific oxidative stress-inducing conditions resulted in prooxidative activity in leafy spices like thyme, marjoram, basil, sage, or summer savory; however, the expected antioxidative effect was revealed when stored in the dark (Pokorny et al. 2001). Medicinal plants, most of them known as aromatic herbs, improve digestion and have some antibacterial, anti-inflammatory, antiviral, and anticarcinogenic activities due to bioactive compounds with functional properties (Charles 2013). Aromatic herbs are also used in food and beverage preservation because of antioxidant and antimicrobial properties (Salgueiro et al. 2010; Wong and Kitts 2006).

Spices are widely used as ingredient in food preparation and processing with their distinctive flavor, color, and aroma. They are considered safe due to their low water activity; however, there is a potential of microbial contamination in spices during preharvesting, postharvesting (Tainter and Grenis 2001), drying, transporting, and storing under poor sanitary conditions in warm and humid regions (Mckee 1995; Banerjee and Sarkar 2003), which may create a risk to public health since they are consumed raw by adding to food products with no further processing. Besides, spores of microorganisms might find a suitable environment to grow when they contacted with water-rich food products (Tainter and Grenis 2001; Banerjee and Sarkar 2003). Many cases of microbial contamination of spices have been reported. Banerjee and Sarkar (2003) isolated *Clostridium perfringens*, *Bacillus* spp., and *Staphylococcus* spp. from black pepper, and Christensen et al. (1967) isolated *Clostridium perfringens*, *Bacillus cereus*, *Enterobacteriaceae*, and a variety of fungi from red pepper. Furthermore, addition of red pepper and black pepper to salami after pasteurization step caused *Salmonella* Montevideo contamination (CDC 2010). Satisfactory results were obtained in spices by using fumigation with

ethylene oxide and other chemicals; however, it is prohibited in many countries due to its possible toxic residues remaining after treatment, thereby revealing chronic or carcinogenic effects (Hayashi 1998; Esa-spice 2004). Consequently, alternative food processing technologies has been investigated to reduce contamination in spices with minimal processing. This chapter aims to overview the applications of common nonthermal and thermal food processing technologies on herbs, spices, medicinal and aromatic plants. The general characteristics of treatments are described. Efficacy of applications on microbial inactivation, product quality, and nutrition is discussed.

10.2 Nonthermal Processing Technologies for Minimal Processing of Herbs, Spices, Medicinal and Aromatic Plants

Nonthermal food processing technologies such as chemical sanitizers, ozone, electrolyzed water, high hydrostatic pressure (HHP), irradiation, ultrasound, pulsed electric field (PEF), and pulsed light (PL) have been used in research and development of minimally processed herbs and spices for fresher tasting, good nutrition, and microbial safety. They were either applied to fresh herbs or dried herbs and spices to improve the quality without nutritional loss and extend the shelf life.

10.2.1 Chemical Sanitizers

There have been numerous chemical sanitizers tested for decontamination of minimally processed food produces (Sapers et al. 2003; Obaidat and Frank 2009). In chemical sanitation of fresh produce, concentration of antimicrobial and time of exposure are important factors affecting decontamination ability of an antimicrobial.

Chlorine is the most widely known sanitizer that is used for produce surface and processing equipment sanitation in food industry (Walker and LaGrange 1991; Cherry 1999). Chlorine is convenient, inexpensive, and generally used in liquid form between 50 and 200 ppm concentration range with a 1–2 min contact time to reduce microbial populations. However, studies indicated that less than 200 ppm is not sufficient for microbial reduction (Parish et al. 2003). Lopez et al. (1988) were able to reduce coliform bacteria 81% on parsley and 85% on coriander by using 300 ppm chlorine solution with 10 min contact time. Wu et al. (2000) were able to reduce more than 7 log CFU/g *Shigella sonnei* populations inoculated onto whole parsley leaves with 250 ppm chlorine solution treatment for 5 min.

The pH value below 4.5 prevents growth of pathogenic bacteria in food produce; therefore, acidification may be an alternative treatment to inactivate microorganism

(Parish et al. 2003). Higher than 7 log reduction was determined in *Yersinia enterocolitica*-inoculated parsley leaves treated with 2% acetic acid or 40% vinegar solutions for 15 min contact time (Karapinar and Gonul 1992). Similar result was obtained in treatment of *Shigella sonnei*-inoculated whole parsley leaves with 7.6% acetic acid solution for 5 min (Wu et al. 2000). Due to its convenience, simple household sanitizers such as vinegar and lemon juice can be used in sanitation.

Ethyl pyruvate (EP) is an antioxidant, which is a stable lipophilic ester derivative of pyruvate, known as a therapeutic agent (Woo et al. 2004). Tornuk and Durak (2015) treated *Escherichia coli* O157:H7- and *Staphylococcus aureus*-inoculated parsley with vaporized EP at concentration of 100, 400, and 1,000 μL in 2.6 L closed-lid food containers. EP treatments with a concentration of 1,000 μL completely inhibited the *Escherichia coli* O157:H7 bacterial population. EP treatments with concentrations of 400 or 1,000 μL had significantly lowered *Staphylococcus aureus* populations compared to control samples. Although EP treatment delayed spoilage, some yellowness was observed in parsley leaves (Tornuk and Durak 2015).

10.2.2 Ozone

Ozone (O_3) or triatomic oxygen is a potent oxidant, affirmed by US Food and Drug Administration (USFDA) as a GRAS (generally recognized as safe) chemical, which can be used as an antimicrobial additive for direct contact with all types of foods (USFDA 2015a) and does not leave any residual component on products. Gaseous ozone usage in food processing is also recognized as allowable by organic certification and regulatory bodies (Selma et al. 2008). It has been approved in many developed countries including the USA, Japan, Australia, France, and Canada (O'Donnell et al. 2012). Ozone destroys cellular components by oxidizing sulfhydryl groups and amino acids of enzymes, peptides, and proteins to shorter peptides (Victorin 1992) and then reacts with the double bonds of unsaturated lipids in the cell envelope, causing leakage of cell contents and eventually microbial lysis (Scott and Lesher 1963). However, microorganisms can display different vulnerabilities to ozone, for example, bacteria are more sensitive than fungi, Gram-positive bacteria are more sensitive than Gram-negative ones, and bacterial spores are more resistant than vegetative cells. Surface area is also an essential parameter in surface decontamination by using ozone. Higher ozone concentration and longer exposure time are required to achieve the same degree of microbial decontamination for smaller particles with larger surface area (Zagon et al. 1992).

Akbas and Ozdemir (2008) were able to reduce 2 log of *Escherichia coli* on inoculated flaked red pepper by a 360 min exposure to ozone at concentration of 1 mg/L. Moreover, Zhao and Cranston (1995) were able to reduce *Salmonella* and *Escherichia coli* populations to 3–4 CFU/g in ground black pepper after 60 min treatment with gaseous ozone (6.7 mg/L at a flow rate of 6 L/min). In addition, researchers also recorded volatile oil changes in gaseous ozone-treated ground

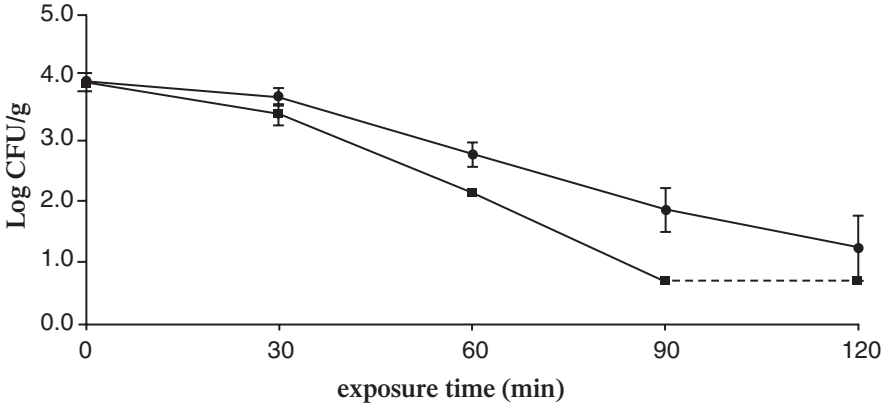

Fig. 10.4 Reduction of aerobic plate count on dried oregano by gaseous ozone treatment at two different concentrations (▲ 2.8 mg/L, ■ 5.3 mg/L) for 120 min. (——) indicates below the detection limit (From Torlak et al. 2013)

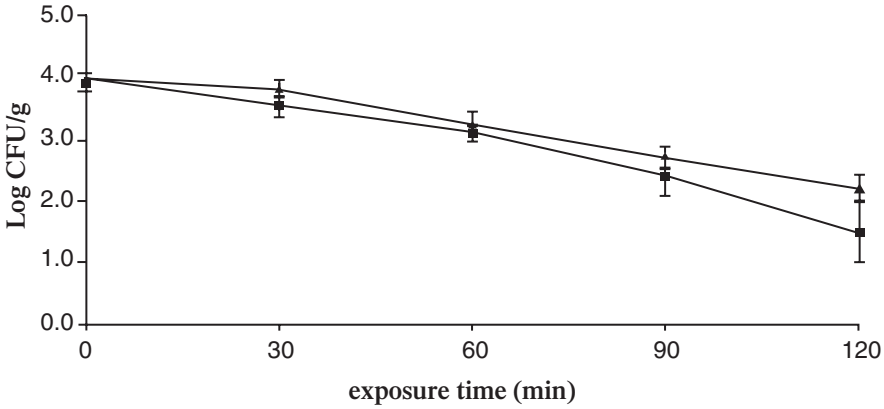

Fig. 10.5 Reduction of yeast and mold counts on dried oregano by gaseous ozone treatment at two different concentrations (▲ 2.8 mg/L, ■ 5.3 mg/L) for 120 min (From Torlak et al. 2013)

black pepper; however, it was not significant in whole black peppercorns. Sengun (2013) achieved 0.28–2.57 log reductions in *Salmonella typhimurium*-inoculated parsley samples by treating with aqueous ozone (0.5, 1.0 and 1.5 ppm) for 3, 5, and 10 min. Torlak et al. (2013) determined significant reductions of 2.7 and 1.8 log in aerobic plate counts and yeast and mold counts of oregano samples after ozonation at 2.8 mg/L for 120 min, respectively (Figs. 10.4 and 10.5). In addition, initial population of a cocktail of *Salmonella* serotypes (S. Typhimurium, S. Newport, and S. Montevideo) on inoculated oregano samples decreased from 5.8 log CFU/g to 2.8 and 3.7 log after gaseous ozone treatment at 2.8 and 5.3 mg/L, respectively, for 120 min (Fig. 10.6). Although gaseous ozone treatment of dried oregano at 2.8

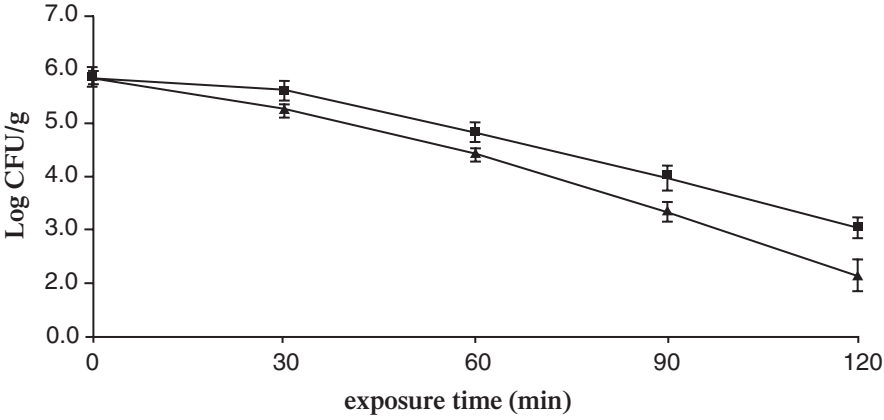

Fig. 10.6 Reduction of *Salmonella* on dried oregano by gaseous ozone treatment at two different concentrations (▲ 2.8 mg/L, ■ 5.3 mg/L) for 120 min (From Torlak et al. 2013)

mg/L for up to 120 min did not affect the sensorial quality including taste, flavor, and appearance, increasing ozone concentration to 5.3 mg/L affected appearance significantly (Torlak et al. 2013). According to the following study, there were no significant changes observed in organoleptic properties of ozone-treated flaked red pepper at the concentration of 5 mg/L for 360 min; however, increasing ozone concentration resulted in significant color changes in samples (Akbas and Ozdemir 2008). Ozone treatment breaks conjugated double bonds in foods (Sarasa et al. 1993) and then chromophores; hence color degradation occurs (Nebel 1975). That is why higher ozone concentration with the prolonging treatment time resulted in higher color degradation in spices (Zagon et al. 1992).

Brodowska et al. (2014) treated cardamom seeds with ozone concentration from 160 to 165 g/m^3 for 30 min three times at 24 h intervals, at a 0.1 L/min flow rate and 0.5 atm pressure. The study indicated contamination of seeds with mesophilic bacteria decreased from 10^5 to 10^3 CFU/g, while the total fungal count varied between 10^2 and 10 CFU/g and seeds retained its original color. Moreover, compared to control samples, the total phenolic content and total antioxidant capacity of ozone-treated seeds reduced 41.2% and 16.2%, respectively. However, improved ferric-reducing antioxidant power activity and better radical scavenging activity were determined in cardamom samples. The reaction mechanism between ozone and polyphenols is not well understood, but reduction in polyphenol content might be caused by oxidation (Gurol and Vatistas 1987). Most probably, it is because of carbon-carbon double bond breakage by ozone and consistence of a new carbonyl compound. Besides, oxidation of phenolic compounds may result in series of aldehyde acids and diacids with ten possible six-carbon compounds. Furthermore, hydroxylation of the benzene ring of phenol can create dihydric phenols (Gould and Weber 1976).

10.2.3 Irradiation

Irradiation is a nonthermal process that inactivates food-borne pathogens, reduces spoilage microorganisms, inhibits ethylene production, and retards the ripening process (Thayer and Rajkowski 1999; Lacroix and Vigneault 2007). Irradiation doses up to 1 kGy have been permitted by USFDA for use on fresh produce (Federal Register 1986); however, doses up to 4 kGy have been approved for iceberg lettuce and spinach only to control food pathogens (USFDA 2008). In irradiation treatment of dried aromatic herbs, spices, vegetable seasonings, and blends of these aromatic vegetable substances, USFDA allowed doses up to 30 kGy (USFDA 2015c).

Low-dose gamma irradiation of *Escherichia coli* O157:H7- and *Salmonella*-inoculated fresh mint at 1–2 kGy caused more than 5 log reduction; however, the effect of irradiation on MS2 bacteriophage was not significant (Hsu et al. 2010). Researchers indicated that visual quality and percent decay improved at 2 kGy and chlorophyll content increased at doses higher than 0.6 kGy. Zaied et al. (1996) found gamma irradiation treatment at 10 kGy resulted in loss of major flavor components such as anethol, anise-aldehyde in anise, β-pinene, and cineol in black pepper. In gamma irradiation of ground liquorice roots with a dosage of 5–20 kGy, there was no microbial growth during 12 months of storage at room temperature. Irradiation did not affect the sensorial quality of ground liquorice roots, but lowered glycyrrhizinic acid concentration in the extracts and the viscosity of suspensions produced from roots (Al-Bachir and Lahham 2002).

Some researchers focused on influence of irradiation to the antioxidant activity of herbs and spices. Calucci et al. (2003) applied gamma irradiation to the nine aromatic herbs and spices (basil, bird pepper, black pepper, cinnamon, nutmeg, oregano, parsley, rosemary, and sage) at the dose of 10 kGy and found a general increase of the quinone radical content in all samples and significant decrease of total ascorbate and carotenoids content of some spices. In another study, Murcia et al. (2004) performed irradiation treatment to seven dessert spices (anise, cinnamon, ginger, licorice, mint, nutmeg, and vanilla) at the doses from 1 to 10 kGy, and it did not affect the antioxidant activities compared to non-irradiated spices. Mishra et al. (2006) evaluated the effect of gamma irradiation on packed dry tea leaves at a dosage of 1–10 kGy with a dose rate of 80 Gy/min, and there was no change in antioxidant, biochemical, antimicrobial, and sensory properties of tea samples. Kim et al. (2009) treated cumin seeds with gamma irradiation at a dosage of 1–10 kGy at ambient temperature. The gamma irradiation did not change DPPH radical scavenging effect, ferrous-reducing antioxidant power, and total polyphenolic content of cumin seeds, thereby significantly protected the natural antioxidants. Irradiation of sun-dried and dehydrated paprika samples at a dose of 10 kGy increased contents of capsaicin, dihydrocapsaicin, and homodihydrocapsaicin by 10% in samples (Topuz and Ozdemir 2004). Rico et al. (2010) also compared treatments of steaming at 1020 mbar and 100 °C for 16 min and gamma irradiation at 10 kGy with a dose rate of 2.5 kGy/h on dried red pepper powder (*Capsicum annum L.*) and evaluated physicochemical and microbiological properties during 6 months of

storage at refrigerated and room temperatures. There was 1 log reduction in steam-treated red pepper powder; however, irradiation resulted in 5 log reduction with minimal effects on the physicochemical properties, except for the decreased content of capsanthin in the irradiated samples. Similarly, a dosage of 10 kGy reduced the total aerobic bacteria by 4–5 logs in powdered hot pepper (Farag et al. 1995), red chili pepper (Munasiri et al. 1987), and ground black pepper (Waje et al. 2008). Rico et al. (2010) recommended refrigerated storage for irradiated red pepper powder to minimize physicochemical changes. Consumer acceptance of irradiated food still remains questionable.

Ultraviolet (UVC) treatment is also a nonthermal method approved to use as a disinfectant for surface treatment of food products (USFDA 2015b), which effectively and rapidly inactivates pathogen microorganisms by transferring the electromagnetic energy from a source without any residue on products (Koutchma et al. 2009). UVC irradiation treatment of *Escherichia coli* O157:H7- and *Salmonella typhimurium*-inoculated powdered red pepper at 20.4 kJ/m^2 for 10 min reduced 0.22 and 0.29 log CFU/g, respectively. However, combined treatment with mild heating at 65 °C reduced surviving numbers of each pathogen by 2.88 and 3.06 log CFU/g, respectively (Cheon et al. 2014).

10.2.4 Electrolyzed Oxidizing Water

Electrolyzed oxidizing water (EOW) is an alternative to traditional chlorine sanitizer with strong antimicrobial effect in food industry. EOW is divided into two types based on the acidity level: slightly acidic electrolyzed water (SAEW) with a pH value of 5.0–6.5 and available chlorine concentration (ACC) of 10–30 mg/L and acidic electrolyzed water (AEW) with a lower pH value (<3.0), a high oxidation-reduction potential (ORP, >1,000 mV), and an ACC of 80–200 mg/L (Hao et al. 2015). Usage of AEW may cause corrosion on surfaces and rapid loss of chlorine followed by HClO decomposition, thereby decreasing the bacterial effectiveness of the solution (Guentzel et al. 2008). For this reason, SAEW is a promising alternative to AEW with neutral pH and lower ACC, which does not cause corrosion of processing equipment or irritation of hands (Abadias et al. 2008; Cao et al. 2009).

Hao et al. (2011) evaluated efficacy of AEW (acidic electrolyzed water), SAEW (slightly acidic electrolyzed water), and NaClO solutions on *Escherichia coli*- and *Bacillus subtilis*-inoculated fresh-cut cilantro. Dipping into AEW, SAEW, and NaClO solutions for 5 min resulted in a reduction in populations of *E. coli* from 6.38 to 4.93, 3.89, and 4.88 log10 cfu/g and in populations of *Bacillus subtilis* from 6.52 to 5.02, 4.98, and 4.63 log10 cfu/g, respectively. Although AEW wash is effective on initial microbial count reduction in fresh-cut cilantro, there might be tissue damage in cilantro due to electrolyte leakage (Wang et al. 2004). Mild heating to 50 °C increased disinfectant ability of AEW compared to SAEW in fresh-cut cilantro, but decreased sensorial quality of products (Hao et al. 2011).

10.2.5 Packaging and Storage

Modified atmosphere packaging (MAP) is generally used in minimally processed fruits and vegetables by adjusting gas composition –O_2 and CO_2 levels in the package to reduce the respiration rate of fresh produce, thereby inhibiting the spoilage mechanisms and extending the shelf life. Respiration activity of the product, storage temperature, and permeability characteristic of packaging material are some of the important parameters affecting the final gas concentration in the package (O'Beirne 1990). MAP delays the browning and spoilage by reducing O_2 and increasing CO_2 level in the surrounding atmosphere of fresh produce (Cameron and Smyth 1997); however, not only MAP but also good hygiene practices and refrigeration are required (Willocx et al. 1994).

Chives, coriander, parsley, and spearmint leaves were washed, cut, packed, and stored for 10 days at refrigerated storage (Santos et al. 2014). Coriander and parsley showed less than 10% variation in color values (L*, a*, b*, C) in storage days 1 and 10 (Fig. 10.7). During the storage, there was no change in flavonoid content of chives and parsley; however, 29 and 12% reductions were observed in coriander and spearmint, respectively. Furthermore, total soluble phenolic content increased 20% in chives, 16% in parsley, and 10% in spearmint leaves and decreased 7.5% in coriander (Santos et al. 2014).

Refrigerated storage is preferred in minimal processing to prevent deterioration due to physiological ageing, biochemical changes and microbial spoilage, and quality losses including color (discoloration and yellowing), texture (loss of crispness or juiciness), and flavor (off-flavors), hence extending the shelf life. Catunescu et al. (2012) found the maximum shelf lives for minimally processed parsley, dill, and lovage are about 23, 14, and 15 days, respectively, under refrigerated conditions based on sensory evaluation.

10.3 Thermal Processing Technologies for Minimal Processing of Herbs, Spices, Medicinal and Aromatic Plants

Steaming is the most common thermal technology used for decontamination of produces in Europe (Schweiggert et al. 2007). However, steam treatment and most of the thermal technologies are not preferred for minimally processed fresh herbs, dried herbs, and spices due to negative efficacy on the fresh quality, sensorial values, and nutritional losses. On the other hand, nonthermal technologies have disadvantages from a health perspective (ethylene oxide) or poor consumer acceptance (gamma irradiation) (Eliasson et al. 2015). Hence, thermal processing technologies have been tested in decontamination of herbs, spices, medicinal and aromatic plants as alternative processing technologies.

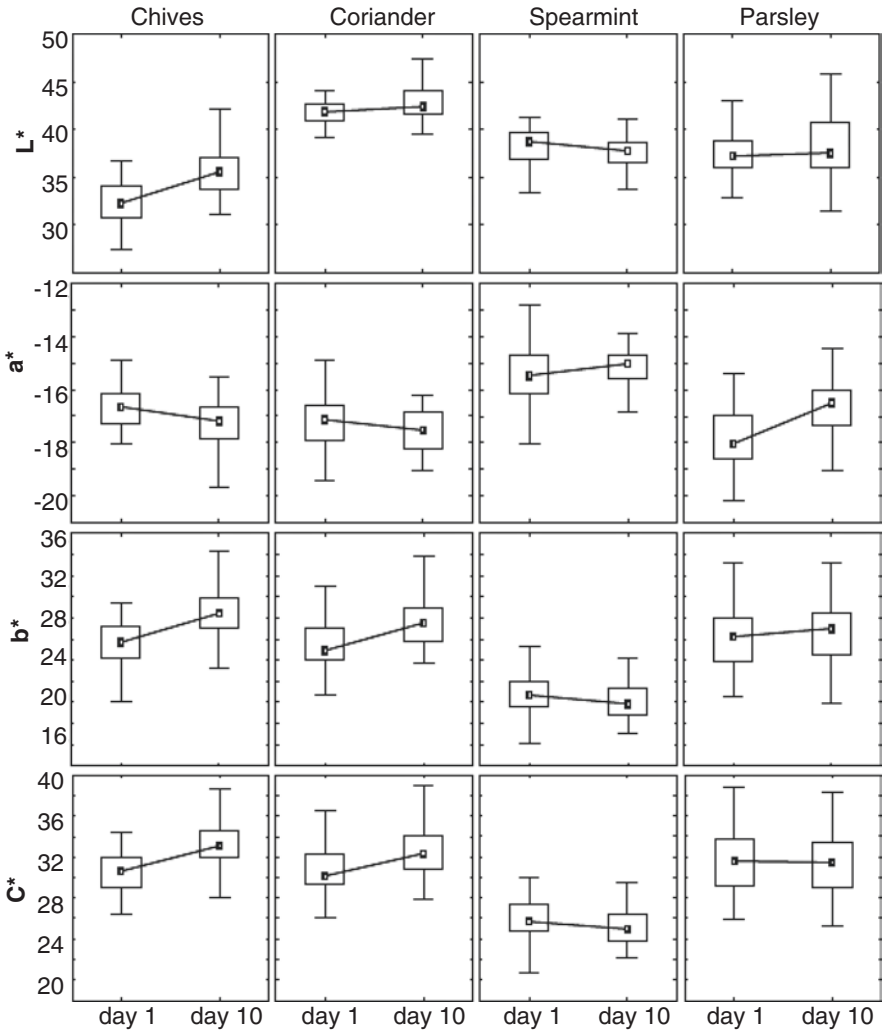

Fig. 10.7 Color values (L*, a*, b*, C*) of fresh-cut aromatic herbs during 10 days at refrigerated conditions (From Santos et al. 2014)

Far infrared radiation is used for surface decontamination of food products by changing vibrational state of molecules and increasing heat at the surface after infrared energy dispersion (Sakai and Hanzawa 1994; Fasina and Thomas 2001; Krishnamurthy et al. 2008). In FIR, the heat transfer in interior side of the food products is slow due to low thermal conductivity; therefore, heat accumulates at the surface. FIR improves the sensory characteristics of food products with rapid surface heating, which enhances sealing moisture, flavor, and aroma compounds (Sakai and Hanzawa 1994; Fasina and Thomas 2001; Huang 2004). Erdogdu and Ekiz (2011) evaluated the effect of FIR and UVC treatments (Fig. 10.8) on reducing the

Fig. 10.8 Lab scale far infrared (FIR) tunnel and ultraviolet (UVC) cabinet (From Erdogdu and Ekiz 2011)

number of microorganisms in cumin seeds. Treatment of cumin seeds with UVC for 60 min under a constant intensity of 10.5 mW/cm^2 caused about 0.6 log unit decrease which is not efficient. Other studies indicated that UVC treatment alone is not sufficient in reducing microorganisms (Sharma and Demirci 2003; Fine and Gervais 2004), but increasing treatment time in UVC treatments increased the microbial reduction (Hidaka and Kubota 2006). Erdogdu and Ekiz (2011) were able to eliminate coliform and fecal coliform bacteria in cumin seed samples by increasing treatment time to 2 h under an intensity of 10.5 mW/cm^2. Combined treatment of UVC light (for 2 h under intensity of 10.5 mW/cm^2) with FIR at different treatment times and temperatures ranged from 1.82 and 6.03 min at 200 °C, 1.57 to 4.4 at 250 °C, and 1.57 to 2.98 min at 300 °C resulted in additional reductions ranged from 0.54 to 1.65, 0.6 to 1.37, and 0.76 to 1.36 at 200, 250, and 300 °C FIR temperatures, respectively. Even though FIR treatment of cumin seeds is sufficient for microbial reduction, combining with UVC treatment decreased the treatment time of FIR, thereby decreasing the quality loss due to thermal effect including changes in volatile oil content and color (Erdogdu and Ekiz 2011). Microwave energy has been used in food processing applications because of its ability to cause fast volumetric heating that penetrates into the bulk of the material with wavelengths ranging from 1 mm to 1 m and frequencies between 300 MHz and 300 GHz.

The heating mechanism of microwave treatment is depending on the interaction between their electric fields and sample. The movement of dipoles and ions results in final heating, which depends on many factors such as food-related properties—moisture content, density, dielectric properties, and temperature—and the actual design of the microwave heating device. Even though microwave heating reduces processing time due to fast direct heating of material, the main problem is variations of temperature within the treated foods (Meredith 1998).

Eliasson et al. (2015) compared the effect of infrared (IR) and microwave thermal treatments on decontamination of naturally contaminated paprika powder. Researchers applied IR and microwave (650 W for 60 s) heating to paprika powder with a water activity 0.88 to 98 °C. Then, paprika samples were hold in a conventional oven at 98 °C for additional 20 min. They achieved 4.8 and 3.8 log reduction of total mesophilic bacteria in paprika powders with microwave and IR treatments, respectively. According to the thermal images, IR generated the highest temperature and homogeneous heating over the entire surface; however, heating was not homogeneous over the entire surface in microwave-treated samples, which explains the reason of hot and cold spot occurrence (Eliasson et al. 2015). Besides the microbial decontamination, Plessi et al. (2002) focused on changes in volatile compounds during microwave treatment of white and black pepper. According to this study, microwave treatment preserved the main aroma compounds in samples. Kiralan (2012) compared microwave and conventional roasting and then evaluated volatile compounds in cumin seeds. The compounds 2-ethyl-3-methylpyrazine, 2-ethenyl 5-methylpyrazine, 2-acetylpyrazine, 3-ethyl-2,5-dimethylpyrazine, and 1-(5-methyl-2-pyrazinyl)-1-ethanone were found in microwave-roasted samples only. Higher 3-methylbutanal and furfural compounds were determined in conventional-roasted samples compared to microwave-roasted samples.

10.4 Conclusion

Researchers have been investigating both thermal and nonthermal processing technologies to prevent contamination and ensure high quality in minimally processed herbs, spices, medicinal and aromatic plants. Although nonthermal treatments are preferred in minimally processed produces due to its fresh-like quality, alternative thermal treatments are also being evaluated. There should be additional investigations for new technologies to prevent the growth of microorganisms, improve physical quality and extend shelf life. Furthermore, it is important to do research focused on new packaging systems for the safety and quality of herbs, spices, medicinal and aromatic plants.

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Chapter 11
Sprouts, Microgreens and "Baby Leaf" Vegetables

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11.1 Introduction

In recent years, people have developed a substantial interest for the consumption of fruits and vegetables characterized by a high content of bioactive substances. It is known that these are beneficial because besides providing essential nutrients for the human body, they have positive effects on human health (Galaverna et al. 2008). Sprouts, microgreens and ready-to-eat "baby leaf" vegetables constitute a growing market segment within the sector of the vegetable products. In many countries around the world, restaurant chefs and consumers use these products both for their sensorial and nutritional properties.

According to the Commission Implementing Regulation (EU) 208/2013, the term "sprouts" indicates "the product obtained from the germination of seeds and their development in water or another medium, harvested before the development of true leaves and which is intended to be eaten whole, including the seed" (European Union 2013). The sprouts have additional regulations concerning their production and marketing, due to their relatively high risk of microbial contamination. According to the Commission Regulation (EU) 752/2014, the term "baby leaf" indicates "the young leaves and petioles of any crops (including Brassica) harvested up to 8 true leaf stage". Instead, "microgreens" is a marketing term used to describe a category of product that has no legal definition, yet (Treadwell et al. 2010). To better understand the distinctive traits of these three types of product, Table 11.1 reported the main differences among sprouts, microgreens and baby leaf vegetables.

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Table 11.1 Differences among sprouts, microgreens and "baby leaf" vegetables

	Sprouts	Microgreens	Baby leaf
Growing cycle	4–10 days	7–28 days	20–40 days
Edible portion	Whole sprout including the rootlets	Shoots with cotyledons and first hint of true leaves without roots	True leaves and petioles without roots
Growth system	Soilless: only water is required without the use of a growing medium	Mainly soilless: a growing medium is required	Soil or soilless: a growing medium is required
Growth environment	Do not require light	Require light	Require light
Nutrient requirement	Not required	Required in small amount if the growing medium does not provide nutrient	Always required
Use of agrochemicals	Not required	Not required	Required
Plant development stage at harvest	Before fully development of the cotyledon leaves	Between fully development of cotyledons and appearance of the first true leaves	Between full development of the first true leaves and eight true-leaf stage
Harvest	Without cutting	Optional by or without cutting	By cutting

Translated from Di Gioia et al. (2015b)

Sprouts and microgreens can easily be grown also in urban or peri-urban settings, where land is often a limiting factor, by either specialized vegetable farmers or the consumers themselves. Given their short growth cycle, sprouts are usually grown in the dark, without a growing medium and without external inputs such as fertilizers and agrochemicals, and are characterized by a very short cycle, and the edible portion is constituted by the entire sprout, including the rootlets. By contrast, baby leaf vegetables are grown in the presence of light, either in soil or in soilless systems using alternative media. Moreover, having a longer growth cycle, baby leaves require the use of fertilizers and agrochemicals and are harvested after the development of true leaves. Microgreens have common traits with both sprouts and "baby leaf" crops; however, they differ from the sprouts because they require light and a growing medium and have a longer growth cycle, and the edible portion is constituted only by the shoots. While compared to baby leaves, microgreens have a shorter cycle being harvested before the development of the true leaves, do not require the use of agrochemicals and can be marketed even without cutting the seedlings. At any rate, sprouts, microgreens and baby leaf vegetables are principally consumed as raw products. Therefore, since only mild treatments (i.e. cleaning, cutting, packaging and refrigerated storage) are required, there is no loss or degradation of bioactive compounds through heat processing such as cooking and sterilization. Thus, precisely for this reason, an important quality aspect of these vegetable productions is their microbiological safety, especially in the case of sprouts.

Nevertheless, applying pre- and postharvest techniques, it is possible to preserve and/or enhance the quality of these minimally processed vegetables, including the microbiological safety.

11.2 Sprouts

11.2.1 Description

Sprouts are constituted by shoots and rootlets, deriving from totally or partially germinated seeds, usually produced in the dark and soaked in water, with a production cycle of just a few days (Table 11.1). From a biological point of view, the sprout represents the first stage of growth of a plant that starts with the seed germination. How a seed transforms into a sprout is a very complex tale that begins before the seed itself is produced. In developing the seeds, a plant stores all vitamins, minerals, proteins, fats and carbohydrates necessary to give birth to another plant of its species. As seeds sprout, some significant chemical changes take place. Seeds begin to produce enzymes that are vital in converting the stored and concentrated nutrients into simpler nutrients and energy that are essential to carry on the life cycle, before the new plantlets are able to start the photosynthesis and be self-sufficient. So, sprouts are what is called "biogenic food", which means they are a living food (Helweg 2011), characterized by calories that are full of vitamins, minerals, protein and many healthy compounds. Sprouts can be easily produced from seeds of numerous species, all year round, either at home or on a large scale at commercial level. Untreated seeds of good quality, characterized by high germination rate, are washed and soaked in lukewarm water for 6–12 h at room temperature. The seeds are then densely packed into sprouting cells or vessels (glass jar, plastic pan) and covered with cheese cloth or a greenhouse tent to maintain temperature and moisture (Meyerowitz 2010). The seeds need to be rinsed or sprinkled several times per day to keep high humidity levels and facilitate the sprouting process. Well, spring or distilled water should be used for rinsing, as chlorinated water may result in poor sprouting (Bass and Sanders 1999). Sprouts take five to 14 days to grow, depending on the species (Meyerowitz 2010). Humans have been eating sprouted seeds, grains, legumes and nuts for thousands of years. There are records indicating that the Chinese have been growing and consuming bean sprouts for more than 5000 years. Bean sprouts, as used by the Chinese, have long been considered to have great health benefits and have been used to prevent and cure many ailments (Helweg 2011). Sprouts have been used to reduce inflammation, cure rheumatism and produce a laxative effect (Helweg 2011). Sprouts are a happy addition to many sandwiches, noodle dishes and side plates at restaurants from decades. On the other hand, sprouting is also applied on a large scale to barley as a part of the malting process. Malted barley is an important ingredient in beer and is used in huge quantities (Fig. 11.1).

Fig. 11.1 Professional equipment for sprouting

11.2.2 Utilized Species

Crop groups used for sprouting include legumes (i.e. alfalfa, azuki bean, blackgram, chickpea, lentil, mungbean, soybean), cereals (i.e. barley, maize, oat, rice, rye, wheat), pseudo-cereals (i.e. amaranth, buckwheat, quinoa), oilseeds (i.e. almond, hazelnut, linseed, sesame, sunflower) as well as vegetables (i.e. broccoli, cabbage, carrot, celery, clover, fennel, kale, leek, lettuce, mustard, parsley, radish, arugula, snow and garden peas, spinach, spring onion, turnip, watercress) (Ebert 2012).

Among the bean sprouts, mungbean (*Vigna radiata* (L.) Wilczek) sprouts are the most common and most consumed and are now found worldwide. Mungbean and, to a lesser extent, soybean sprouts have long been a staple of Oriental and vegetarian diets. Mungbean sprouts are sold in market stalls in most of Asia, Central America and East Africa and in recent years have become quite popular also in Western countries where the main food chain stores stock them. Mungbeans can be grown in about 5 days to 5 cm long mature sprouts. Within 8 days they can grow up to 8–9 cm long sprouts, but longer growth over 10 cm should be avoided as sprouts then tend to become bitter (Bass and Sanders 1999). Mature sprouts are placed in a water-filled container for washing and removal of the seed coats and fibrous roots. The sprouts sink to the bottom and the seed hulls float to the top and can then easily be removed with a wire strainer. Sprouts are then allowed to drain.

Soybean sprouts are known for their appealing nutty taste and good texture. They are produced from special small-seeded soybean varieties and are the most popular sprouts in Korea. In Japan, blackgram (*Vigna mungo*) is preferred as its sprouts are whiter and stay fresh for longer than mungbean sprouts. Sprouts are best when consumed immediately after washing, but they can also be kept inside closed glass and plastic containers or freezer bags for a few days up to 1 week in a refrigerator. Sprouts can be eaten raw or cooked (Ebert 2012). The crispy sprouts are often added fresh to appetizers, salads, soups, sandwiches and even desserts to add texture

and flavour. Cereal sprouts are also used in casseroles, pasta and baked products (Lorenz 1980). Sprouts can also be canned or frozen if there is excess produce for fresh consumption, but this might lead to a deterioration of their nutritional value.

11.2.3 Nutritional Traits

Sprouts are commonly considered highly nutritious and are sometimes called "miracle food" (Meyerowitz 2010). In fact, they are outstanding sources of proteins, vitamins and minerals and have high content of beneficial compounds such as glucosinolates, phenolic and selenium-containing components in the *Brassica* species or isoflavones in the soybean (Meyerowitz 2010). As the sprouts are consumed few days after the germination, their nutrient concentration remains very high. Compared to the seed, it was established that the sprout, due to its transformed protein content, which is of higher biological value, and to the higher polyunsaturated fatty acid content, higher vitamin content and the better bioavailability of minerals, has a higher nutritional value. During the germination, the polysaccharides degrade into oligo- and monosaccharides and the fats into free fatty acids, whereas the proteins are reduced into oligopeptides and free amino acids, whose processes support the biochemical mechanisms in our organism. They improve the efficiency of both the protein decomposing and the carbohydrate- and fatty acid-decomposing enzymes. Therefore, the germination can be considered as one kind of predigestion that helps to break down the high-molecular complex materials into their building blocks (Marton et al. 2010). Soybean sprouts have the highest level of protein (28%), followed by lentil and pea sprouts with 26% (Meyerowitz 2010). Soybean sprouts thus have twice the protein content of eggs, but only a tenth of the fat, and are the valuable dietary supplements that may promote health and well-being in many parts of the world and particularly in the rural areas of Asian countries, where seasonal fruits and vegetables are not available all year round (Mbithi et al. 2001). The flatulence-producing carbohydrates in legumes largely disappear during sprout formation resulting in low levels of stachyose and raffinose (van Hofsten 1979). Due to the biochemical changes during germination, sprouts contain significantly higher levels of vitamins than the respective dry seeds. Some sprouts such as mungbeans are very good sources of ascorbic acid reaching over 50 mg/100 g of fresh weight (van Hofsten 1979). Vitamins of the B group increase from 100 to 300% during germination, and therefore, sprouts are often a good source of vitamin B12. Moreover, during germination, the phytic acid present in the seed is degraded, due to phytase enzyme activity, resulting in higher availability of the trace minerals compared to the dry seed (van Hofsten 1979). Isoflavonoids, which protect against cancer, cardiovascular disease and osteoporosis, are found in relatively high concentrations in soybean sprouts, but their level is much lower in other legume species (Nakamura et al. 2001). The composition of isoflavonoids differs significantly between soybean sprouts, immature beans and mature beans. The highest levels of isoflavonoids in aglycone form – known to be of stronger biological activity than the

glycoside form – are found in soybean sprouts and progressively decrease from sprouts via immature beans to mature beans, the latter having the lowest levels. The percentage of the glucoside form of isoflavonoids increases in reverse order. Some health-protecting phytochemicals can be found in the sprouts in a much higher concentration than in the developed plant (Fernàndez-Orozco et al. 2006). For example, studies on broccoli revealed that broccoli sprouts contain on a gramme-fresh-weight basis up to 50 times more glucoraphanin, the glucosinolate precursor of sulphoraphane, compared with mature broccoli plants (Fahey et al. 1997, 2001; Fahey and Stephenson 1999). Therefore, sprouts can be considered as new foodstuffs rich in nutrients and phytonutrients beneficial for human health. Finally, sprouts may be biofortified to increase the content of essential micronutrients (Zou et al. 2014), and other strategies like the application of ultrasounds on seeds, before starting germination, may be used to enhance their nutritional quality (Yang et al. 2015).

11.2.4 Production

The sprout production takes a few days and can be done at home manually, as a semiautomated process, or industrially on a large scale at commercial level. It is important to highlight that seeds for sprout production should be obtained under good agricultural practices (GAPs) and should be stored properly to minimize any possibility of contamination of the seeds with pathogens. For example, seeds should be stored in closed or covered containers in a clean dry area exclusively dedicated to seed storage. Containers should be positioned off the floor and away from walls to reduce the possibility of contamination by rodents or other pests and to facilitate regular monitoring for pest problems (Food and Drug Administration 1999). Typically, the seeds for sprout production are first rinsed to remove soil, dirt and the mucilaginous substances produced by some seeds when they come in contact with water. Anyway, seeds for sprouting should be treated with one or more antimicrobial (such as calcium hypochlorite) that have been approved for reduction of pathogens in seeds or sprouts. Some treatments can be applied at the sprouting facility, while others will have to be applied earlier in the seed production process. However, at least one approved antimicrobial treatment should be applied immediately before sprouting (Food and Drug Administration 1999). Then they are soaked for 20 min to 12 h, depending on the type and size of seed, in order to increase the water content in the seeds and bring them out of quiescence. Considering that currently approved antimicrobials have not been shown to be capable of eliminating all pathogens from seeds, sprout producers should conduct microbiological testing of spent irrigation water from each production lot to ensure that contaminated product is not distributed. Because testing for pathogens can be done with irrigation water as early as 48 h into what is generally a 3- to 10-day growing period, producers who plan accordingly can obtain test results before shipping product without losing product shelf life (Food and Drug Administration 1999). After draining and then rinsing seeds at regular intervals, they germinate or sprout. For home sprouting, the seeds

are soaked (big seeds) or moistened (small) and then left at room temperature in a sprouting vessel. Many different types of vessels can be used. One type is a simple glass jar with a piece of cloth or nylon window screen secured over its rim. "Tiered" clear plastic sprouters are commercially available, allowing a number of "crops" to be grown simultaneously. By continuous sowings, a constant supply of fresh sprouts can be ensured. Any vessel used for sprouting must allow water to drain from it, because sprouts that sit in water will rot quickly. The seeds swell, may stick to the sides of the jar and begin germinating within a day or two. Another sprouting technique is to use a pulse drip method; it is a one-way watering system with micro-sprinklers providing intermittent pulses of fresh water to reduce the risk of bacterial cross-contamination during the sprouting process. In this context, it is important to highlight that the conditions for the production of sprouts may pose a potentially high public health risk, as they may result in a significant multiplication of food-borne pathogens. Sprouts are rinsed two to four times a day, depending on the climate and the type of seed, to provide them with moisture and prevent them from souring. Each seed has its own ideal sprouting time. After 3–5 days, the sprouts may be grown 5–8 cm in length and may be ready for consumption. Refrigeration can be used as needed to slow or halt the growth process of any sprout. Mungbeans can be sprouted either in light or more frequently in a dark environment. When sprouted in the dark, they will be crisper in texture and whiter, as in the case of commercially available Chinese bean sprouts, but these have less nutritional content than those grown in partial sunlight. Growing in full sunlight is not recommended, because it can cause the beans to overheat or dry out. Subjecting the sprouts to pressure, for example, by placing a weight on top of them in their sprouting container, will result in larger, crunchier sprouts similar to those sold in grocery stores. Another effective way to sprout beans, like lentils or azuki, is in colanders. Soak the beans in water for about 8 h, and then place the soaked beans in the colander and wash them twice a day until they are ready to eat.

11.2.5 Microbiological Risk

Sprouts should be considered as ready-to-eat food, as they can be consumed without the need for cooking or other processing, which would otherwise be effective in eliminating or reducing to an acceptable level pathogenic microorganisms. For commercial production, sprouts typically germinate from seeds in rotary drums or other types of containers with high humidity and frequent or constant watering. The conditions for sprouting, including relatively high temperature, high humidity, low (or no) light and abundance of nutrients from sprouting seeds, may determine rapid bacterial growth. It has been reported that the *Escherichia coli* population on the final products of sprouts could exceed 7 log cfu/g without negatively affecting the appearance of products (Taormina et al. 1999). In addition, the high volumes of water used during sprout production offer opportunities for pathogens to be spread between sprouts within the production batch, especially in systems where sprouts

are exposed to a common "water bath" and frequently or continuously mixed, like in a rotary drum. Therefore, even if sprouts have gained popularity worldwide as they are perceived as healthy sources of carbohydrates, proteins, minerals and vitamins, their consumption has been implicated in several high-profile food-borne illness outbreaks. An outbreak of enterohaemorrhagic *E. coli* (EHEC) O157:H7, which affected over 6000 people in Japan in 1996, was linked to the consumption of contaminated radish sprouts (Taormina et al. 1999). More recently, sprouts from an organic farm in Germany were determined to be the source of an outbreak of enteroaggregative *E. coli* (EAEC) O104:H4, which infected nearly 4000 people in 2011 and caused 53 deaths (Uphoff et al. 2014). These outbreaks have heightened public health concerns over the safety of sprouts from consumers and from officials at federal and local regulatory agencies and prompted many food retail and service establishments to institute policies restricting the availability of sprouts. On 20 October 2011, the European Food Safety Authority (EFSA) adopted a Scientific Opinion on the risk posed by Shiga toxin-producing *E. coli* STEC and other pathogenic bacteria in seeds and sprouted seeds (European Food Safety Authority 2011). In its Opinion, EFSA concludes that the contamination of dry seeds with bacterial pathogens is the most likely initial source of the sprout-associated outbreaks. In addition, the Opinion states that, due to the high humidity and the favourable temperature during sprouting, bacterial pathogens present on dry seeds can multiply during sprouting and result in a public health risk. Moreover, EFSA recommends, inter alia, that microbiological criteria should be strengthened as one of the components of a food safety management system for the sprouted seed production chain. That recommendation concerns the existing microbiological criteria on *Salmonella* for sprouted seeds and the consideration of microbiological criteria on other pathogens. EFSA considers different options for microbiological criteria for pathogenic *E. coli* for seeds: before the start of the production process, during sprouting and in the final product. In that context, EFSA states that the detection and mitigation of a contamination problem earlier in the sprouted seed production chain may have advantages as it avoids contamination being amplified during the full sprouting process. It also acknowledges that testing seeds alone does not allow for the detection of contamination, which may come at a later stage in the production process. EFSA therefore concludes that microbiological criteria could be useful during the sprouting process and/or for the final product. When considering a microbiological criterion for the final sprouted seeds, EFSA notes that the time required for the detection methods for pathogenic bacteria combined with the short shelf life may not allow withdrawing the product in the event of non-compliance. In its opinion, EFSA considers that it is currently not possible to evaluate the extent of public health protection provided by specific microbiological criteria for seeds and sprouted seeds. This highlights the need for data collection to conduct quantitative risk assessment. Therefore, this criterion should be reviewed taking into account progress in science, technology and methodology, emerging pathogenic microorganisms in foodstuffs and information from risk assessment.

11.3 Microgreens

11.3.1 Description

Microgreens are young and tender edible seedlings produced using the seeds of different species of vegetables, herbaceous plants, aromatic herbs and wild edible plants (Di Gioia and Santamaria 2015a, b). Depending on the species that has been used, they can be harvested 7–21 days after germination, when the cotyledon leaves have fully developed and the first true leaves have emerged (Xiao 2013). Microgreens are harvested by cutting the single seedlings just above the growing media line when their height is 3–9 cm. The edible portion is constituted by the single stem, the cotyledon leaves and, often, by the emerging first true leaves (Table 11.1). In some cases, when small and tender, also the integuments of the seeds that remain attached to the cotyledons may be considered edible (Di Gioia and Santamaria 2015a). Despite small in size, microgreens – also known as "vegetable confetti" (Treadwell et al. 2010) or "microherbs", when referring to aromatic herbs – can provide a wide variety of intense flavours, bright colours and a good texture; therefore, they may be proposed as a new ingredient to enhance and garnish drinks, salads, appetizers, main and second courses, soups, sandwiches and desserts (Treadwell et al. 2010; Xiao et al. 2012). Microgreens represent a new category of vegetables with different traits as compared to the already known sprouts and the common fresh-cut leafy vegetables. Moreover, they should not be confused with mini vegetables, also known as miniature, petite and Lilliputian vegetables, that can be produced by means of specific cultivation techniques (high plant density or anticipated harvest) or refer to genetic material at reduced growth and development. Microgreens first appeared in the menus of the chefs of San Francisco, in California, at the beginning of the 1980s (USDA 2014), and they have been grown in the southern part of California since the second half of the 1990s. As compared to the sprouts, microgreens are grown in greenhouse or in open environment, in soil or alternative growing substrates and in the presence of light; moreover, microgreens have a longer growing cycle and only the aerial part without the roots is edible (Table 11.1). Unlike the classical baby leaf vegetables, whose edible portion is constituted only by the true leaves and is harvested necessarily through a cut, microgreens have the advantage that they can be sold even before being harvested with the cut, keeping the plantlets alive with all the growing media, so that the chef or the final consumer can actually cut the product in their kitchen, even just a few minutes before using them (Di Gioia and Santamaria 2015b). Such possibility to sell the product while it is still growing represents a great innovation as it can guarantee a longer shelf life of the product on the market and assures a high quality in terms of both freshness and nutritional value (Di Gioia and Santamaria 2015b). On the other hand, one of the reasons of the success of this new category of products is represented by the reduction of the time needed or the alternative use of the free time off work, which is more and more leading towards the consumption of vegetables that do not involve

Fig. 11.2 "Rainbow" mix of Swiss chard (*Beta vulgaris* subsp. *vulgaris*) microgreens grown on peat

particular difficulties or too much time in the preparation phase (Di Gioia et al. 2015b). An aspect that makes microgreens particularly interesting, also from a gastronomic and nutritional point of view, is the possibility of using species and varieties whose cotyledon leaves and first true leaves are characterized by a vast array of shapes, colours (green, yellow, red, purple), textures (tender, crunchy, juicy) and tastes (sweet, neutral, slightly sour, spicy) (Di Gioia et al. 2015a) (Fig. 11.2).

11.3.2 Utilized Species

The species of vegetables most commonly used to produce microgreens belong to several botanical families, among which the Brassicaceae (e.g. cauliflower, broccoli, cabbage, Chinese cabbage, kale, Savoy cabbage, rappini or brassica raab, watercress, mizuna, radish, arugula, mustard and tatsoi), Asteraceae (e.g. lettuce, endive, escarole, chicory, radicchio), Apiaceae (dill, carrot, fennel, celery), Amaryllidaceae (garlic, onion, leek), Amaranthaceae (amaranth, red orach, Swiss chard, beet, spinach) and Cucurbitaceae (melon, cucumber, squash) (Di Gioia et al. 2015a). Other herbaceous species commonly used to produce microgreens are cereals (oat, soft wheat, durum wheat, corn, barley, rice), quinoa (that often is assimilated to cereals but belongs to the Amaranthaceae family), leguminous plants (chickpea, alfalfa, bean, green bean, fenugreek, fava bean, lentil, pea, clover), oleaginous plants (sunflower) and even fibre plants like flax, as well as many aromatic species such as basil, chives, cilantro and cumin (Di Gioia et al. 2015a). For all these species, it is possible to use either commercial varieties, some of them particularly selected for the production of microgreens, or local varieties and populations, possibly characterized by seedlings with a particular shape, colour, texture and taste and by a high content of phytonutrients (Di Gioia et al. 2015a). Lastly, there are plenty of wild species, traditionally used in the folk cookery (Sánchez-Mata et al. 2012;

Boari et al. 2013), that can be valorized through the production of microgreens and that can potentially provide a wide range of colours, shapes, tastes and, above all, essential nutrients beneficial for the consumers' health (Di Gioia et al. 2015b). Among the wild edible plants, some of the most interesting species that may be considered for the production of microgreens are, for example, common amaranth (*Amaranthus retroflexus* L.), blood amaranth (*Amaranthus cruentus* L.), sea beet (*Beta vulgaris* L. subsp. Maritima (L.) Arcang.), smooth golden fleece (*Urospermum dalechampii* (L.) F.W. Schmidt), prickly golden fleece (*Urospermum picroides* (L.) Scop. ex F.W. Schmidt), borage *(Borago officinalis* L.), pigweed (*Chenopodium album* L.), wild chicory (*Cichorium intybus* L.), sea fennel (*Crithmum maritimum* L.), white wall rocket (*Diplotaxis erucoides* (L.) DC.), wild rocket (*Diplotaxis tenuifolia* (L.) DC.), wild fennel (*Foeniculum vulgare* Mill.), watercress (*Nasturtium officinale* R. Br. subsp. *officinale*), common purslane (*Portulaca oleracea* L.), wild radish (*Raphanus raphanistrum* L.), salicornia (*Salicornia patula* Duval-Jouve), white mustard (*Sinapis alba* L.), Mediterranean mustard (*Hirschfeldia incana* (L.) Lagr.-Foss.), common dandelion (*Taraxacum officinale* Weber) and goatsbeard (*Tragopogon porrifolius* subsp. *Australis* (Jord.) Nyman) (Di Gioia et al. 2015a). On the other hand, it is important to pay particular attention in the choice of the species that can be used to produce microgreens, by assessing attentively the edibility of each species at the seedling stage. Indeed, it is possible to use all those species whose edibility is well known, whereas all the wild or domesticated species whose seedlings are not edible must be excluded. Among those, for example, the species belonging to the Solanaceae family such as tomato, pepper and eggplant at the seedling stage contain anti-nutrients and therefore cannot be considered edible (Di Gioia et al. 2015a). For this reason, the selection of species that may be used to produce microgreens is firstly linked to their edibility at the seedling stage. Once assessed that a species is edible, the product should also have a good palatability, to be fully acceptable and attractive for the consumer. Flavour, smell, texture and colour are in fact fundamental traits for the consumer acceptability of the product, and companies producing microgreens at commercial level are always searching new species characterized by attractive shapes, bright colours and new and particular flavours (Di Gioia et al. 2015a). Depending on the flavour, it is possible to distinguish microgreens with neutral (rappini, spinach), slightly sour (beet and salicornia) and spicy taste (watercress, radish and arugula), whereas microgreens of Cucurbitaceae are often bitter. As compared to the standard vegetables, the flavour of most of the microgreens is not "micro" but strong and concentrated (Di Gioia et al. 2015a). For other species such as cereals, leguminous, sunflower or flax, whose standard fresh plant is usually not edible, the taste will be new to most consumers and, however, is distinctive of the species. The smell of microgreens can be intense, as for many aromatic herbs, and delicate or barely perceptible as in the case of many species of vegetables. Based on the texture, it is possible to distinguish species with juicy (salicornia, sea fennel, beet, sunflower), crunchy (celery) and regular texture (Brassicaceae and Asteraceae) (Di Gioia et al. 2015a). Depending on the colour, it is possible to distinguish species of microgreens that are green, yellow, red, crimson or multicolour (Di Gioia et al. 2015a). Different colours may depend from species,

Table 11.2 Some types of microgreens grouped for colours

Colour	Microgreens
Green	Broccoli, radish, arugula, celery, spinach
Yellow	Etiolated pea, etiolated corn
Red	Red orach, amaranth, chenopodium
Crimson	Red cabbage, red basil, radish
Multicolour	Beet, sorrel, mustard

cultivar or plant physiological state, such as in the case of the etiolated microgreens (Table 11.2). From an agronomic and commercial point of view, the selection of the species for the production of microgreens is strongly dependent from the availability of seeds of good quality, characterized by high and homogenous germinability, not treated with chemicals, hygienically safe and, at the same time, available at a low cost. Moreover, it is important to choose species that can be grown all year round and that do not have particular thermal and environmental needs, especially during the germination phase. Finally, a critical aspect at commercial level is the shelf life of the product (Di Gioia et al. 2015a).

11.3.3 Nutritional Traits

Over the past few years, microgreens have gained popularity as a new culinary trend, being served as an edible garnish to embellish a wide variety of dishes or as new salad ingredient (Xiao et al. 2012; Pinto et al. 2015). Microgreens are considered "functional foods" or "super foods" (Treadwell et al. 2010). In addition to the intake of nutrients, they can provide bioactive compounds able to improve some functions of the organism and/or reduce the risk of diseases. A recent study, conducted by a group of researchers of the US Department of Agriculture (USDA) and the University of Maryland, analysing the concentration of vitamins (C, E and K) and carotenoids (β-carotene, lutein and zeaxanthin) in 25 varieties of microgreens, demonstrated that as compared to regular vegetables harvested at the standard commercial ripening stage, microgreens have a content of antioxidant compounds even ten times higher (Xiao et al. 2012). For instance, in the case of the red cabbage, comparing the amount of the above-mentioned vitamins in the microgreens with those reported in the literature for the same species harvested at a regular ripening stage, microgreens showed an average content of vitamin C six times higher (147 vs 23.5 mg/100 g of fresh product (FP)), a 400 times higher value of vitamin E (24.1 vs 0.06 mg/100 g of FP) and a 60 times higher content of vitamin K (2.4 vs 0.04 μg/g of FP) (Xiao et al. 2012). Considering the content of vitamins C, E and K estimated in the same study and the daily intake levels recommended by the European Food Safety Authority (EFSA) for vitamin C (60 mg), vitamin E (13 mg) and vitamin K (70 μg) for an adult of medium weight, Di Gioia and Santamaria (2015a) estimated that for some of the species analysed, even few grammes of microgreens can entirely

Table 11.3 Content of ascorbic acid (vitamin C), α-tocopherol (vitamin E) and phylloquinone (vitamin K) in some species of microgreens and relative amount of fresh product (FP) necessary to satisfy the recommended daily intake of each vitamin for an adult (Di Gioia and Santamaria 2015a)

		Vitamin content (mg/100 g FP)			Grammes of FP necessary to satisfy the recommended daily intake of vitamin		
Microgreens		C	E	K	C	E	K
Garnet amaranth	*Amaranthus hypochondriacus* L.	131.6	17.1	4.1	46	76	17
Opal basil	*Ocimum basilicum* L.	90.8	24.0	3.2	66	54	22
Red beet	*Beta vulgaris* L.	46.4	34.5	2.0	129	38	35
Red cabbage	*Brassica oleracea* L. var. *capitata*	147.0	24.1	2.8	41	54	25
Cilantro	*Coriandrum sativum L.*	40.6	53.0	2.5	148	25	28
Peppercress	*Lepidium bonariense* L.	57.2	41.2	2.4	105	32	29
Pea tendrils	*Pisum sativum* L.	50.5	35.0	3.1	119	37	23
Green radish	*Raphanus sativus* L.	70.7	87.4	1.9	85	15	37
Arugula	*Eruca sativa* Mill.	45.8	19.1	1.6	131	68	44
Celery	*Apium graveolens* L.	45.8	18.7	2.2	131	70	32
Popcorn shoots	*Zea mays* L.	31.8	7.8	0.9	189	167	78
Golden pea tendrils	*Pisum sativum* L.	25.1	4.9	0.7	239	265	100

Average value of vitamins C, E and K measured by Xiao et al. (2012)
The daily intake recommended by the EFSA for adults is 60 mg for vitamin C, 13 mg for vitamin E and 70 μg for vitamin K

satisfy the recommended daily intake of these three vitamins (Table 11.3). For example, for an adult of medium weight, the consumption of about 41 g of red cabbage microgreens would be enough to fulfil the recommended daily intake of vitamin C, or 15 g of green radish microgreens would satisfy the daily intake of vitamin E, and just 17 g of garnet amaranth microgreens would be enough to fulfil the daily intake of vitamin K (Table 11.3). Moreover, it is worth of note that, compared to the conventional vegetables often used cooked, the consumption of raw microgreens has the advantage of avoiding the loss of nutrients or the degradation of thermolabile vitamins. In addition to the high content of vitamins and antioxidant compounds, microgreens have a good content of minerals (Di Gioia et al. 2015b). Analysing the content of the main minerals in few microgreens, it is possible to observe that microgreens represent a good source of potassium and calcium (Table 11.4). Nevertheless, like other leafy vegetables, microgreens may be characterized also by a high content of nitrates (Di Gioia and Santamaria 2015a), which are considered anti-nutritional factors (Di Gioia et al. 2013; Santamaria 2006). Analysing the mineral composition of several microgreens, it was observed that, especially for basil and the Brassicaceae, in the presence of exceeding supply of nitric nitrogen and under low levels of sunlight, the content of nitrates can increase over 4000 mg/kg of fresh product (Table 11.4). On the contrary, the content of sodium seems to be

Table 11.4 Nitrates and mineral content of some species of microgreens (Di Gioia and Santamaria 2015a)

Microgreens		NO_3^-	Na^+	K^+	Ca^{2+}	P	Mg^{2+}
		(mg/100 g of fresh product)					
Arugula	*Eruca sativa* Mill.	305	8.8	**301**	116	13.2	30.5
Green basil	*Ocimum basilicum* L.	429	11.9	**299**	107	13.2	26.9
Red basil	*Ocimum basilicum* L.	462	8.3	289	105	14.0	26.8
Brassica raab	*Brassica rapa* L., Broccoletto group	355	9.8	230	114	18.4	28.8
Broccoli	*Brassica oleracea* L. var. *italica*	267	8.4	255	**126**	20.1	28.7
Red cabbage	*Brassica oleracea* L. var. *capitata*	368	8.2	167	**126**	32.6	32.1
Mizuna	*Brassica rapa* L. var. *nipponsinica*	400	6.6	256	96	17.0	24.1
Red mustard	*Brassica juncea* L. Czern.	405	14.6	**383**	116	17.0	31.4
Pea tendril	*Pisum sativum* L.	127	7.9	**436**	106	54.4	26.4
Green radish	*Raphanus sativus* L.	226	8.2	189	76	25.0	23.8

Values reported in bold indicates that a portion of 100 g of FP provide over 15% of the recommended daily intake for an adult of mean weight

Table 11.5 Contents of fibre, protein and iron in microgreens and regular brassica raab (*Brassica rapa* L., Broccoletto group)

Vegetable types	Fibre	Protein	Iron
	(mg/kg of fresh product)		
Microgreens	4.1	23	7.7
Adult			
Inflorescence	9.6	56	12.8
Leaves	4.3	38	12.5

Average value by Di Gioia and Santamaria (2015a)

generally very low (Table 11.4). Thus, microgreens can also be considered as low-sodium food (Di Gioia and Santamaria 2015a). On the other hand, the content of minerals in microgreens is strongly determined by the availability of the same minerals in the growing media or in the nutrient solution provided. Therefore, it is possible to obtain microgreens with a high content of essential macro- and microelements or with a low content of undesired elements such as nitrates and sodium, by modifying the composition and the management of the nutrient solution. In this perspective, the application of eco-sustainable production techniques and process innovations able to enhance the nutritive value of microgreens can help to satisfy also consumers that have specific diet needs (Di Gioia and Santamaria 2015a). So far, very limited information are available on the proximate profile of microgreens; however, analysing microgreens of broccoli raab (*Brassica rapa* L., Broccoletto group), Di Gioia and Santamaria (2015a) verified that the content of fibres and proteins, as well as the concentration of essential microelements, was lower in microgreens than in the regular broccoli raab (Table 11.5). Undoubtedly, further studies are needed to examine these aspects more deeply and to verify if microgreens can really be considered as "super food". Moreover, more attention should be focused to evaluate also potential correlation between nutritional composition and sensory attributes. For example, Xiao et al. (2015b) found that total phenolic content (TPC)

was strongly correlated with flavour attributes, such as sourness, astringency and bitterness. Therefore, TPC values could be used by microgreen growers and food service uses (i.e. industry) as indicators of sensory information and predictors of consumer acceptability, providing the industry the possibility of predicting the consumer acceptability and likability for microgreens especially in ethnically unique food markets (Xiao et al. 2015b). Thanks to their distinctive peculiarities, microgreens represent a rich food source also for categories of consumers particularly demanding, like vegetarians and vegans, that can diversify and enrich their diet by using a large variety of microgreens available. Moreover, being the microgreens usually consumed raw, they can also satisfy the specific needs of the so-called raw foodists. Lastly, the chance of growing microgreens in a very simple way, without the use of fertilizers (e.g. when using the peat) and pesticides, even in very little spaces like a terrace, a balcony or a windowsill, allows the production of "zero-mile" and "low-cost" food that may be used to prepare appetizers, salads, main and second courses, soups, sandwiches and desserts, improving the flavour, the colour, the texture and the nutritive value and enhancing the compliance with the World Health Organization recommendations that, to prevent degenerative diseases, suggests the intake of at least 400 g of fruit and vegetables per day (Di Gioia and Santamaria 2015a).

11.3.4 Production

Microgreens are usually produced using soilless cultivation systems (Table 11.1), in which the soil is replaced by a substrate or the plants are grown in a liquid culture and are fed through a nutrient solution containing all the elements needed by a plant to live (Di Gioia et al. 2015b). The commercial production of microgreens is usually performed under controlled environment, inside greenhouses or high tunnels provided either with simple or advanced technologies, depending on the size of the farm and the more or less favourable climatic conditions, and using soilless growing systems that can be essentially attributable to three types. One possibility is to grow microgreens in "containers" constituted by plastic trays having different sizes, with height variable from 3 to 5 cm (Fig. 11.3). Depending on the case, the bottom of the container holding the growing media can be intact, with no holes, or more often with holes, in order to enhance the drainage of the excess of water (or nutrient solution) and avoid water stagnation that can lead to the development of diseases and compromise the production and the quality of microgreens (Di Gioia et al. 2015b). Usually, the containers are placed on growing channels or benches, unmovable or movable, perfectly levelled and complete of a system to recover the excess water or nutrient solution. Irrigation water and nutrient solution can be delivered from the top by means of a nebulization system, activated manually or automatically, or from the bottom, through subirrigation. In the latter case, it is essential that containers have holes at the bottom. The cultivation in containers generally allows the commercialization of the product along with the growing media, thus avoiding the need

Fig. 11.3 Red mustard *(Brassica juncea* L. Czern.) microgreens grown in a plastic tray

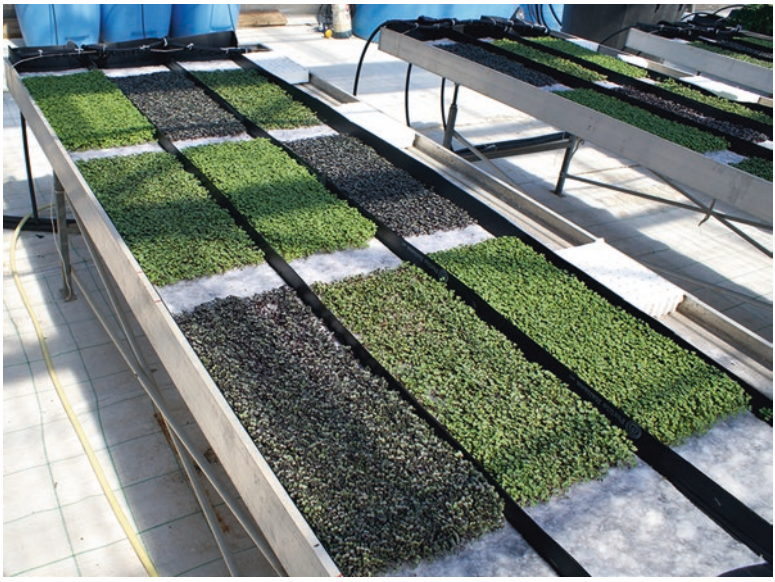

Fig. 11.4 Microgreen production on benches

to cut the product before it is shipped in the market. Such possibility prevents all the issues associated with the cut of a vegetable, with consequent advantages for the shelf life and the quality of the product, that will be harvested by the final consumer, just a few minutes before actually using the product in the kitchen (Di Gioia et al. 2015b). In order to minimize the environmental impact, today, it is also possible to use biodegradable containers (polylactic acid (PLA) and others) rather than those made of plastic deriving from oil (Di Gioia et al. 2015b). A second possibility is to grow microgreens on "channels" or on benches (made of plastic, aluminium, galvanized iron, wood) of different sizes, by placing the growing media right inside the channels or on the benches (Fig. 11.4). Moreover, in this case, channels and benches

can be movable or unmovable and have to be perfectly levelled, to realize a slight slope in order to enhance the flow of the water or the nutrient solution from one end of the channel or bench to the other, enabling if possible also the recovery and recycling of the surplus water or nutrient solution. Like for the previous growing system described, water and nutrient solution can be delivered from the top by means of a nebulization system or preferably from the bottom by subirrigation. With this growing system, once the optimal stage of growth is achieved, microgreens are harvested by cutting the seedlings at the base. After the cut, the product is usually washed and dried and can be packed and marketed as fresh-cut, ready-to-eat product (Di Gioia et al. 2015b). A third growing system, quite simple but less common at commercial level, is the "floating system". In this case, polystyrene plug trays of different sizes float on the nutrient solution contained in a basin or a bench, so that the growing media contained in the cells can be soaked from the bottom. Being this a static growing system, in which the nutrient solution does not circulate, in order to maintain a good level of oxygen, it is essential to enrich the nutrient solution with air. The scarce use of this growing system at commercial level is mainly due to the fact that in liquid culture, it is difficult to produce microgreens with a good dry matter content and, thus, with a good shelf life (Di Gioia et al. 2015b). Besides the cultivation of microgreens in regular greenhouses, in the last few years, with the availability of more efficient lamps, several companies have implemented very advanced and intensive indoor growing systems, in which trays, channels or benches can be placed on different levels, one on top of the other, in "multi-layer" growing systems, to produce microgreens even without natural light, by integrating or entirely replacing the sunlight with an artificial lighting system, using lamps with an adequate spectrum for the photosynthesis of the plant. It is important to highlight that, also microgreens, require an adequate level of radiation (at least 100 μmol m^{-2} s^{-1} of photosynthetically active photons for the less demanding species) to assure the achievement of a good commercial, hygienic-sanitary and nutritional quality of the product. In fact, in North Europe and North America, even in greenhouse, sometimes it is necessary to integrate the sunlight with supplemental lighting. When using supplemental radiation, the possibility to control the intensity and quality (wavelength) of the radiation can be exploited at commercial level even to modify and increase the nutritional value of microgreens (Kopsell and Sams 2013; Samuoliene et al. 2013) (Fig. 11.3).

One of the most critical aspects involved in the production of microgreens is the selection of the growing media as it plays a fundamental role in determining the productivity and quality of microgreens, as well as the sustainability of the production process (Di Gioia et al. 2015b). In order to assure a good germination and an optimal growth of the seedlings, a good growing media should have in terms of physical properties: a porosity over 85% of the total volume, an adequate ratio between macro- and micropores to guarantee at the same time a good water holding capacity (55–70% of the total volume) and a good level of aeration (20–30% of the total volume) of the root system (Abad et al. 2001). As for the chemical properties, a good growing media for the production of microgreens should have a pH value ranging from 5.5 to 6.5 and an electrical conductivity below 500 μS/cm; yet, the

substrate should not contain heavy metals or polluting compounds (Di Gioia et al. 2015b). Yet, it is of fundamental importance that the growing media is not microbiologically contaminated. Especially materials of organic origin can contain microorganisms pathogenic to the human being, like *Salmonella* and *E. coli* (Di Gioia et al. 2017). To prevent hygienic-sanitary issues, it is important to choose substrates whose microbiological quality is guaranteed or materials that have undergone sterilization treatments (physical or chemical). There are many solutions available on the market, and the selection of the substrate is generally based on availability at local level; cost; adequate physical, chemical and biological properties; and environmental sustainability (Di Gioia et al. 2015b). Growing media can be classified in organic and inorganic, the first are made of natural and biodegradable materials, like the peat, while the inorganic ones like perlite are usually inert. The growing substrates most commonly used for the production of microgreens, either at commercial or non-professional level, are peat, perlite and vermiculite, which can be used individually or in mix (Di Gioia et al. 2015b). Mixing in adequate proportions substrates having different properties, it is possible to obtain a growing media with optimal physical-chemical and, thus, agronomic properties or at least with better properties as compared to the single substrates. An organic material alternative to peat is the coconut coir, which has the advantage of being produced from a renewable source. Of course, when selecting the coconut coir, it is important to consider that, based on the size of the particles (fibre or pith), the properties of the substrate can remarkably change. In the case of the coconut coir or other organic substrates deriving from composting processes, it is important to verify that the content of salts is not too high, as this may limit the germinability of the seeds. Among the substrates that have been specifically developed for the production of microgreens, there are mats constituted by fibrous materials of natural origin (coconut coir, jute fibre, cotton fibre, algae fibre and paper pulp) or synthetic (produced by means of polyethylene terephthalate (PET) (Fig. 11.5). Usually, commercial mats have

Fig. 11.5 Radish (*Raphanus sativus* L.) microgreens grown on an inert media

well-defined and standardized physical, chemical and agronomic properties and have a good balance between water holding capacity and air capacity and, not less important, have good hygienic-sanitary quality. Nevertheless, the high cost of commercial substrates has led many producers of microgreens to look for alternative materials, including discarded or recycled materials (possibly of natural origin and renewable) and to develop their own growing media, whose composition is often secret. For instance, many producers of microgreens buy discarded fibrous materials deriving from industrial processes, such as the low-cost cellulose pulp, cotton and jute fibres, and they use these materials, usually characterized by limited water holding capacity after improving their agronomic properties (Di Gioia et al. 2015b).

11.3.5 Harvest and Postharvest

The harvest of microgreens can be performed by cutting the seedling few millimetres above the growing media, either manually using a pair of scissors or a blade or using an electric knife, avoiding to include particles of the growing media and, if possible, the integuments of the seeds that, in many species, cannot be easily excluded since they often remain attached to the cotyledons. Being highly perishable, microgreens should be washed and cooled (1–5 °C) immediately after the harvest, and of course they should be handled following all the good practices to preserve the hygienic-sanitary quality (cleaning harvesting tools, packing and storage room, using gloves, etc.) as for regular fresh-cut vegetables (Kyriacou et al. 2016). An alternative to the harvest of the microgreens by cut is to market the product directly into a tray or a package with the whole growing media, while the plants are still growing. In this case, although the producer must still ensure a good hygienic quality, the final consumer will need to wash the product. The critical point in this case is to provide enough water to the seedlings in order to assure their survival and a good shelf life of the product once it exits the farm (Di Gioia et al. 2015b). Extending shelf life (by optimized postharvest handling conditions) is a permanent goal of all those who produce and sell horticultural products of any kind. It should be noted that recently Sasuga (2014) patented a method for providing a microgreen product with a shelf life of at least 10 days. As Hodges and Toivonen (2008) noticed, the two most important storage parameters for postharvest shelf life are storage temperature and atmospheric composition. For example, Kou et al. (2013) indicated that for buckwheat microgreens storage temperature significantly affected the changes in O_2 and CO_2 composition, tissue electrolyte leakage and microbial growth during storage. The same authors suggest that buckwheat microgreens should be stored at 5 °C with moderately high O_2 (14.0–16.5 kPa) and moderately low CO_2 (1.0–1.5 kPa) content to maintain optimal quality and maximal shelf life. Another useful measure to extend the microgreens shelf life is controlling respiration rates (Berba and Uchanschi 2012). Postharvest experiments on rocket (*Eruca vesicaria* (L.) Cav.), radish (*Raphanus sativus*) and red cabbage (*Brassica oleracea* var. *rubra*) are designed to lower respiration rates and increase shelf life which could allow for transportation

towards far markets. Storage temperature, packaging film and wash treatment were investigated by Xiao et al. (2014) on daikon radish (*Raphanus sativus* L. var. *longipinnatus*) microgreens. Accordingly, studies conducted in dynamics during storage highlighted that storage temperature significantly affected package atmosphere, product quality and shelf life. The facts reviewed here indicate that there are promising results in terms of measures to influence microgreens postharvest physiology and extend shelf life. Moreover, as suggested by Artées-Hernández et al. (2013), attention should be focused on "eco-innovative emerging alternative" to prolong the shelf life without losing quality characteristics specific to the fresh product. Finally, as regards microbiological risk, although it is likely that microgreens could also serve as vehicle of bacterial pathogens, to date, no food-borne outbreak associated with consumption of microgreens has been reported (Xiao et al. 2015a). The lack of food safety incidence could be attributed to the low production and consumption, high consumer geographic and demographic selectivity or purported intrinsically safe characteristics of microgreens (Xiao et al. 2015a). At any rate, since there is a lack of data pertaining to the microbiological safety of microgreens, more attention should be focused also to investigate the survival and proliferation of food-borne bacterial pathogens on microgreens grown and stored under conditions simulating commercial production (Figs. 11.4 and 11.5).

11.4 Baby Leaf Vegetables

11.4.1 Description

More specifically "baby leaf" may be considered as leafy vegetables that are harvested beyond the seedling stage (after true leaves have formed) but before they are fully grown (Table 11.1). In other words, any vegetable crops harvested before the eight true leaves may be classed as baby leaf vegetables. Minimally processed baby leaf vegetables are gaining popularity among consumers worldwide, as they represent a good source of minerals, vitamins and phytochemicals of considerable antioxidant potential (Subhasree et al. 2009). So, ready-to-eat baby leaf vegetables are arousing more and more interest in consumers and are requested mostly for mixed salads (Gonnella et al. 2002). It is generally accepted that the physical damage that occurs during the preparation of whole heads causes an increase in respiration rates, biochemical changes and microbial spoilage, which may result in degradation of colour, texture and flavour of the fresh-cut produce and development of browning during storage (Cantwell 1996). In the case of the ready-to-eat baby leaf vegetables, the product can be processed without any further preparation because the entire leaf is harvested. Because the stem diameter is small, a lower wound response can be expected, with less bruising and minimal oxidation when compared with the whole heads. All these characteristics suggest a relatively longer storage potential of ready-to-eat baby leaf vegetables in terms of better colour and nutritional and microbiological qualities (Martínez-Sánchez et al. 2012) (Fig. 11.6).

Fig. 11.6 Mechanical harvest of "baby leaf" wild rocket (*Diplotaxis tenuifolia* L.) grown under protected environment

11.4.2 Utilized Species

Lettuce (*Lactuca sativa* L.) is one of the most important species used as baby leaf vegetable, and many types of lettuce, with attractive colours and shapes, combining the best quality characteristics from all varieties are used in salad mixes called *mesclun* in France, "spring mix" in the United States, *mezclar* in Spain or *misticanza* in Italy. *Mesclun* is also known as boutique salad or field greens. Traditionally, in the Provence region of France, *mesclun* consisted of four items: chervil, arugula, lettuce and endive (in precise proportions). It is made up principally of small whole leaves, harvested when they are 10 cm long or less (Ryder 2002). Since consumers are looking for softer textures, baby leaf vegetables have been one of the most promising fresh-cut developments. Regarding ready-to-eat products, some benefits of baby leaf lettuce when compared with whole-head lettuce include greater efficiency with higher percentage of usable product, easier and faster processing, more attractive presentation in packaging because of the 3D structure and minimal oxidation due to smaller stem diameter (Martínez-Sánchez et al. 2012). Anyway, many species from different botanic families may be used as baby leaf crops both raw and cooked. For example, spinach (*Spinacia oleracea* L.), Swiss chard (*Beta vulgaris* L.), mustard (*Brassica juncea* L.) and kale (*B. oleracea* L.) are mainly used as cooked vegetables (Ryder 2002); however, they are used also as raw especially in mixed salad. Regarding Chenopodiaceae, orach (*Atriplex hortensis* L.) is used as a spinach substitute having similar flavour, but without the puckering effect from oxalic acid (Ryder 2002). Species mainly used as a raw "baby leaf" especially in mixed salads are endive (*Cichorium endivia* L.), chicory (*C. intybus* L.),

corn salad (*Valerianella locusta* L.), common purslane (*Portulaca oleracea* L.), chervil (*Anthriscus cerefolium* Hoffm.), perilla (*Perilla frutescens* [L.] Britt.) and some species of the Brassicaceae such as rocket (*Eruca vesicaria* L.), wild rocket (*Diplotaxis tenuifolia* L.), mizuna (*Brassica rapa* L. Mizuna group), tatsoi (*B. rapa* Tatsoi group) (Ryder 2002) and watercress (*Nasturtium officinale* R. Br.). Chicory and endive are much less used than lettuce, but they are anyway among the most known and popular horticultural products in the world and, although with great differences in cultural practices and type of utilization, they are diffused in almost every country and are included in the diet of most Western as well as Eastern populations (Lucchin et al. 2008). Mizuna, tatsoi and perilla are Oriental vegetables, while corn salad is popular in Western Europe (Ryder 2002). Common purslane has a cosmopolitan distribution, but it is more present in the Mediterranean area, mainly in arid and semiarid lands of the northern Africa and southern Europe (Gonnella et al. 2010). Apart from lettuce, rocket and wild rocket are two of the most popular baby leaf vegetables since they have particular sensorial traits such as the spicy flavour. These species, as well as common purslane, are probably better known as weeds but have been selected for cultivated use. In Italy, one of the most successful attempts of cultivation is that of wild rocket which, since the first attempts in early 1990s, is currently grown over a 1000 ha area, mainly in greenhouses. Since 1990 there has been an increase in its consumption also due to the initiatives of some great chefs who proposed wild rocket in various recipes. Thus, for example, wild rocket is used as an ingredient in *risotto*, different types of pasta, pizzas and *piadine* as well as with and *bruschetta* and to prepare *carpaccio* (Renna et al. 2015).

11.4.3 Nutritional Traits

It is well known that leafy vegetables are rich in vitamins as well as in fibre and minerals and have appreciable amounts of other phytochemicals. However, it is true that the biosynthesis, composition and concentration of health-promoting compounds vary widely among leafy vegetables and carry the influence of genetic and environmental factors, growing conditions, harvest practices, postharvest handling conditions (Rouphael et al. 2012) and maturity stage. The contents of nutrients and phytochemicals are also highly affected by genetic factors, with large variability between species as well as between cultivars and populations of the same species. For example, lettuce is a good source of ascorbic acid, folate and, like all green vegetables, chlorophyll. Moreover, most cultivars are rich sources of carotenoids (β-carotene, lutein and zeaxanthin) and assorted flavonoids. The latter include flavanols (mostly quercetin) and flavones (apigenin and luteolin); in addition red cultivars contain anthocyanins (Hedges and Lister 2005). Therefore, the antioxidant activity of red baby leaf lettuce cultivars could be even 11-fold higher than in green baby leaf lettuce (Fadda et al. 2015). Rocket contains similar core nutrients to those in lettuce and shares many of the same phytochemicals except anthocyanins (Cefola and Pace 2015). Besides its characteristic nutty flavour, what is distinctive about rocket is that it also provides glucosinolates like other baby leaf Brassicaeae species.

When the plant tissue is damaged, by cutting or chewing, these compounds are enzymatically converted to other compounds, including isothiocyanates. The latter protect against cancer through a number of mechanisms, particularly the induction of phase 2 enzymes and encouraging apoptosis. Therefore, when baby leaf Brassicaceae species are eaten raw, the enzyme (myrosinase) that converts glucosinolates into isothiocyanates is not destroyed during cooking, ensuring that conversion to isothiocyanates is optimized (Hedges and Lister 2005). Although microgreens may have a higher content of phytochemicals compared to vegetables harvested at the regular stage (Xiao et al. 2012), the literature available regarding the effect of the baby leaf stage (intermediate between microgreens and regular stage) on phytochemicals is not clear. For example, in the case of spinach, Pandjaitan et al. (2005) found that the midmature stage (medium-sized leaves – leaf width 3.81–6.35 cm) showed highest total phenolic, total flavonoids and ORAC values with respect to mature stage (large leaves – leaf width > 6.35 cm). Conversely, in another study, spinach had higher antioxidant capacities at mature head stage relative to baby leaf stage (Zhao et al. 2007). Among the environmental factors, light plays a crucial role in driving phytochemical photosynthetic activity (Bian et al. 2015). It has been demonstrated that phytochemical biosynthesis and accumulation in plants are well correlated with the amount of photosynthates (Wu et al. 2007). Therefore, optimal light conditions are of vital importance for maximizing phytochemical accumulation (Bian et al. 2015). For example, Colonna et al. (2016) demonstrated that antioxidant activity and total phenols in ten species of baby leaf vegetables were higher, respectively, with low and high photosynthetically active radiation (PAR) at the time of harvest. At any rate, some species used as baby leaf vegetables such as rocket, cress, lamb's lettuce, lettuce, spinach and Swiss chard are also hyperaccumulator of nitrates (Santamaria et al. 1999; Di Gioia et al. 2013). Nitrate per se is relatively non-toxic, but its reaction products and metabolites, such as nitrite, nitric oxide and N-nitroso compounds, have raised concern because of their implications for adverse health effects, most notably methaemoglobinaemia or "blue baby syndrome" (Santamaria 2006). More than 300 nitrosamines and N-nitroso compounds were proven to be carcinogenic to more than 40 animal species (Gangolli et al. 1994). However, the significance on human health is still equivocal, since several epidemiological studies (Milkowski et al. 2010) did not confirm any direct correlation between nitrate concentration in food and the incidence of cancer (Speijers and Van Den Brandt 2003). On the other hand, McKnight et al. (1999) demonstrated that nitrite, nitric oxide and N-nitrosamines exert an important antimicrobial role in the stomach against gastrointestinal pathogens. Nitrate metabolites were also shown to have important physiological and pharmacological function, such as vasoregulation, as well as tissue protective properties (European Food Safety Authority 2008). Despite the debate on the effects of nitrate on human health, the production and commercialization of some leafy vegetables are subject to regulatory limitations, since some population groups (e.g. vegetarians) could be at higher risk for developing cancer when consistently exposed to elevated dietary intake of nitrate (Cavaiuolo and Ferrante 2014). Therefore, the World Health Organization (WHO) has set an acceptable daily intake (ADI) for nitrate of 3.7 mg/kg body weight (Santamaria 2006).

11.4.4 Production

The production of baby leaf vegetables may be considered as the first step of a complex production process, which begins in field up to establishment for the processing and packaging. Cropping system and environmental conditions may have a great impact on plant metabolism, which can influence leaf quality at harvest and during postharvest life (Weston and Barth 1997; Nicola et al. 2009). Therefore, the choice of the growing environment and systems is very important. In order to produce baby leaf vegetables suitable to obtain ready-to-use products, growing systems are required that can shorten growing cycles and improve uniformity of growth and automation of cultural techniques, in addition to providing high hygienic and quality levels (Gonnella et al. 2002). Today baby leaf vegetables are mainly grown under protected environment, where it is possible to obtain the best guarantee of product cleaning as well as a better management of the pest control and fertilization (Fig. 11.6). Moreover, under protected environment, it is possible to plan the production during the whole year, obtaining a greater number of crop cycles compared to the cultivation in open field. Since the cultivation begins with direct sowing, it follows that the essential first step is the good preparation of the soil. Sowing is done with mechanical or pneumatic seeder, which guarantee a continuous distribution of the seeds along the row. Crop density, climate, irrigation and nutrient management are the most important parameters for a satisfactory baby leaf vegetable yield. In fact, while a too low crop density results in a decrease in yield and makes it hard for mechanical harvesting, it is equally true that with a too high crop density, you could obtain light-coloured, elongated and very tender leaves, which are unsuitable to be handled and stored. Similarly, not suitable climatic conditions as well as too high or too low moisture and nutrients in the soil may have negative effects regarding yield and quality of the baby leaf produce. The production of baby leaf vegetables as ready-to-use salads requires high-quality raw material from an organoleptic and hygienic viewpoint (no presence of pesticide, crop or substrate residues). Another important quality feature for leafy vegetables, and especially for minimally processed products, is a low nitrate content. In line with the World Health Organization (WHO), the nitrate commercial threshold is fixed by Commission Regulation (EU) 1258/2011 for lettuce, spinach and rocket (European Union 2011). For minimally processed vegetables, nitrate content should be more carefully controlled because of the particular environmental conditions that occur in the packaging (low oxygen level, high humidity and presence of cut portions of tissues) enhancing nitrate reduction to nitrite, which could be dangerous in the case of elevated dietary intake. A valid alternative is to produce baby leaf vegetables in hydroponic systems that allow a direct control of the nutrient supply, making possible the instant modification of the composition and concentration of the nutrient solution in order to reach a fixed qualitative standard as regards the dry matter (or crunchiness), nitrate content or other organoleptic and aesthetic features of products (Santamaria et al. 2001). Different strategies can be applied for reducing the nitrate content in vegetables grown by using hydroponic systems: (i) removing part of leaf petioles, (ii) removing part or all of the nitrate nitrogen from the nutrient solution a few days before

harvesting and (iii) using nutrient solutions with NO_3-N and NH_4-N rather than nitrate nitrogen only (Santamaria et al. 2001). Moreover, hydroponic systems provides higher sanitary quality than conventional soil-based culture, without soil contaminants (Fontana and Nicola 2008), and usually reduces nutrients and water use with high environmental benefit (Vernieri et al. 2005). Among hydroponic methods to produce baby leaf vegetables, the floating system is the easiest and cheapest because of its low installation and manpower costs; weeds are avoided and harvesting is straightforward (Rodríguez-Hidalgo et al. 2010; Nicola et al. 2009). In addition, the resulting products are almost clean and practically ready to be packed as minimally processed vegetables. This system shows high water and fertilizer efficiency and a very low environmental impact (Gonnella et al. 2002). At any rate, growing conditions could have an impact on the nutritional profile of the vegetables. For example, Romani et al. (2002) found that for an Italian cultivar of lettuce (Audran), all open-air grown samples had higher levels of polyphenol compounds than those grown in greenhouse. Similarly, field-grown curly lettuces had higher carotenoid levels than those grown hydroponically (Kimura and Rodriguez-Amaya 2003). On the other hand, it is important to highlight that in recent years several studies have been published regarding the possibility of enriching micronutrients in the baby leaf vegetables, using floating systems as a tool of biofortification (D'Imperio et al. 2016). Moreover, the use of supplemental light, like light-emitting diodes (LED), in controlled environments could increase beneficial phytochemical levels while reducing levels of harmful substances in leafy vegetables (Bian et al. 2015).

11.4.5 Postharvest

Since baby leaf vegetables are harvested before they are fully grown, differences in the maturity stage between baby leaf, as immature leaves, and mature leafy vegetables can affect the shelf life of the processed produce. Baby leaf of kale as immature leaves have almost double the respiration rate of full-sized leaves when stored at different temperatures (Cantwell and Suslow 2004). Higher respiration rates indicate a more active metabolism and usually a faster deterioration rate. A higher respiration rate can result also in a more rapid loss of organic acids, sugars and other components that determine flavour quality and nutritional value (Cantwell and Suslow 2002). Martínez-Sánchez et al. (2012) hypothesized that baby leaf vegetables could be more appropriate as raw material for the fresh-cut industry, because of a lower degree of cutting which can decrease respiration rate and the adverse reactions of browning during storage. However, the maintenance of excellent sensory properties, microbial quality and pigments, such as anthocyanins, may be difficult due to their tender texture (Martínez-Sánchez et al. 2012). Anyway, by using suitable preharvest strategies, it is possible to affect the quality during postharvest. For example, red baby leaf lettuce cultivars show a lower respiration rate than green ones; therefore, the choice of red species for growing could enhance the marketability of the ready-to-eat products. (Fadda et al. 2015). Conversa et al. (2014)

reported that the azoxystrobin application on baby spinach during growth may result in a higher ascorbic acid content at harvest and after 7 days of refrigeration at 5 °C. Moreover, also by postharvest treatments, it is possible to affect the quality of baby leaf vegetables during storage. For example, after 8 days of shelf life, fresh-cut baby spinach stored under N_2O-enriched modified atmosphere packaging (MAP) showed the lowest microbial growth, with good sensory quality and preserved antioxidant capacity (Rodríguez-Hidalgo et al. 2010). Such results suggest that N_2O-enriched MAP is a useful tool to preserve the quality of fresh-cut spinach leaves for longer than conventional passive and super-atmospheric MAP (Rodríguez-Hidalgo et al. 2010). Cefola and Pace (2015) reported that the dipping of rocket and baby spinach leaves in an oxalic acid (OA) solution significantly delayed deterioration during storage, with clear benefits for overall quality. Specifically, OA reduced the visual quality loss and yellowing of the leaves, slowing down the respiration rate, ammonia production, chlorophyll degradation and electrolyte leakage. In addition, the antioxidant activity and total phenol content were preserved by OA treatment, and the mesophilic aerobic count was reduced, with a performance similar to that of hypochlorite washing. In conclusion, both pre- and postharvest treatments could be easily implemented during the production of the baby leaf vegetables in order to give advantages to processors and consumers in terms of quality and shelf life extension.

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Chapter 12
Minimally Processed Mushrooms

Hatıra Taşkın and Saadet Büyükalaca

12.1 Introduction

During the period between 1940s and 1970s, which is known as "Green Revolution," it was aimed to increase the agricultural activities in order to feed the world population. During that period, the increase in productivity was the only thing that came forth; that is why such agricultural activities as the use of chemicals and fertilizers were implemented without any limitations. At the end of that period, the environment and human health problems began to emerge, and then quality, aroma, taste, and nutrition gained significance. As for today, not only have the agricultural activities, which pay importance on both environment and human health in general, been widely accepted, but also authorities have started to support the matters such as high nutritive food. As a result of this, recently the term "functional food" started to be pronounced frequently. This term can simply be defined as "food providing contributions to health rather than basic diet" (Boyacıoğlu 2016). The term can also be defined as "the modified food consumed as part of standard diet and similar to conventional food yet it is aimed to gain physiological roles rather than addressing the needs for ordinary diet" (Boyacıoğlu 2016). As for some kinds of mushrooms with nutritious and health benefits, they can be counted among the functional food. The role of mushrooms as food and medical uses dates back ancient times. In the scientific world, a number of studies have been conducted on nutritional values of mushrooms for a long time. According to the results of the studies, the researchers have agreed upon a general opinion (including all mushroom species that can be consumed) that mushrooms contain a great amount of protein despite not having so much as found in animal products, yet they have low fat and no cholesterol; instead they contain

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ergosterol, which is the initial item for the synthesis of vitamin D in human body (Manikandan 2011). They are also rich regarding some macro- and microelements and several vitamins. Moreover, another significant outcome found as a result of the studies is that the protein in mushrooms have high rate of digestibility. It is important not only high protein content in food but also digestibility of this protein. Bernaś and Jaworska (2010) reported using the results of Shah et al. (1997) that the digestibility of protein in fresh *A. bisporus* was 84%, whereas in *P. ostreatus* it was 77%.

In the book *Mushrooms* written by Chang and Miles (2004), they included the detailed results of the studies conducted on the nutritional values of especially the most commonly cultured mushrooms. According to those studies, the protein amount in fresh weight of four most popular and cultured mushrooms *Agaricus bisporus*, *Lentinula edodes*, *Pleurotus* and *Volvariella volvacea* was between 1.75% and 3.63%. This amount was reported 5.9% in maximum and 3.5–5% on average by Flegg and Maw (1976). In other words, the amount of protein in mushrooms is twice as much as asparagus and cabbage have, and it is four times as much as orange has, and finally it is six times as much as apple has. When dry basis is examined, mushrooms contain 19–35% protein, while rice, wheat, soybean, and milk contain 7.3%, 13.2%, 39.1%, and 25.2% protein, respectively. The crude protein content in mushrooms is lower than that of in meat; however, they have higher amount of crude protein than several types of food such as milk. Protein consist of different combinations of more than 20 amino acids having different amounts. Some amino acids can be converted into other amino acids; however, nine amino acids are basic (lysine, methionine, tryptophan, threonine, valine, leucine, isoleucine, histidine, phenylalanine). Although animal products contain higher amount of and well-balanced proteins, plant products are lack of essential amino acids. The mushrooms which are commonly cultured contain such nine essential amino acids. Dry basis of mushrooms contains 1.1–8.3% fat, being 4% on average. Mushrooms generally contain lipid compounds such as free fatty acids, monoglycerides, diglycerides, triglycerides, sterols, sterol esters, and phospholipids. Since mushrooms contain unsaturated fatty acids, they are important for healthy diet (Holman 1976), and high linoleic acid content is another advantage. They are essential since mushrooms contain unsaturated fatty acids (Holman 1976), and they have some certain advantages as they also have high amount of linoleic acid. It is determined that this amount is 76% in *L. edodes*, 70% in *V. volvacea*, and 69% in *A. bisporus*. Ergönül et al. (2013) determined that the amount of C18:2 cis-linoleic acid and C18:3 linolenic acid in *P. ostreatus* was 65.29% (dry basis, % of total fatty acid) and 0.03% (dry basis, % of total fatty acid), respectively. Animal products mostly contain saturated fatty acids, and they are unhealthy. Edible mushrooms are great source of essential vitamins such as thiamine (B1), riboflavin (vitamin B2), niacin, biotin, and ascorbic acid (vitamin C) (Crisan and Sands 1978). It was determined that the content of thiamine was 0.35 mg per 100 g in *V. volvacea*, 1.14 mg per 100 g in *A. bisporus*, 1.16–4.80 mg per 100 g in *Pleurotus*, and 7.8 mg per 100 g in *L. edodes*. It was reported that the content of niacin was 54.9 mg per 100 g in *L. edodes*, 55.7 mg per 100 g in *A. bisporus*, 64.88 mg per 100 g in *V. volvacea*, and 46.0–108.7 mg per 100 g in *Pleurotus*. As for the content of riboflavin, it was observed that *A. bisporus* (5.0 mg

per 100 g) and *L. edodes* (4.9 mg per 100 g) were higher than that of in *V. volvacea* with 1.63–2.98 mg per 100 g. When it comes to vitamin C, it was determined that the highest amount was in *L. edodes* with 9.4 mg 100 g dry sample followed by *P. sajor-caju* with 7.4 mg 100 g dry sample, *A. bisporus* with 1.8 mg 100 g dry sample, and *V. volvacea* with 1.4 mg 100 g dry sample. The total amount of ash consists of 56–70% Potassium (K), Phosphorus (P), Calcium (Ca), and Magnesium (Mg). The content of K was the highest with 45%, while the amount of Na and Ca was almost the same in the cultured mushroom species. The amount of Ca in *L. edodes* is high. The amount of Cu in *Pleurotus* is higher than the other cultured mushroom species and is between 12.2 and 21.9 ppm. The heavy metals in cultured mushrooms are below the levels determined by regulations (Source for all information in this paragraph: Chang and Miles 2004).

Mushrooms can be classified as cultured and collected from nature or edible, medical, and poisonous. Mushroom cultivation has several advantages compared with the other agricultural activities as it does not depend on climate, it does not require farm land since it is performed indoors and family members can work in mushroom growing rooms altogether. When the data of Food and Agriculture Organization of the United Nations (FAO) of 2014 concerned, the leading country in mushroom production was China, which was followed by Italy, the USA, the Netherlands, Poland, Spain, and France (the leading countries in mushroom production and their import values see Table 12.1, for the export values see Table 12.2) However, since the data given by FAO was labeled as "mushrooms and truffles," the production value of each mushroom species was not evident. It is likely that such information can simply be obtained from the national database of the countries. When the data related to import of 2013 was investigated, the first five leading countries in importing mushroom were United Kingdom, Germany, Russian Federation, the USA, and France; on the other hand, the first five leading countries in importing canned mushroom were Germany, Russian Federation, the USA, France, and Japan. As for export of "mushroom and truffles," the leading countries were Poland, the Netherlands, China, Lithuania, and Ireland, while regarding the export of canned mushroom, the leading countries were China, the Netherlands, Spain, Poland, and France. It has been clear for several years that the most popular three mushrooms species are *Agaricus bisporus, Lentinula edodes*, and *Pleurotus ostreatus*. These three species of mushrooms are easily obtainable at markets in different shapes (Fig. 12.1).

12.2 The Nutritive Value, Consumption, and Extending Shelf Life of *Agaricus bisporus* (J.E. Lange) Imbach

So far there have been various studies regarding nutrition value, extending shelf life and increasing quality of *A. bisporus*. The studies regarding the nutritive value of *A. bisporus*, protein, fat, carbohydrate, fiber, macro- and microelements, and vitamin content, have been investigated and given in Tables 12.3 and 12.4 (Goyal et al. 2006; Koyyalamudi et al. 2013). As can be seen from Table 12.4, *A. bisporus* is rich in P, Sodium (Na), and K.

Table 12.1 The leading countries in mushroom production (FAOSTAT 2014) and their import values (FAO 2013)

Countries	Production (tonnes)	Import mushrooms and truffles (tonnes)	Import canned mushroom (tonnes)
China	7626791.00	341.00	889.00
Italy	600114.00	17607.00	14250.00
USA	432100.00	44771.00	38635.00
Netherlands	310000.00	22629.00	18832.00
Poland	254224.00	3358.00	94.00
Spain	149854.00	4660.00	4983.00
France	108540.00	44723.00	34210.00
Canada	87675.00	9619.00	11221.00
United Kingdom	94857.00	117687.00	7195.00
Iran	80239.00	0	145.00
Ireland	69600.00	3235.00	260.00
Japan	65811.00	5613.00	26639.00
Australia	60023.00	2200.00	5594.00
Germany	59923.00	80916.00	72430.00
Belgium	41754.00	22793.00	11037.00
Turkey	38767.00	0	5.00
Indonesia	37410.00	1201.00	1956.00
India	28000.00	2.00	32.00
Republic of Korea	27130.00	6538.00	13100.00
Hungary	22603.00	438.00	2769.00
Vietnam	22000.00	0	0
South Africa	17299.00	122.00	769.00
Lithuania	12200.00	21873.00	187.00
Denmark	10113.00	6135.00	4143.00
Israel	10000.00	229.00	15427.00
Romania	9758.00	2681.00	3779.00
Switzerland	8155.00	3643.00	4217.00

A. bisporus is marketed in different sizes and shapes. These are fresh packed, fresh unpacked, sliced packed, canned and sliced, canned whole, and frozen (Figs. 12.2 and 12.3). There have been studies related to nutrition losses in different marketing types of mushrooms. One of the relevant studies was conducted by Vetter (2003) in which fresh and canned*A. bisporus* was compared in terms of nutrition content. The results obtained at the end of the study are given in Tables 12.5 and 12.6. The protein content, which is essential for the basic diet, is not lost during the canning process. It was observed that there were some changes in mineral levels. For instance, there were some decreases in the amount of K, Mg, Selenium (Se), Copper (Cu), and Boron (B) in canned products, whereas there were some increases in the amount of certain minerals. The researcher stated that the decreases in the amount of some minerals may have been due to the techniques used in the canning process.

Table 12.2 Export values for the leading countries in mushroom production (FAO 2013)

Countries	Export mushrooms and truffles (tonnes)	Export canned mushroom (tonnes)
China	47008.00	284224.00
Italy	3351.00	2734.00
USA	9511.00	1749.00
Netherlands	69320.00	231130.00
Poland	205200.00	31635.00
Spain	5065.00	41287.00
France	2098.00	20121.00
Canada	31938.00	709.00
United Kingdom	217.00	146.00
Iran	190.00	0
Ireland	32729.00	76.00
Japan	1184.00	92.00
Australia	80.00	27.00
Germany	10187.00	4089.00
Belgium	28199.00	2309.00
Turkey	301.00	174.00
Indonesia	1020.00	5164.00
India	2313.00	2310.00
Republic of Korea	16290.00	30.00
Hungary	8013.00	2390.00
Vietnam	5.00	4352.00
South Africa	2100.00	243.00
Lithuania	33419.00	35.00
Denmark	126.00	1051.00
Israel	–	6.00

The findings obtained from a study conducted by Bernaś and Jaworska (2010) were summarized in Tables 12.7 and 12.8. The results of the study clearly revealed that the frozen products of *A. bisporus* were still rich in terms of protein content. Amino acids and their amounts in *A. bisporus* were clearly summarized through the study. The information about amino acids in *A. bisporus* is important in terms of determining the quality of protein and digestibility.

The most commonly studied subjects on *A. bisporus* are increase of the mushroom quality and shelf life. The storage duration of mushrooms following the harvest is about 2 weeks. Mushroom has very high respiration rates, so it has a very short shelf life in the market chain (Yildiz et al. 1999). Just after harvesting, the symptoms that reduce marketing value, such as darkness, cap opening, and losing the rigidity of mushroom, will start. The short storage life of mushrooms is one of the factors limiting the mushroom production. Considering all the circumstances above, it is essential that there should be a ready market for the mushroom producers and the mushrooms should be transported to market immediately. Moreover,

Fig. 12.1 Different mushroom species in a supermarket

Table 12.3 Nutritional content of *A. bisporus* (in dry weight (dw))

Nutrient	Content
Moisture (%)	90.09 ± 0.07
Fat (%)	3.06 ± 0.03
Ash (%)	9.17 ± 0.52
Crude fiber (%)	10.24 ± 0.05
Crude protein (%)	24.43 ± 0.10
Total carbohydrates (%)	53.10 ± 0.56
Energy (k cal)	337.68
Total nitrogen (g/100 g on dw)	5.58 ± 0.02
Protein nitrogen (g/100 g on dw)	4.77 ± 0.11
True protein (g/100 g on dw)	20.88 ± 0.48
Non-protein nitrogen (g/100 g on dw)	0.81 ± 0.09
Phytic acid (g/100 g on dw)	32.5 ± 0.03
Oxalates (g/100 g on dw)	220 ± 0.00
Protein digestibility (in vitro) g/100 g on dw	81.52 ± 1.23

Source: Goyal et al. (2006)

most supermarkets make deal with mushroom producers on condition that they can return the products in case the mushrooms are not sold in time. One of the applications used in the researches regarding the delay of the problems encountered after harvesting of *A. bisporus* is to add some compounds containing calcium, especially calcium chloride ($CaCl_2$) into irrigation system several times during the production

Table 12.4 Mineral and vitamin content of *A. bisporus*

Nutrient	Content
Magnesium (Mg) (mg/100 g fresh weight)	15.39
Calcium (Ca) (mg/100 g fresh weight)	17.82
Manganese (Mn) (μg/100 g fresh weight)	105.66
Iron (Fe) (mg/100 g fresh weight)	1.00
Cobalt (Co) (μg/100 g fresh weight)	1.26
Copper (Cu) (μg/100 g fresh weight)	533.73
Zinc (Zn) (μg/100 g fresh weight)	438.42
Selenium (Se) (μg/100 g fresh weight)	11.23
Molybdenum (Mo) (μg/100 g fresh weight)	4.64
Sodium (Na) (mg/100 g fresh weight)	100.41
Lead (Pb) (μg/100 g fresh weight)	39.06
Cadmium (Cd) (μg/100 g fresh weight)	1.84
Silicon (Si) (mg/100 g fresh weight)	3.75
Potassium (K) (mg/100 g fresh weight)	84.53
Boron (B) (μg/100 g fresh weight)	1298.74

Source: Koyyalamudi et al. (2013)

Fig. 12.2 **a** *A. bisporus* production using bunk growing system. **b** Mushroom packaging. **c** *A. bisporus* production using bag growing system. **d** *A. bisporus* mushrooms had different sizes and cap opening features

Fig. 12.3 Whole and sliced *A. bisporus* mushrooms

Table 12.5 Nutrient content of fresh and conserved *A. bisporus* (DM: dry matter)

Chemical constituents	1	2	3	4
DM (%)	9.62 ± 1.04	9.37 ± 1.43	8.82 ± 0.54	8.23 ± 0.73
Crude protein (DM%)	34.84 ± 0.5	39.30 ± 0.7	35.1 ± 0.45	40.6 ± 0.4
Crude fat (DM%)	2.28 ± 0.02	2.11 ± 0.05	1.63 ± 0.06	2.30 ± 0.015
Crude ash (DM%)	9.23 ± 0.06	9.48 ± 0.03	5.28 ± 0.25	8.34 ± 0.12

Source: Vetter (2003)
1. *A. bisporus* var. 333, fresh. 2. *A. bisporus* var. 229, fresh, sliced. 3. *A. bisporus*, conserved, sliced. 4. *A. bisporus*, conserved, whole

process. In a study conducted by Philippoussis et al. (2010), irrigation process was performed with seven different doses of $CaCl_2$ (0.15%, 0.25%, 0.35%, 0.45%, 55%, 75%, and 1.00%). They achieved such positive results as delay in softening, better color scheme, and extending of the shelf life. Kılıç et al. (2004) tested three different doses of $CaCl_2$ (0.05%, 0.10%, and 0.15%) and reported good results in terms of hardness with 0.10% dose, and the increase in the dose caused losses in crop yields.

Khan et al. (2014) investigated effect of 1 mmol L^{-1} Na_2EDTA + 10 mmol L^{-1} $CaCl_2$ and 1 mmol L^{-1} Na_2EDTA + 2.5% $CaCl_2$ + 0.5% citric acid + 2.5% sorbitol implementations. In the second implementation, they achieved better results such as the continuity of hardness during the storage, maintenance of natural color, and less

Table 12.6 Mineral content of fresh and conserved*A. bisporus* (in parts per million of DM: dry matter)

Mineral elements	1	2	3	4
Aluminum (Al)	21.2 ± 1.66	18.6 ± 2.74	73.6 ± 9.4	59.4 ± 3.2
Arsenic (As)	<0.05	<0.05	<0.05	<0.05
Boron (B)	3.73 ± 0.4	3.57 ± 0.51	<0.05	<0.05
Barium (Ba)	2.37 ± 0.56	2.12 ± 0.34	6.93 ± 0.4	7.78 ± 0.14
Calcium (Ca)	888 ± 10.3	860 ± 72	2064 ± 142	2563 ± 33.2
Cadmium (Cd)	0.22 ± 0.03	0.12 ± 0	0.22 ± 0.03	0.22 ± 0.05
Cobalt (Co)	<0.002	0.09 ± 0.03	0.86 ± 0.07	0.37 ± 0.04
Chromium (Cr)	0.73 ± 0.24	0.85 ± 0.38	1.95 ± 0.36	1.59 ± 0.47
Copper (Cu)	57.7 ± 1.87	64.7 ± 1.16	15.4 ± 0.4	15.1 ± 0.65
Iron (Fe)	49.9 ± 4.6	44.5 ± 4.12	116 ± 5.3	125 ± 5.4
Potassium (K)	38,105 ± 788	39,566 ± 658	1287 ± 77	448 ± 14
Magnesium (Mg)	1099 ± 38	1115 ± 25	391 ± 20.5	470 ± 22.2
Manganese (Mn)	5.70 ± 0.41	6.03 ± 0.20	11.7 ± 0.36	6.02 ± 0.07
Sodium (Na)	861 ± 23.3	849 ± 25	16,065 ± 1246	24,811 ± 109
Nickel (Ni)	0.35 ± 0.05	0.73 ± 0.07	1.56 ± 0.21	1.32 ± 0.15
Phosphorus (P)	11,235 ± 290	10,430 ± 142	3789 ± 67	4508 ± 85
Selenium (Se)	1.88 ± 0.10	3.75 ± 0.9	<0.05	<0.05
Strontium (Sr)	6.70 ± 0.89	7.47 ± 1.19	15.7 ± 1.0	34.2 ± 0.34
Titanium (Ti)	<0.03	0.09 ± 0.04	0.74 ± 0.09	0.83 ± 0.10
Vanadium (V)	<0.05	<0.05	0.35 ± 0.03	0.25 ± 0.09
Zinc (Zn)	60.5 ± 0.47	62.4 ± 0.7	60.1 ± 1.2	86.3 ± 3.65

Source: Vetter (2003)

Table 12.7 Nutrient content of frozen*A. bisporus*

Nutrient	g/100 g dry matter
Total carbohydrates	58.11 ± 1.38
Ash	7.45 ± 0.31
Crude fat	3.14 ± 0.25
Total nitrogen	7.14 ± 0.26
Protein nitrogen	4.40 ± 0.15

Source: Bernaś and Jaworska (2010)

water loss. In order to delay cap opening in *A. bisporus*, Braaksma et al. (2001) used benzyladenine (BA) from cytokinin group, and they achieved fruitful results. Qin et al. (2015) tried a different technique called PLA/PCL/cinnamaldehyde antimicrobial packaging. Samples of *A. bisporus* were stored at 4 ± 1 °C for 16 days in order to investigate the effects of biobased poly (lactic acid) (PLA)/poly (ε-caprolactone) (PCL) blend films with different cinnamaldehyde contents (0, 3, and 9 wt%) on physicochemical and microbial quality of mushroom. PLA/PCL/C9 implementation produced better results in terms of hardness, color, and the reduction in number of microbials. 1-Methylcyclopropene (1-MCP) is also one of the implemantations

Table 12.8 Amino acid content of frozen *A. bisporus*

Amino acids	mg/100 g fresh matter	g/100 g protein
Alanine	109.8 ± 5.3	6.24 ± 0.03
Arginine	101.1 ± 2.3	6.46 ± 0.04
Asparagine	186.6 ± 6.0	11.29 ± 0.11
Glutamine	296.6 ± 2.7	18.51 ± 0.02
Glycine	72.5 ± 2.6	3.92 ± 0.06
Proline	77.2 ± 2.7	4.64 ± 0.12
Serine	91.9 ± 4.9	5.42 ± 0.14
Amino acids	**mg/100 g fresh matter**	**g/16 g protein**
Cysteine	18.2 ± 0.8	1.11 ± 0.01
Histidine	50.1 ± 1.7	3.39 ± 0.08
Isoleucine	57.6 ± 2.2	3.54 ± 0.01
Leucine	117.6 ± 2.3	7.23 ± 0.03
Lysine	102.7 ± 1.1	6.41 ± 0.05
Methionine	31.1 ± 0.8	1.95 ± 0.02
Phenylalanine	76.0 ± 1.0	4.82 ± 0.13
Threonine	82.7 ± 0.8	5.00 ± 0.11
Tyrosine	46.0 ± 1.9	2.95 ± 0.03
Valine	69.3 ± 1.5	4.18 ± 0.04

Source: Bernaś and Jaworska (2010)

used to extend shelf life. Therefore, Huang et al. (2010) investigated the effect of 1-MCP on the chemical and physiological characteristics of *A. bisporus*. While 10 μg L^{-1} dose of 1-MCP increased peroxidase activity (POD), superoxide dismutase activity (SOD), catalase activity (CAT), soluble sugar, and protein contents, there was a decrease in the contents of malondialdehyde (MDA). As for 100 μg L^{-1} concentration, it was reported that POD, CAT, MDA, soluble sugar, and protein contents increased, and there was a decrease in the SOD value. Other implementations used to extend shelf life of *A. bisporus* are washing in antimicrobial compounds, stipe trimming, and storage in a modified atmosphere.

12.3 Nutritive Value, Consumption, and Extending Shelf Life of *Pleurotus ostreatus* (Jacq.) P. Kumm

One of the most commonly cultured mushrooms in the world is the genus *Pleurotus*, and the most commonly produced species of this genus is *Pleurotus ostreatus* known as "oyster" mushroom. The factors that led the common production of *Pleurotus* after *A. bisporus* are the ability of fast biological conversion of various agricultural wastes (Philippoussis et al. 2000), no need for compost in farming, which enables saving in time and labor; cheaper and easier production; and relatively lower initial investment cost that is ideal for families with limited financial support (Kurt 2008; Kurt and Buyukalaca 2010). The nutritive value of this

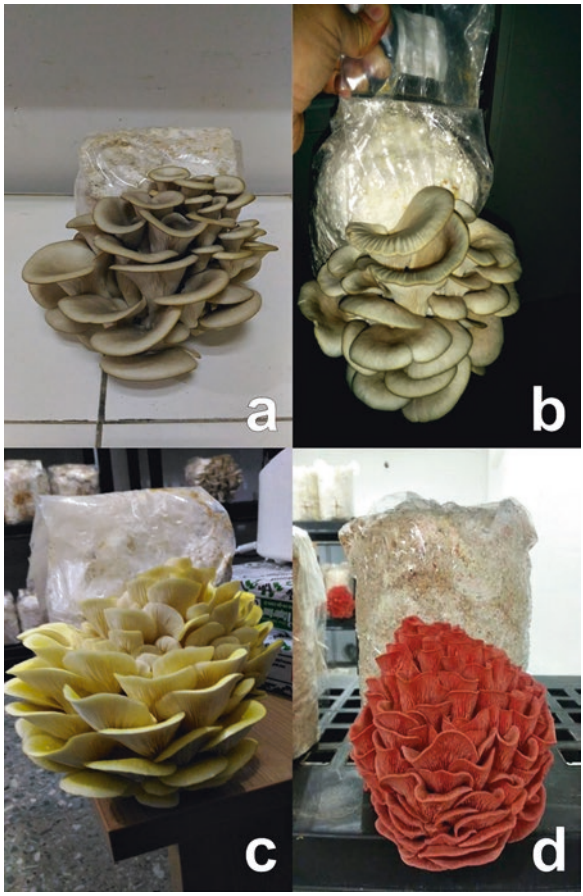

Fig. 12.4 **a**, **b** *P. ostreatus*production in the sawdust substrate supplemented tea waste at least 30%. **c** *P. citrinopileatus* production in the sawdust substrate supplemented tea waste at least 30%. **d** *Pleurotus djamor* production in the sawdust substrate supplemented tea waste at least 30%

mushroom was identified as good source of non-starchy carbohydrates, high content of dietary fiber, and moderate quantity of proteins, including amino acids, minerals, and vitamins (Croan 2004; Ahmed et al. 2013). The nutritive value of *Pleurotus* species varies with the mixture of substrate used by the growers. In production of *Pleurotus* species, sawdust which is obtained from different tree species is generally used in the world. *Pleurotus* producers have started to seek for alternative substrate materials due to the increasing demand for sawdust (Hwang et al. 2015) (Fig. 12.4). Besides, using the waste produced by most common farming practices of the region provides great value in terms of recycling agricultural wastes.

So far, there have been various studies conducted on the use of different agricultural wastes as substrate. Some examples of such studies are as follows:

Coffee wastes in *P. ostreatus* and *P. pulmonarius* (Velázquez-Cedeño et al. 2002)
Banana leaves in *P. ostreat*us and *P. sajor-caju* (Reddy et al. 2003)
Coffee grounds in six different species of *Pleurotus* spp. (Mata et al. 2005)
Rice stalk and coir fiber in *P. florida* (Shashirekha and Rajarathnam 2007)

Table 12.9 The nutritive contents of *P. ostreatus* collected from nature and grown in different substrates

Nutrient	A	B	C	Wild PO
Potassium (mg/100 g)	11.34 ± 0.02	9.42 ± 0.15	10.33 ± 0.025	–
Sodium (mg/100 g)	4.39 ± 0.012	4.03 ± 0.02	4.11 ± 0.01	–
Calcium (mg/100 g)	8.87 ± 0.006	5.37 ± 0.01	6.85 ± 0.017	–
Magnesium (mg/100 g)	3.57 ± 0.01	1.69 ± 0.015	2.22 ± 0.015	–
Phosphorus (mg/100 g)	56.77 ± 0.015	51.97 ± 0.01	53.24b ± 0.04	–
Ash (%)	4.75 ± 0.05	8.19 ± 0.01	6.76 ± 0.03	7.25
Protein (%)	20.11 ± 0.05	20.03 ± 0.017	20.06 ± 0.02	16.96
Carbohydrate (%)	45.74 ± 0.06	41.8 ± 0.05	45.74 ± 0.06	62.27
Moisture content (%)	9.25 ± 0.03	10.72 ± 0.03	2.22 ± 0.02	10.31
Fat (%)	3.09 ± 0.02	2.31 ± 0.02	2.76 ± 0.05	3.21
Dietary fiber (%)	17.51 ± 0.02	17.35 ± 0.02	17.42 ± 0.03	–

Source: Oyetayo and Ariyo (2013); Akata et al. (2012)
A*: P. ostreatus* cultivated on *Pycnanthus ongoleubis*
B: *P. ostreatus* cultivated on *Ceiba pentandra*
C: *P. ostreatus* cultivated on *Canarium* sp.
Wild PO: wild *P. ostreatus*

Table 12.10 General nutrition content of frozen *P. ostreatus*

Nutrient	g/100 g dry matter
Total carbohydrates	76.97 ± 0.62
Ash	4.09 ± 0.22
Crude fat	4.34 ± 0.08
Total nitrogen	3.55 ± 0.12
Protein nitrogen	2.62 ± 0.11

Source: Bernaś and Jaworska (2010)

Date palm wastes in *P. ostreatus* (Alananbeh et al. 2014)

Pruned wax apple and Indian jujube branches in *P. eryngii* (DC.:Fr.) Quél. (Hwang et al. 2015)

The nutritive contents of *P. ostreatus*, collected from nature by Akata et al. (2012) and grown in different substrates by Oyetayo and Ariyo (2013), are given in Table 12.9. The findings obtained through the study can enlight the growers regarding the nutritive value of *Pleurotus*. As it can be seen clearly in the Table, different substrates do not cause considerable differences in the nutritious contents of the mushroom.

The results of the study regarding nutritious and amino acid contents of *P. ostreatus* conducted by Bernaś and Jaworska (2010) are given in Tables 12.10 and 12.11. The researchers conducted the same study on *A. bisporus*, for which the results were given in Tables 12.6 and 12.7. When the two mushroom types were compared in terms of protein nitrogen, higher results were obtained from *A. bisporus*. However, when total nitrogen was considered, it was seen that *P. ostreatus* produces higher values. Crude fat was found to be higher in *A. bisporus*, while total carbohydrates were determined to be higher in *P. ostreatus*.

Table 12.11 Amino acid content of frozen *P. ostreatus*

Amino acids	mg/100 g fresh matter	g/100 g protein
Alanine	116.7 ± 4.9	6.35 ± 0.06
Arginine	132.0 ± 4.6	8.08 ± 0.33
Asparagine	170.9 ± 2.0	10.08 ± 0.07
Glutamine	197.6 ± 1.9	11.82 ± 0.12
Glycine	87.0 ± 3.7	4.51 ± 0.04
Proline	79.2 ± 8.7	4.55 ± 0.40
Serine	92.3 ± 2.3	5.22 ± 0.05
Amino acids	mg/100 g fresh matter	g/16 g protein
Cysteine	24.3 ± 1.1	1.41 ± 0.02
Histidine	44.7 ± 2.5	2.90 ± 0.20
Isoleucine	85.1 ± 2.9	4.49 ± 0.05
Leucine	144.2 ± 1.2	8.49 ± 0.08
Lysine	113.2 ± 3.4	6.77 ± 0.24
Methionine	40.4 ± 2.4	2.42 ± 0.03
Phenylalanine	83.8 ± 1.4	5.09 ± 0.10
Threonine	91.5 ± 1.3	5.30 ± 0.13
Tyrosine	67.3 ± 2.7	4.13 ± 0.04
Valine	103.3 ± 1.8	5.96 ± 0.06

Source: Bernaś and Jaworska (2010)

Fig. 12.5 **a** *P. ostreatus*. **b** *P. citrinopileatus*. **c**, **d** Different species of *Pleurotus* with other mushroom species

Pleurotus species are consumed as fresh, dried, and frozen forms and can be found in the shelves of supermarkets in the world (Fig. 12.5). There have been various studies conducted on extending the shelf life of the most commonly cultured and edible species of *Pleurotus*, especially *P. ostreatus*. Xiao et al. (2011) conducted

experiments with fresh samples of *P. ostreatus* in order to extend the shelf life of this mushroom species using modified atmosphere packaging (MAP) and chemical treatments. At the end of the study, it was determined that low-density polyethylene (LDPE) was more suitable than polyvinyl chloride (PVC) and LDPE-PVC. Moreover, the MAP in combination with chemical treatments (sorbitol 0.05 g/100 g, $CaCl_2$ 1.0 g/100 g, and citric acid 3.0 g/100 g) showed inhibitory effect on weight loss and mushrooms cell permeability. The results of polyphenol oxidase (PPO) activity, texture, and organoleptic analysis revealed that active MAP, with the combination of 1.5% O_2 and 20% CO_2 as well as chemical treatments were found to be useful on the quality and extending shelf life of oyster mushroom.

12.4 The Nutritional Value, Significance, and Consumption of *Ganoderma lucidum* (Curtis) P. Karst

The increase of diseases due to the increased stress and changes in eating habits has led people to nature. Some plants and mushrooms found in nature can make people more resistant to certain diseases by strengthening the immune system. *Ganoderma lucidum* known as medicinal mushroom is called by different names in different countries (Japan, "reishi"; China, "lingzhi"; Korea, "Hangul or Yeongji"; England, "glossy ganoderma" or "shiny polyporus"; Nigeria, "Leman kwado" or "Burtuntuna" (Shamaki et al. 2012); Turkey, "mushroom of immortality").

It is one of the most important medicinal mushrooms and is known since ancient times. The results of the study conducted by Shamaki et al. (2012) on the nutrient content of *G. lucidum* collected from nature (Lafia in Nassarawa State, North Central Nigeria), dried, pulverized, and analyzed are given in Table 12.12. As clearly seen from the Table, this mushroom species is rich in protein, fiber, and various mineral elements. In addition to rich content, *Ganoderma lucidum* contains also significant amount of germanium (e.g., 489 μg/g Ge in the freeze-dried *Ganoderma* extract powder (Chiu et al. 2000)). This element is known for antimutagenic and anticarcinogenic properties (Chiu et al. 2000). Therefore, this mushroom has attracted the attention of especially the cancer patients in recent years due to high germanium content. In a study conducted by Chiu et al. (2000), germanium was found as fifth highest element among analyzed minerals with 489 μg/g (K, 84,650; Ca, 9449; Mg, 4480; Na, 1612; Ge, 489; Zn, 257; Ga, 246; Mn, 179; Fe, 115; Ni,133; Pb, 86; Cr, 69; Bi, 71; Cu, 47; Co, 31; Sr, 28; Be,4; Ba, 2 μg/g in the freeze-dried *Ganoderma* extract powder). The ganoderic acid, which is determined as a triterpene component, regulates the blood pressure, blood sugar, and fat in the body, reduces the levels of cholesterol, and prevents blood clotting. Ganoderic acids A and B were isolated to be the first triterpene component of *G. lucidum* by Kubota et al. (1982). Then, over 150 lanostane-type triterpenoids of *Ganoderma* were divided into ten groups by defining their features (Kim and Kim 2002; Chang and Miles 2004). Morigiva et al. (1996) determined that ganoderic acid F has a strong

Table 12.12 The nutritive content of *G. lucidum* (crude extract of *G. lucidum* powder)

Nutrient	Content
Moisture (%)	10.54
Total ash (%)	5.93
Crude protein (%)	17.55
Crude fat (%)	2.60
Crude fiber (%)	30.25
Carbohydrates (%)	33.13
Nitrogen (%)	23.52
Sodium (mg/kg)	192.5
Calcium (mg/kg)	322.6
Magnesium (mg/kg)	8.7
Iron (mg/kg)	44.6
Potassium (mg/kg)	317.1
Lead (mg/kg)	0.106
Copper (mg/kg)	0.843
Zinc (mg/kg)	14.65
Manganese (mg/kg)	1.03
Chromium (mg/kg)	0.140
Phosphate (mg/kg)	197.1
Arsenite (mg/kg)	1.230
Molybdenum (mg/kg)	0.090
Nickel (mg/kg)	0.095
Carbon (mg/kg)	68.2
Fluorine (mg/kg)	0.0039
Silicon (mg/kg)	4.10
Aluminum (mg/kg)	0.20
Cobalt (mg/kg)	0.026

Source: Shamaki et al. (2012)

antihypertension activity, while ganoderic acids B, D, H, and Y have a weaker impact (Chang and Miles 2004). Ganoderic acids R and S, which have antihepatotoxic activity, were identified by Hirotani et al. (1986). Ganoderial F and ganodermanontriol, as anti-HIV components, were isolated and determined by El-Mekkawy et al. (1998) (Chang and Miles 2004). Beta-glucan, which is known one of polysaccharides in the *Ganoderma* (Mizuno et al. 1995), promotes formation of T cells fighting against infected cell by stimulating the immune system. It was reported that *G. lucidum* consisted two types of steroids, ergosterol and cholesterol (20 steroids were isolated (Ha et al. 2000; Ma et al. 2002), and these steroids showed lipid-lowering and anti-atherosclerotic effect (Kimura et al. 1988) by different researchers (Chang and Miles 2004). Many scientific studies about the positive effects of this mushroom species on human health were conducted, and over 900 scientific studies were found in PubMed. The majority of these studies have demonstrated the medicinal value of this mushroom species and produced promising results about

applicability of *Ganoderma* in treatment of certain diseases. Although all of these studies produced promising results, in the use of this mushroom for treatment of any disease, a doctor should be consulted.

G. lucidum can be consumed as tea prepared using dried mushroom. Mushroom tea is prepared as follows: add water into the ceramic or glass teapot, put the mushroom pieces into the water (2–3 g L^{-1} for healthy person, 6–9 g L^{-1} for sick person), and boil the mixture on low heat. Consumers can also follow the instructions given by the prospectuses prepared by the producer companies. Metal and aluminum pots and tools should not be used during preparation of the mushroom tea. Metal appliances cause the decline of important substances in mushroom. This mushroom can also be consumed as instant coffee, instant tea spore powder, or pill. In the recent years, different products of *Ganoderma* such as shampoo, body lotion, soap, and toothpaste are on the market. The price of 1 kg dried *Ganoderma* mushroom sold by Turkish companies varies between 800 and 1000 TL (Turkish Lira, Approximatelly 250 to 350 USD). Although all of the positive effects, it should be accepted as a medicine and used with advices of doctors due to its medical properties (it has many components at high dose and side effects can be seen).

G. lucidum is one of the cultured mushrooms. The substrate preparation used in the cultivation of *G. lucidum* is not difficult and complex as the substrate used in *A. bisporus* cultivation. The studies on cultivation of *Ganoderma* were conducted using different substrate mixtures by different researchers (Fig. 12.6). For example, in a study carried out by Yen (2008) in Turkey, oak sawdust, wheat bran, molasses, and calcium sulfate ($CaSO_4$) were used. The other studies can be summarized as follows: tea waste, hornbeam sawdust, and wheat bran by Peksen and Yakupoglu (2009), hornbeam and oak sawdust supplemented with wheat bran by Peksen et al. (2011), the mixtures of sawdust and wood chip of oak with tea manufacture waste by Yakupoğlu and Pekşen (2011), and beech sawdust, olive oil, and mineral salts by Berovic et al. (2012). *Ganoderma* can also be produced in logs by mycelia inoculation to logs.

Fig. 12.6 **a** Cultured *G. lucidum*. **b** *G. lucidum* in the nature

12.5 The Importance, Nutritive Value, and Consumption of *Morchella*

Morels have a cap in various colors ranging from pale yellow to dark brown and have a thick stipe in color white or whitish, and their inside is empty (Tüzel and Boztok 1987). True morels are consumed widely because of their flavor (Figs. 12.7 and 12.8). Due to the increasing demand of them in exclusive restaurants across the world, their trading value keeps increasing each year. Therefore, countries such as China, Pakistan, India, the USA, and Turkey, which are rich with morels, export morels to other countries (Pilz et al. 2007) (Fig. 12.9). It is known that 1 kg of fresh morels is bought for about 70–80 TL (Turkish Lira), while a kilo of dried morels is bought for about 250–300 TL from collectors by local mushroom companies in Turkey. However, the export price of morels is not clearly expressed by the local companies.

The first patent regarding the cultivation of *Morchella* was taken by Ower from the USA. Ower, prior to the application regarding the patent, published a paper explaining briefly how the mushroom was grown artificially (Ower 1982). Jim Malachowski and Dr. Gary Mills from Neogan Anonymous Company published the first USA patent of the morel cultivation in 1986 (Ower et al. 1986). Jim Malachowski and Gary Mills continued to develop the process and took two additional patents (Ower et al. 1988, 1989). *M. esculenta* collected from San Francisco State University campus was used for these patents. However, Kuo (2006) speculated that this species was actually *M. rufobrunnea*. Although cultivation technique was developed

Fig. 12.7 Consumption morel mushroom with pepper as grill

Fig. 12.8 Morel mushroom dish with onion and pepper

Fig. 12.9 **a** Morel mushroom in the nature. **b** Dried morel mushroom in Spain. **c**, **d** From mushroom export company in Turkey

with just one species, it was claimed that the patents could be applied to all *Morchella* species. However, the procedure was not clear enough to be repeated by the others. In 1990, Neogan Anonymous Company merged with Domino's Pizza under the name "Morel Mountain" for testing the production. Then, the rights for the cultivation process were bought by Terry Farms from Illinois in 1993 and in 1995; a growing facility was constructed at Auburn Technology Park in Auburn, Alabama. However, the first complaints regarding the morels cultivated were related to the lack of flavor and taste. Against all these developments, it is known that nobody has not been successful in producing the mushroom on a larger scale using the applications of the methods found by Ower, and developed by Mill (Source for all information in this paragraph: Pilz et al. 2007).

Tables 12.13 and 12.14 show the results of two different studies regarding the content of mineral and heavy metal of the *Morchella* species (Ozturk et al. 2010; Sarikurkcu et al. 2011; Uzun et al. 2011). As clearly seen in the results of the studies, *Morchella* species, which are rich in the elements that are essential parts of nutrition such as K, P, Mg, and Ca and whose heavy metal content is not above the standard, can be considered as a potential functional food in terms of nutrition (Ozturk et al. 2010).

Studies on *Morchella*, which is admiringly consumed due to its flavor and taste and therefore whose commercial value is high, focus mostly on its molecular taxonomy (Taşkın et al. 2010, 2012; Işiloğlu et al. 2010; O'Donnell et al. 2011; Du et al. 2012a, b; Kuo et al. 2012; Clowez 2012; Elliott et al. 2014; Richard et al. 2015; Clowez et al. 2014; Loizides et al. 2015, 2016; Taşkın et al. 2015; Voitk et al. 2016;

Table 12.13 Mineral and heavy metal content of some *Morchella* species (mg/kg dw)

Element	*M. conica* (Ozturk et al. 2010)	*M. vulgaris* (Sarikurkcu et al. 2011)
Cobalt (Co)	0.33 ± 0.01	3.62 ± 0.06
Molybdenum (Mo)	0.10 ± 0.001	–
Copper (Cu)	39 ± 0.03	28 ± 0.3
Manganese (Mn)	41 ± 2	77 ± 1.1
Zinc (Zn)	90 ± 3	146 ± 0.5
Iron (Fe)	340 ± 4	1714 ± 10
Calcium (Ca)	875 ± 4	–
Magnesium (Mg)	1600 ± 10	–
Sodium (Na)	490 ± 30	–
Potassium (K)	20,400 ± 960	–
Phosphorus (P)	13,250 ± 400	–
Arsenic (As)	0.25 ± 0.004	–
Mercury (Hg)	0.06 ± 0.002	–
Cadmium (Cd)	0.20 ± 0.002	0.89 ± 0.01
Lead (Pb)	1.20 ± 0.008	4.2 ± 0.1
Chromium (Cr)	0.70 ± 0.01	7.0 ± 0.03
Nickel (Ni)	1.10 ± 0.07	4.0 ± 0.1

Sources: Ozturk et al. (2010); Sarikurkcu et al. (2011)

Table 12.14 Mineral and heavy metal content of different *Morchella* species (mg/kg dw)

Element	*M. crassipes*	*M. rigida*	*M. vulgaris*
Calcium (Ca)	1415	1180	250
Iron (Fe)	100	48	110
Potassium (K)	9290	10,280	13,830
Magnesium (Mg)	1045	760	790
Zinc (Zn)	81	60	240
Cadmium (Cd)	1.01	0.48	1.08
Copper (Cu)	52	49	43
Manganese (Mn)	12.8	7.4	17.7
Nickel (Ni)	0.81	0.60	2.42
Lead (Pb)	1.5	0.9	<0.01

Sources: Uzun et al. (2011)

Fig. 12.10 **a** *Gyromitra* **b** *Verpa conica*

Taşkın et al. 2016). In these studies, species from different regions and continents have been discovered, and common species have been identified in collaboration with researchers from various countries. The main purpose of all these systematic studies is to find out geographical distribution of *Morchella* species by correct taxonomy, provide the sustainability of the species and protect the important and commercial species.

Morchella species are consumed as fresh, frozen, or dried (Fig. 12.9). However, dried consumption is more common due to the necessity that the fresh food should be consumed immediately. *Morchella* species can be dried easily as they are hollow inside. This mushroom cannot be canned because their low acid content requires high temperature and pressure to avoid bacterial contamination (Weber 1988; Pilz et al. 2007). On a worldwide known shopping website, the price for 2 oz. (about 56 g) of dried *Morchella esculenta* is 30 USD.

True morels may be confused with *Gyromitra* and *Verpa* species (Fig. 12.10), which are among Pezizales ordo. Although some species of these genera may upset one's digestive system or cause further damages. However, they are traditionally eaten or even sold. In many countries, people think of *Gyromitra* as edible and do not know that it is toxic. This mushroom contains ethylidene

gyromitrin, acetaldehyde-N-methyl-N-formylhydrazone, and according to IUPAC, 2-ethylidene-1-methylhydrazide is toxic (Arshadi et al. 2006; Pilz et al. 2007). Gyromitrin is volatile, and it evaporates during the cooking period because its melting point is 189.5 °F (87.5 °C). However, some of it may not evaporate while being cooked and stay in the food, affecting the person who cooks it (Pilz et al. 2007). Fresh, raw, and not completely processed *Gyromitra* has toxic effects (Pyysalo and Niskanen 1977; Pilz et al. 2007).

12.6 The Importance, Nutritive Value, and Consumption of Some Edible Species of Tricholoma Genus

Tricholoma genus that contain many species and some of this species such as *Tricholoma anatolicum* H.H. Doğan and Intini, *Tricholoma matsutake* (S. Ito et Imai) Sing., and *Tricholoma magnivelare* (Peck) redhead, have a big importance as edible and commercial. Successful results have not been obtained yet in the cultivation of these species because they are ectomycorrhizal. Therefore, the some edible species of *Tricholoma* have a high commercial value. *T. anatolicum* named by Intini et al. (2003), one of *Tricholoma* species, was found in Turkey and described as endemic to Turkey. This species spread in Mediterranean region of Turkey, especially in Taurus Mountain, and while the other *Tricholoma* species form ectomycorrhizas with *Pinus*, *T. anatolicum* forms ectomycorrhizal relationship with *Cedrus*. The ecological requirements of *T. anatolicum* are as follows: should be in cedar forests (*Cedrus libani*), about 30 years old, well drained, sandy, in non-fertile soils, in the months of October–November, at the Mediterranean climate, 1400–1700 m of elevation, and ectomycorrhizal with cedar (*Cedrus libani*) (Doğan and Akata 2011). This *Tricholoma* species has extremely high commercial value, and it is consumed in domestic market and exported also. Some species of *Tricholoma*, especially *T. matsutake*, are consumed by Japanese people due to their flavor, medicinal properties, and iconic significance (Vaario et al. 2011). In the recent years, matsutake mushroom collected from pine forests in Japan has declined rapidly due to pine nematode (*Bursaphelenchus lignicolous*) found and spread in Japanese pine forests (Vaario et al. 2011). This fungus plays an important role in forest ecosystems due to its ectomycorrhizal relationship with forest trees and fructification to be completed under the ground.

In many countries, in situ conservational strategies are commonly applied to preserve commercially important mushrooms that are found in nature and also cannot be cultivated. As a result of improper collection of these mushrooms, sustainability of them in their native habitat is decreasing year by year. The most important mistakes of *Tricholoma* collectors are as follows: collecting immature mushrooms; using tools such as stick while harvesting mushrooms destroying their growing area; neglecting to close the opened areas, which prevent reforming of mushrooms; and storing the collected mushrooms in plastic bags. The errors in the morels collected are hand-drawn collection, collecting very small mushrooms, the use of

plastic bags for the collected mushrooms, and not leaving any mushroom, which causes less spore production for future. These mistakes made by collectors cause reduction in their incomes, losses of country' economy, and most importantly deficiency of genetic resources. Therefore, mushroom collectors should be educated with practical training for proper or correct mushroom collection, and also authorities should implement some regulations about improper harvesting of mushrooms.

The nutritional contents of some species of *Tricholoma* were given in Tables 12.15 and 12.16 (Kalmış et al. 2011; Doğan et al. 2012). The mycelia of *T. anatolicum* was compared with its young and old fruiting bodies in terms of nutritional content. In Table 12.17, the samples of *T. matsutake* collected from different regions of China were also compared in terms of their mineral contents (Li et al. 2013).

In Japan, while the price for 1 kg of matsutake mushroom is 20,000 ¥ (JPY) (about 171.2 USD) in a good collecting season, this price can go up to 100,000 ¥ (JPY) (about 856.00 USD) in poor collecting seasons. The price of the *Tricholoma* species imported from different countries varies between 3000 ¥ (JPY) (about 25.68 USD) and 9000 ¥ (JPY) (about 77.04 USD). The reason for the higher prices for

Table 12.15 Nutrient composition of *T. anatolicum* (for minerals: mg/kg-dry sample)

Nutrient	Mycelium	Young fruiting body	Mature fruiting body
Moisture (%)	33.69 ± 2.51	14.75 ± 0.16	11.03 ± 0.16
Ash (%)	0.26 ± 0.06	2.67 ± 0.12	1.30 ± 0.08
Protein (%)	97.02 ± 0.52	90.24 ± 0.55	93.95 ± 0.42
Crude oil (%)	0.97 ± 0.05	0.56 ± 0.03	0.53 ± 0.17
Total carbohydrate (%)	1.74 ± 0.56	6.71 ± 0.58	4.40 ± 0.54
Energy (kcal)	403.8 ± 0.6	391.2 ± 1.6	396.6 ± 2.0
Energy (kJ)	1714.9 ± 2.0	1668.9 ± 6.4	1691.6 ± 4.6
Iron (Fe)	73.4 ± 5.91	77.0 ± 4.08	68.4 ± 0.82
Sodium (Na)	127.7 ± 6.5	69.0 ± 2.48	79.3 ± 0.69
Potassium (K)	40.6 ± 3.87	53.2 ± 3.04	58.3 ± 3.91
Zinc (Zn)	66.4 ± 0.59	65.41 ± 0.61	61.4 ± 0.97
Copper (Cu)	38.0 ± 0.17	38.88 ± 1.34	46.68 ± 2.77
Calcium (Ca)	88.13 ± 1.39	77.86 ± 3.44	81.86 ± 2.35

Source: Kalmış et al. (2011)

Table 12.16 Metal concentrations in some *Tricholoma* species (mg/kg dw)

Species	Mn	Fe	K	Na	P
T. anatolicum	51 ± 2.2	1711 ± 75	35,660 ± 1343	3033 ± 132	4546 ± 102
T. cedretorum	36 ± 1.3	983 ± 65	37,090 ± 1120	2909 ± 124	5786 ± 145
T. columbetta	71 ± 5.4	3215 ± 157	50,614 ± 1230	3206 ± 135	10,883 ± 321
T. imbricatum	16 ± 1.2	744 ± 56	24,217 ± 989	3209 ± 143	7922 ± 243
T. orirubens	113 ± 8.9	2450 ± 132	65,266 ± 1500	3450 ± 124	7755 ± 233

Source: Doğan et al. (2012)

Table 12.17 Element contents in fruiting bodies of *T. matsutake* samples (mg/kg)

	Ca	Cu	Fe	K
TM1	380 ± 130	5.6 ± 4.1	39 ± 10	890 ± 350
TM2	250 ± 79	27 ± 8	42 ± 12	1100 ± 360
TM3	510 ± 180	21 ± 9	56 ± 9	830 ± 250
TM4	300 ± 95	25 ± 7	42 ± 16	1200 ± 430
TM5	300 ± 53	32 ± 6	37 ± 12	1100 ± 360
TM6	260 ± 83	25 ± 4	38 ± 6	1100 ± 400
TM7	280 ± 94	34 ± 6	27 ± 8	1000 ± 340
TM8	310 ± 96	28 ± 5	29 ± 7	1200 ± 330
TM9	430 ± 130	26 ± 7	39 ± 13	980 ± 260
TM10	270 ± 69	28 ± 7	43 ± 9	1100 ± 280
TM11	270 ± 86	29 ± 10	44 ± 14	950 ± 330
TM12	320 ± 56	27 ± 7	30 ± 7	1200 ± 350
Total	320 ± 120	26 ± 10	39 ± 13	1100 ± 350
	Mg	Mn	Na	Zn
TM1	350 ± 130	2.4 ± 1.0	410 ± 120	71 ± 28
TM2	300 ± 36	3.3 ± 0.6	330 ± 72	36 ± 8
TM3	200 ± 33	2.5 ± 2.3	400 ± 130	100 ± 51
TM4	290 ± 51	3.2 ± 1.3	350 ± 87	33 ± 7
TM5	260 ± 32	3.1 ± 0.5	320 ± 43	51 ± 18
TM6	270 ± 55	2.4 ± 0.7	320 ± 75	33 ± 7
TM7	270 ± 30	2.7 ± 0.7	320 ± 71	48 ± 20
TM8	240 ± 34	3.6 ± 1.1	320 ± 65	35 ± 9
TM9	130 ± 33	3.6 ± 1.8	440 ± 150	88 ± 31
TM10	300 ± 53	2.3 ± 1.2	430 ± 130	35 ± 7
TM11	260 ± 40	3.5 ± 1.4	380 ± 120	42 ± 14
TM12	270 ± 40	3.1 ± 0.5	310 ± 79	38 ± 8
Total	260 ± 73	3.0 ± 1.2	360 ± 110	51 ± 30

Source: Li et al. (2013)
TM1 (Lanping, Nujiang, Yunnan), TM2 (Deqin, Diqing, Yunnan), TM3 (Shangri-la, Diqing, Yunnan), TM4 (Shangri-la, Diqing, Yunnan), TM5 (Yulong, Lijiang, Yunnan), TM6 (Jianchuan, Dali, Yunnan), TM7 (Binchuan, Dali, Yunnan), TM8 (Xiangyun, Dali, Yunnan), TM9 (Daocheng, Ganzi, Sichuan), TM10 (Xiangcheng, Ganzi, Sichuan), TM11 (Muli, Liangshan, Sichuan), TM12 (Xichang, Liangshan, Sichuan)

domestic ones is explained as quality losses in imported mushrooms during transport (Matsutani 2010). It is known that 1 kg of fresh *T. anatolicum* is bought for about 60–70 TL (Turkish Lira) from collectors by local mushroom companies in Turkey (Fig. 12.11). However, it is not clear yet that how much the local companies sell it to abroad countries. Because the export price is not clearly expressed by the local companies. It is also known that Japanese people like *T. anatolicum* due to its aroma and odor similar to the smell of calendula (specific odor and aroma as a result of association with cedar).

Fig. 12.11 **a**, **b** *Tricholoma anatolicum* in cedar forests of Feke, Adana region of Turkey. **c** *T. anatolicum* harvested by local collectors of Feke region of Turkey for exporting to Japan. **d** After a national edible mushroom congress of Turkey organized by Cukurova University, Adana, *T. anatolicum* barbecue party

12.7 The Importance, Nutritive Value, and Consumption of *Boletus edulis* Bull: Fr

Boletus genus which contained many species is known as ectomycorrhizal edible mushroom, and, therefore, it cannot be cultivated. The natural habitat for *Boletus* genus is determined as deciduous coniferous forests, especially in symbiosis with oak (*Quercus* sp.), beech (*Fagus sylvatica*), hornbeam (*Carpinus betulus*), and chestnut (*Castanea sativa*) (Šırıć et al. 2014). The edible species of *Boletus* genus collected from nature are consumed by European countries. *B. edulis* and close species are known as porcini in the world. Porcini group can form ectomycorrhizal symbiosis with some families such as Pinaceae, Fagaceae, and Dipterocarpaceae (Cui et al. 2015). The cultivation of edible ectomycorrhizal mushrooms cannot be achieved because of the need to establish relationship between mushroom and tree. *Boletus edulis* is the most consumed and preferred species in *Boletus* genus due to its aroma, sensory qualities, texture, and biologically active compounds (Wang et al. 2015).

Besides *Boletus* species is rich in terms of proteins, carbohydrates, vitamins, and essential elements, its anti-inflammatory, antimicrobial, antioxidant, antiviral, antitumor, and immunomodulatory activities and reduction of blood glucose levels effect were reported (Heleno et al. 2011; Wang et al. 2015). The nutritional value of *B. edulis* was summarized in Tables 12.18 and 12.19 (Gyar and Owaku 2011; Wang

Table 12.18 Nutrient content of *Boletus edulis* (on a dw)

Nutrient	Content
Moisture content (%)	28.99 ± 0.11
Ash content (%)	22.06 ± 0.13
Crude fat content (%)	07.44 ± 0.02
Crude fiber content (%)	10.34 ± 0.10
Crude protein content (%)	5.22 ± 0.01
Carbohydrate (%)	25.95 ± 0.11
Calcium (Ca) (mg/L)	14.94 ± 0.01
Magnesium (Mg) (mg/L)	12.3 ± 0.03
Manganese (Mn) (mg/L)	0.56 ± 0.02
Sodium (Na) (mg/L)	2.44 ± 0.01
Zinc (Zn) (mg/L)	0.10 ± 0.02
Chromium (Cr) (mg/L)	0.27 ± 0.03
Iron (Fe) (mg/L)	2.55 ± 0.10
Potassium (K) (mg/L)	2.01 ± 0.11
Copper (Cu) (mg/L)	0.55 ± 0.22

Source: Gyar and Owaku (2011)

Table 12.19 Element analysis of cap, stipe and underlying soil of *Boletus edulis* (mg/kg dw)

Nutrient	Cap	Stipe	Soil
Calcium (Ca)	320 ± 26	317 ± 33	1624 ± 45
Copper (Cu)	51 ± 34	32 ± 4	152 ± 20
Iron (Fe)	931 ± 82	4200 ± 678	52,613 ± 656
Potassium (K)	9413 ± 567	4865 ± 245	8533 ± 234
Magnesium (Mg)	429 ± 54	297 ± 65	3665 ± 867
Manganese (Mn)	26 ± 11	70 ± 13	945 ± 77
Sodium (Na)	191 ± 53	122 ± 23	1972 ± 45
Phosphorus (P)	6615 ± 343	2429 ± 545	1495 ± 22
Zinc (Zn)	88 ± 4	41 ± 33	118 ± 67

Source: Wang et al. (2015)

et al. 2015). The mineral content of *B. edulis* collected from mixed coniferous and broadleaf forest ground were determined in cap, stipe, and soil separately in Table 12.19 (Wang et al. 2015). The most interesting results obtained from this study can be listed as follows: P, K, Mg, and Zn elements were determined as higher in cap than stipe, and Fe and Mn elements were found to be higher in stipe than cap.

As in the *Morchella*, the researchers worked on *Boletus* focus mostly on its molecular taxonomy. Classical taxonomy methods that contained microscopic and morphologic techniques are not enough to determine species diversity because of the great similarity between the morphology of the species. Therefore, the researchers studied on *Boletus* have started to use the molecular methods as well as morpho-taxonomic techniques for species diversity studies (Lian et al. 2008; Dentinger et al. 2010; Feng et al. 2012; Nuhn et al. 2013; Wu et al. 2014).

Fig. 12.12 *B. edulis* in the nature (Turkey)

Fig. 12.13 Dried *Boletus* mushroom. **a** From Barcelona, Spain. **b** From Germany

It was reported by different researchers that the porcini group mushroom species are consumed 20,000–100,000 tonnes annually; the total price for fresh porcini mushroom in the USA was 60 USD/kg, even rising up to 200 USD/kg (Hall et al. 1998). This mushroom group is consumed as fresh or dried, after collecting from nature (Figs. 12.12 and 12.13). On a worldwide known shopping website, the price for 1 lb (about 450 g) of porcini mushroom is 44.50 USD. In a study carried out by Hall et al. (1998), it was reported that this mushroom can be cooked without loss of its aroma at autoclave temperature, dried, and stored (Ney and Freytag 1980); therefore, it was demanded by soup and stew manufacturers, chefs, and gourmets; also it was used in traditional Chinese medicine (the major component of "shujin wan" used in accelerating the blood circulation and relieving muscle in Taiyuan, Shanxi Province; "shujin wan" is a traditional herbal medicine (Liu 1984).

12.8 Nutritional Value, Medical Significance, and Consumption of *Lentinula edodes* (Berk.) Pegler

Lentinula edodes is called "shiitake" in Japan because it is associated with the shii tree, and Japan ranks first in the production of this species. In Far East countries, especially in Japan, China, Korea, it has been known as nourishment and medication for long years. Dried shiitake mushroom consists of 58–60% carbohydrate, 20–23% protein (the proportion of its digestibility is 80–87%), 9–10% fiber, 3–4% fat, and 4–5% ash. Dried shiitake mushroom contains 1–5% water-soluble polysaccharides (Rahman and Choudhury 2012). Table 12.20 shows the results of a study carried out by Mallikarjuna et al. (2013) on the mineral element content of shiitake mushroom, which contains a great number of vitamins and minerals such as provitamin D2 (ergosterol), B1, B2, and B12 (Rahman and Choudhury 2012). In their study, *L. edodes* was cultivated by the use of sawdust as substrate. As seen in Table 12.20, *L. edodes* is rich in minerals such as K, P, Na, Ca, Mg, Fe, and Zn. In a study by Longvah and Dosthale (1998), while 77.7% of fatty acid of *L. edodes* was determined to be unsaturated, 22.3% was found as saturated, and it was also determined that it has 68.8% C 18.2 linoleic, 19.2% C 16.0 palmitic, 8.3% C 18.1 oleic, 2.7% C 18.0 stearic, 0.6% C 18.0 linolenic, and 0.4% C 20.0 arachidic acids (Bisen et al. 2010). Besides nutritional value of *L. edodes*, there are studies related to its anticarcinogenic, antitumor, hepatoprotective, cardiovascular, hypolipidemic, hemagglutinating, immune-modulating, antithrombotic, antihypercholesterolemic, antihypertensive, anti-obesity, antidiabetic, antifungal, antioxidant, antiviral, antibacterial, and antimicrobial activities (Bisen et al. 2010; Rahman and Choudhury 2012). In a study by Bisen et al. (2010), the components of *L. edodes* that have medical effects and therapeutic effects of them were summarized. Table 12.21 shows the results obtained by the different researchers (Bisen et al. 2010).

Table 12.20 Mineral content of *Lentinula edodes* (mg/100 g on a dw)

Element	Content
Potassium (K)	1302 ± 101
Phosphorus (P)	769.9 ± 64.1
Calcium (Ca)	174.9 ± 34.3
Sodium (Na)	327.4 ± 51.6
Magnesium (Mg)	40.7 ± 1.2
Iron (Fe)	14.8 ± 2.3
Zinc (Zn)	9.44 ± 0.24
Copper (Cu)	1.48 ± 0.03
Manganese (Mn)	1.00 ± 0.32
Selenium (Se)	0.182 ± 0.01
Nickel (Ni)	0.15 ± 0.03
Lead	Below detectable level
Cadmium	Below detectable level

Source: Mallikarjuna et al. (2013)

Table 12.21 Studies on the medical value of *Lentinula edodes*

Therapeutic effects	Bioactive compounds
Antitumor	Lentinan (β-D-glucans), KS-2-α-mannan-peptide, LEM, LAP (heteroglucan protein), EP3
	Chihara et al. (1969); Fujii et al. (1978); Mizuno (1995, 1996); Wasser and Weiss (1999)
Immunomodulation	Mannoglucan, polysaccharide protein complex, glucan, Lentinan, polysaccharide L-II, (1-3)-β-D-glucan
	Chihara et al. (1969, 1970); Hobbs (2000); Zheng et al. (2005); Yap and Ng (2005); Zhou et al. (2009)
Antimicrobial	Lentinamicin
	Komemushi et al. (1996)
Antiviral	Lentinan, LEM, JLS-18, EP3, EPS4
	Hanafusa et al. (1990); Sarkar (1993); Wasser and Weiss (1999)
Antibacterial	LEM, lenthionine, chloroform, and ethyl acetate extracts
	Yasumoto et al. (1971); Yamamoto (1977); Hanafusa et al. (1990)
Antifungal	Lentin
	Ngai and Ng (2003)
Cardiovascular and hypolipidemic	Eritadenine, lentinan, lentysine
	Chibata et al. (1969); Kamiya et al. (1969); Rokujo et al. (1970); Tokita et al. (1972); Breene (1990); Enman et al. (2008)
Hepatoprotective	Lentinan, LEM, hot water extraction, and ethanol extraction
	Zhu (1985); Akamatsu et al. (2004)
Hemagglutinating	Lectin
	Wang et al. (1999); Vetchinkina et al. (2008)
Antioxidant	Methanol and water extracts, polyphenolic compounds
	Cheung and Cheung (2005); Choi et al. (2006); Xu et al. (2008)

Source: Bisen et al. (2010)

The shiitake mushroom consumed can both be collected from the nature and be cultured. In addition to being cultivated on wood logs, they can also be cultivated in plastic bags in growing rooms (Fig. 12.14). In the case of growing in tree logs, they can be cultivated on hardwood logs of oak, nuts, and eucalyptus (Mata and Savoie 1998; Casaril et al. 2011). In their culture, sawdust is the main material as substrate, and different ingredients like straw, corncobs, wheat bran, rice bran, soybean bran, millet, rye, and corn can be added to this main material (Ohga 1992; Royse 2001; Royse and Sanchez 2007; Regina et al. 2008; Casaril et al. 2011). If the need for different sample mixtures arises, follow these mixtures:

Mixture 1: sawdust, 3–4% rice bran, 1% corn meal or wheat bran, and 1% $CaCO_3$

Mixture 2: sawdust, 10–25% corn waste, and 1–2% $CaCO_3$

Mixture 3: 93.5% fermented sawdust, 5% rice bran, 0.4% corn starch, 0.1% magnesium sulfate, and 1% gypsum

Mixture 4: oak (*Quercus* spp.) and beech (*Fagus sylvatica*) sawdust with 10–25% shredded corn, rice bran, and corn flour (Oei 2003)

Fig. 12.14 **a** *L. edodes* cultivation **b** Fresh *L. edodes* for consumption

Shiitake mushroom is produced over 130,000 tonnes each year; 45% of this production is sold fresh (Fig. 12.14), and the rest is sold dried. Additionally, it can be consumed as solution (1 mg/vial), sugar-coated tablet, capsule, concentrate, powdered extract, syrup, tea, wine, and a medicinal dish. Nominal standard dose of dried mushroom for tea and mushroom dishes is 6–16 g, and this is equal to approximately 90 g of dried shiitake fruiting body. As a tablet, 2–4 of 2-gram-tablets can be swallowed daily. The commercial products of shiitake mushroom can be found in healthy food stores and supermarkets in many countries (Bisen et al. 2010; Rahman and Choudhury 2012).

12.9 Conclusion

Healthy nutrition has become more important as a result of increased diseases in recent years. Therefore, the foods containing high protein, vitamin, mineral elements, and low fat are preferred nowadays. The importance of edible mushrooms for human nutrition is increasing every day because they have the good features given above. The medicinal mushrooms (such as *G. lucidum*) consumed in different ways for medical purposes can be considered in the alternative medicine area. In this chapter, some information about the most cultivated and edible mushroom species, *A. bisporus*, *P. ostreatus*, and *L. edodes*; medicinal mushroom species, *G. lucidum*; and wild mushroom species collected from nature that cannot be cultured and consumed, *Morchella*, some edible species of *Tricholoma* genus, and *B. edulis* was given. Especially nutritional value and the use of these mushroom species were summarized. The most important factor limiting the increase of mushroom cultivation is the short shelf life of mushrooms. However, recently, this problem has started to be solved with the developments both in storage conditions and in transport systems. With the introduction of more modern technologies in the future, it would be possible to extend the shelf life of mushrooms, which will impose a positive effect on production and consumption. Sales of hobby kits have become widespread recently for some cultivated mushroom species, especially *Pleurotus*

species. These kits are prepared for hobby mushroom growers and people who wish to consume their own production as fresh. Such kits also attract the attention of children as well as hobby mushroom growers; even they can prepare their school projects with these kits. Mushrooms are an important source of income for the countries rich in the mushrooms that cannot be cultivated and that are exported by collecting from nature. Therefore, regulations for protection and sustainability of these mushroom species, which have both high nutrient content and economic value, should be implemented. Mushroom collectors should be educated by practical training for proper or correct mushroom collection. Some applications such as after inoculation of mycelia of ectomycorrhizal mushroom species such as *Tricholoma* spp. and *Tuber* spp. to the roots of host trees and planting of these trees in the forest have been experimented, and successful results have been obtained. Similarly, planting of mushroom mycelia to their nature as in *Morchella* species is one of the other successful applications. The use of mushrooms for alternative medicine area has been known since ancient years. Many studies related to the medicinal value of the mushroom species such as *G. lucidum* and *L. edodes* have been performed, and important findings have been obtained. Although all of these studies produced promising results, in the use of mushrooms for treatment of any disease, doctor should be consulted.

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Chapter 13
Minimal Processing of Tropical and Subtropical Fruits, Vegetables, Nuts, and Seeds

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13.1 Introduction

Tropical climate zones of the Earth, in all 12 months, have mean temperatures above 18 ^{0}C (64.4 °F). The tropical climate experiences hot, humid weather, and rainfall year round. Tropical vegetation is any vegetation in tropical latitudes. Plant life that occurs in climates that are warm year round is in general more biologically diverse than the other latitudes. Subtropical climates are often characterized by warm to hot summers and cool to mild winters with infrequent or no frost. Subtropical regions should have at least 8 months with a mean temperature greater than 10 °C (50.0 °F) and an average temperature of the coldest month between 6 °C (42.8 °F) and 13 °C (55.4 °F) (Belda et al. 2014). Tropical soils are often several meters deep, but the soils are often washed out, or strongly leached, by heavy rains, with large amounts of nutrients and minerals being removed from the subsoils. But, the very thin topsoils are made up mainly of decaying vegetal and animal remains. An amazing cycle exists between the huge body of vegetation above ground and this thin topsoil. The tropical and subtropical vegetation depends for its nutrients on the constant recycling of its enormous biomass (Tiessen et al. 2001).

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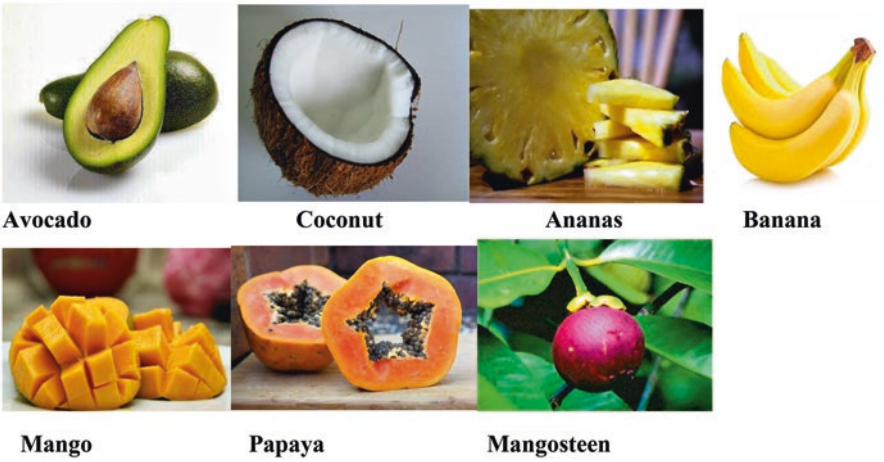

Fig. 13.1 Selected tropical and subtropical fruits

Plants such as palms, citrus, mango, litchi, and avocado are grown within the tropics and subtropics (Fig. 13.1). Most of the edible nuts and seeds are grown in the tropical and subtropical regions of the world.

13.2 Tropical and Subtropical Fruits

Tropical and subtropical fruits are important sources of vitamins and minerals and many of these fruits are also high in dietary fiber. The color, flavor, and texture of these fruits are very attractive to consumers. Research findings (Patil et al. 2013) also indicate that these fruits contain micronutrients, phytochemicals, and antioxidants which could contribute to human well-being and health. However, each kind of fruit is unique in its composition. Banana yields highest calories per unit area and takes least time for digestion. Cashew nut is rich in protein (21.2%), while mango and papaya lead in vitamin A content. In many countries, these fruits are also used for the prevention and as healing remedies for a number of illnesses and diseases. With these properties, there is a vast potential for innovation and development of new products from these fruits in MPR products, the functional foods and nutraceutical industries. Fruits are the rich source of natural bioactive substances that have the property to prevent oxidative chain reaction by removal of free radicals' intermediates and even by oxidizing themselves. Research suggests (Patil et al. 2013; Yildiz 2010) that phytochemicals, working together with nutrients found in fruits, may help slow the aging process, reduce cell damage, stimulate the immune system, and reduce the risk of many diseases.

China, India, the Philippines, Indonesia, Brazil, Latin America, and the Caribbean are the world's major producer of tropical fruits, accounting for 95% of world

Table 13.1 World fruit, vegetable, and nut market, million tons (quantity), and value in billion USD

	Production (million tons)			Value (billion USD)		
Period	Vegetables[a]	Fruits[b]	Tree nuts[c]	Vegetables	Fruits	Tree nuts
1992–1994	613	287				
2002–2004	903	527				
2014–2016	1150	800		1.851	868	
2015–2016			3.78			33

[a]Vegetables and fruits include all organic, fresh, fresh-cut, dried, frozen, canned vegetables and fruits
[b]Fruits include 421 million tons of tropical and subtropical fruits which include banana, grapefruit, oranges, mangoes, plantains, tangerines, pineapples, peaches, apricot, lemon, limes, papaya, dates, persimmons, and avocados
[c]Tree nuts include almonds, cashew, walnut, pistachio, hazelnut, pecan, macadamia, Brazil nuts, and pine nuts as kernel
Table calculated from the data given in references FAO (2015), International Nut and Dried Fruit Council (2015), Kazagro National Marketing Manager (2014), USDA (2016)

production in 2016. World trade and consumption of tropical and subtropical fruits and vegetables are increasing. Most of the growth in trade and consumption is fruit sector, but not in vegetables. The summary of the world trade and consumption is shown in Table 13.1 and major commodity list is given in Table 13.2.

13.2.1 Packaged Fresh-Cut Tropical Fruits

Avocado-based products such as guacamole; avocado flesh, chunks, or paste; avocado-based salsas; etc., have a very short shelf life period (5–10 days) due to the spoilage effects of various enzymes over this product, particularly polyphenol oxidase (PPO) and lipoxygenase (LOX). A reduction in enzymatic activity is achieved through conventional or traditional thermal methods or addition of chemical additives, but this involves a significative loss in product quality, color, smell, and flavor in comparison with the fresh product. High-pressure processing of fresh-cut fruits with cold storage may be suitable for minimal processing. Minimally high-pressure processed avocado products being marketed include:

- Avocado halves
- Avocado chunks
- Avocado puree
- Guacamole (various recipes)
- Avocado-based salsas and sauces

The parameters used for industrial production vary between 5000–6000 bar of pressure and 2–5 min of holding this high pressure up, at refrigeration conditions (3–7 °C) (Purroy et al. 2011). Fresh-cut tropical fruits on the market today include melons, cantaloupe, watermelon, mangoes, mangosteen, rambutan, jackfruit, pummelo, papaya, durian, grapefruit, pineapples, and fruit mixes.

Table 13.2 Economically important, selected tropical and subtropical fruits, vegetables, nuts, and seeds

Tropical and subtropical fruits	Exotic tropical and subtropical fruits
Banana Avocado Pineapple, (*Ananas*) Coconut Papaya Mango Mangosteen	The cashew apple Pitahaya Passion fruit Carambola Litchi Rambutan Longans Durian Tamarinds
Tropical and subtropical vegetables	**Tropical and subtropical nuts**
Amaranth leaves and seeds Chili peppers Cayenne peppers Cassava Taro Yam Yam bean Chayote Artichoke Sweet potatoes	Almonds Macadamia nut Cashews Walnuts Pecans Brazil nuts Cedar nuts/pine nuts Cashews Pistachios Hazelnuts/filberts
Tropical and subtropical seeds	
Flaxseeds Hempseeds Sunflower seeds Pumpkin seeds Sesame seeds Chia seeds	

13.2.2 Banana

Banana (*Musa acuminata*, *Musa balbisiana)* belongs to family Musaceae. Banana fruit has a good flavor and high nutritional properties. It has a good texture and is consumed globally. Banana fruit is minimally processed to meet consumers demand for ready-to-eat and healthy foods. This minimal processing affects the texture and color of banana slices during storage. For minimizing these undesirable effects, different preservation techniques are used. The combined effect of chemical dip and/or edible coating and/or controlled atmosphere (CA) on quality of fresh-cut banana was investigated. Slices of banana were first given treatment of solution containing 1% (w/v) calcium chloride, 0.75% (w/v) ascorbic acid, and 0.75% (w/v) cysteine by dipping slices into solution for 3 min and/or combined with a carrageenan coating and/or

with controlled atmosphere of 3% O_2 and 10% CO_2. The results after storage period of 5 days at 5 °C showed that synergistic effect of dip and CA treatment reduced the product weight loss and activity of polyphenol oxidase. Microbiologically, minimally processed bananas were acceptable during 5 days of storage at 5 °C (Bico et al. 2009). Banana slices are susceptible to browning and softening. To prevent these undesirable changes in fresh-cut banana slices, different techniques have been used. In one study, the effect of atmospheric modification, exposure to 1-methylcyclopropene (1-MCP), and chemical dips on the quality of fresh-cut bananas was investigated. Atmospheric modification with low levels of O_2 (2 and 4 kPa) and high levels of CO_2 (5 and 10 kPa), alone or in combination, did not prevent browning and softening of fresh-cut banana slices. Softening and respiration rates reduced when treated with 1-MCP treatment (1 μL/L for 6 h at 14 °C) of fresh-cut banana slices (after processing), but their ethylene production and browning rates were not influenced. A 2 min dip in a mixture of 1% (w/v) $CaCl_2$ + 1% (w/v) ascorbic acid + 0.5% (w/v) cysteine effectively prevented browning and softening of the slices for 6 days at 5 °C. Dips in less than 0.5% cysteine promoted pinking of fresh-cut banana slices, while concentrations between 0.5% and 1.0% cysteine delayed browning and softening and extended the post-cutting life to 7 days at 5 °C (Vilas-Boas and Kader 2006).

13.2.3 Avocado

Avocado (*Persea americana*) belongs to family Lauraceae. Avocado fruits are rich sources of bioactive compounds such as monounsaturated fatty acids and sterol. The impact of minimal processing on health-promoting properties of avocados has been investigated. Avocados were first cut into slices or halves. These sliced and halved avocados were packaged in plastic bags with nitrogen, air, or vacuum. The filled plastic bags were then stored at 8 °C for 13 days. The stabilities of fatty acids and sterols as well as the effect on antioxidant activity were evaluated. The main fatty acid identified and quantified in avocado was oleic acid (about 57% of total content), whereas β-sitosterol was found to be the major sterol (about 89% of total content). In general, after refrigerated storage, a significant decrease in fatty acid content was observed. Vacuum/halves and air/slices were the samples that maintained better this content. With regard to phytosterols, there were no significant changes during storage. Antioxidant activity showed a slight positive correlation

against stearic acid content. At the end of refrigerated storage, a significant increase in antiradical efficiency (AE) was found for vacuum samples. AE values were quite similar among treatments. Hence, minimal processing can be a useful tool to preserve health-related properties of avocado fruit.

Effect of high-pressure processing on the quality, physiology, biochemistry, and microstructure of avocado slices has been investigated. Avocado slices were given high-pressure treatment of >300 MPa. This treatment was seen to reduce respiration rates and ethylene production 1 h after treatment and particularly after 17 h at 20 °C. HPP treatment also resulted in some peroxidase (POD) activity reduction, particularly at higher pressures, while polyphenol oxidase (PPO) activity was generally increased. HPP (600 MPa) induced changes in the cell wall structure, disruption of the cellular network, and coalescence of oil vesicles. HPP treatment of avocado slices provides potentially beneficial reduced respiratory activity, without obvious color changes. The study of Woolf et al. (2013) demonstrated that HPP has beneficial effects by inactivating respiration and ethylene production in avocado slices, but it moderately effects POD activity and has little effect on PPO activity.

Avocado slices have been given pulsed light treatment to enhance their quality characteristics and for increasing oxidative stability. In this study, avocado slices were given pulsed light treatment on both sides (3.6, 6.0, and 14 J/cm^2 per side), and its effect on microbial burden, color, chlorophyll stability, and lipid oxidation for 15 days of storage at 4 °C was investigated. Exposure of fresh-cut avocado to the highest dose led to the highest reductions in aerobic mesophilic microorganisms (1.20 log CFU/g) and inhibited the proliferation of yeasts and molds for 3 days, prolonging their microbiological shelf life up to 15 days. Hue values of fresh-cut avocados were better maintained after applying PL treatments. PL treatments did not affect the stability of the lipidic fraction of processed samples and thus allowed keeping the oil acceptability for at least 15 days of storage (Aguilo-Aguayo et al. 2014).

13.2.4 Pineapple

Pineapple (*Ananas comosus*) is a non-climacteric tropical fruit and belongs to family Bromeliaceae and subfamily Bromelioideae. It is the third most important tropical fruit in world production followed by banana and citrus. In market, pineapple is available as canned slices, chunks, crush, juice, and fresh fruit. Fresh-cut pineapple is found in various food service chains and supermarkets. Fresh-cut pineapple is well known for its flavor, taste, and juiciness (Gonzalez-Aguilar et al. 2005; Montero-Calderon et al. 2008). The major problems associated with fresh-cut pineapple are microbial growth and browning. So far various preservation techniques have been used to minimize deteriorations in fresh-cut pineapple. Post-cutting life of pineapple is very much dependent on temperature, ranging from a few hours at 20 °C to several weeks at 1 °C (O'Hare 1994). Marrero and Kader (2001) found that storage life of fresh-cut pineapple ranged from 4 days at 10 °C to over 2 weeks at 0 °C. Bierhals et al. (2011) reported that coating of cassava starch effectively reduced the physiological changes in fresh-cut pineapple, but the treatment was not effective in preventing the loss of ascorbic acid during 12 days of storage at 5 °C. Yeoh and Ali (2017) reported that application of ultrasound treatment increased the total antioxidant capacity in fresh-cut pineapple due to increase in total phenolic content and activity of PAL (Phenylalanine ammonia lyase). MAP has been found to increase the storage life of fresh-cut pineapple to 2 weeks at 5 °C, without undesirable changes in quality parameters (Marrero and Kader 2006). Pineapple cubes kept in polypropylene containers at 4 °C retained sensory characteristics up to 7 (O'Connor-Shaw et al. 1994). Application of methyl jasmonate (15 μL/L) to fresh-cut pineapple resulted in a 3 log CFU/g reduction of the native microbiota (Martinez-Ferrer and Harper 2005).

13.2.5 Coconut

Coconut (*Cocos nucifera*) belongs to family Arecaceae. Coconut is a tropical plant native to coastal areas of Southeast Asia. Its mature kernel is eaten as food. Oil from kernel is used for cooking and various other purposes. Nut water from immature nuts is a refreshing drink. Coconut contains good amount of vitamins and minerals. Coconut is an important source of saturated fatty acid known as lauric acid that increases amount of HDL in blood. Cytokinins present in coconut water have anticarcinogenic, antiaging, antithrombotic effect. Coconut meat and coconut water contain good amount of potassium. The optimum storage temperature for freshly

harvested coconuts is 0–15 °C and relative humidity is 75% or less. The white meat of kernel is prone to microbial spoilage due to its low acidity (Sinigaglia et al. 2003). Techniques such as chemical treatments, edible coating, modified atmospheric packaging, irradiation, etc., are used to maintain quality of minimally processed coconut.

In a study by Ferrentino et al. (2012), the effect of supercritical CO_2 treatment for 15 min at 45 °C and 12 MPa on hardness and microbiota on fresh-cut coconut was investigated. The treatment reduced mesophilic microbes, lactic acid bacteria, total coliform, yeast, and molds, and hardness was not affected by the treatment. The dehusked coconuts can be stored for 60 days at 0–5 °C and relative humidity of 75–85% (Muliyar and Marar 1963). Husked nuts can be film wrapped and waxed.

13.2.6 Papaya

Papaya (*Carica papaya* L.) belongs to family Caricaceae. It is a climacteric fruit and is an excellent source of carotenoids, vitamins, proteins, and polysaccharides (Waghmare and Annapure 2013). Due to its convenience, fresh-cut papaya has become popular among consumers. However, the main problems associated with fresh-cut papaya like weight loss, loss of firmness, decrease in nutritional value, and microbial proliferation limit their storage life. In food industry, application of ozone has received a commercial interest due to its effectiveness over the shelf life extension of fresh-cut products by inhibiting the microbial growth (Sothornvit and Kiatchanapaibul 2009) and by preventing fungal decay (Ong et al. 2012). Application of 9.2 ± 0.2 μl/L gaseous ozone for 20 min to fresh-cut papaya resulted in depletion of microbial load without any depletion of major antioxidants except ascorbic acid (Yeoh et al. 2014). Edible coatings have become an important alternative to maintain the quality of fresh-cut fruits. Edible coatings may also act as carriers of food additives such as antibrowning and antimicrobial agents, colorants, flavors, nutrients, and spices (Rojas-Grau et al. 2009). Narsaiah et al. (2015) have reported that alginate (2% w/w) with bacteriocin could be used to store minimally processed papaya for 3 weeks without compromising physicochemical qualities or microbial safety. Fresh-cut papaya stored at lower temperature (14–15 °C) resulted in a shelf life of 3 days (Falaha et al. 2015). Under refrigerated condition, fresh-cut papayas packed in LDPE vacuum-sealed pouches retained a storage life of 12 days. These samples also showed minimum changes

in physicochemical properties and higher sensory scores (Tirkeya et al. 2014). Waghmare and Annapure (2013) reported that MAP in combination with chemical treatment maintained the sensory and quality characteristics up to 25 days of storage in fresh-cut papaya.

13.2.7 Mango

Mango (*Mangifera indica* L.) is a climacteric fruit and belongs family Anacardiaceae. It is an economically important tropical fruit with a global production of about 25 million tons in 2006 (FAO 2007). Fresh-cut fruits are one of the major growing segments in food retail markets. However, marketing of such fruits is limited owing to their limited shelf life, which is due to excessive tissue softening and surface browning (Soliva-Fortuny and Martin-Belloso 2003). The demand for fresh-cut mangoes is increasing, but problems like translucency, browning, and loss of firmness affect their quality and limit shelf life. Although low temperature (<5 °C) has been reported to extend the shelf life up to 5 days (Rattanapanone et al. 2001), shelf life of such a length makes distribution and marketing difficult. So, low temperature should be used in combination with other preservation techniques in order to achieve best results. For the preservation of fresh-cut mangoes, a number of treatments have been used so far which include calcium chloride, low oxygen, high carbon dioxide, and browning inhibitors. Calcium chloride has been reported to maintain firmness (Trindade et al. 2003). Rattanapanone et al. (2001) and Izumi et al. (2003) reported that low oxygen (<5%) minimizes surface darkening associated with browning and translucency. Carbon dioxide (>3%) has been reported to maintain firmness and decrease translucency in fresh-cut mangoes (Poubol and Izumi 2005). Gonzalez-Aguilar et al. (2000) found that a combination of browning inhibitors like hexyl resorcinol, potassium sorbate, and ascorbic acid effectively delayed surface browning of cut slices. Chantanawarangoon and Kader (2000) reported that a combination of calcium chloride, low oxygen, and enhanced carbon dioxide increased the shelf life of "Keitt" and "Kent" mango slices up to 15 days at 5 °C. Combination of calcium and low oxygen has been found to extend the storage life of "Kensington" slices to 15 days at 3 °C (de Souza et al. 2006). A combination of ascorbic acid, citric acid, and calcium chloride resulted in better color retention and retarded

quality loss and increased the antioxidant activity of fresh-cut mango cubes stored at 5 °C (Robles-Sancheza et al. 2009). Vilas-Boas and Kader (2007) reported that application of 1-MCP retarded surface browning and softening in fresh-cut "Kent" and "Keitt" mango slices. This treatment did not influence respiration rate, but only ethylene production was affected toward the end of shelf life.

13.2.8 Mangosteen

Mangosteen (*Garcinia mangostana* L.) belongs to the family Clusiaceae. It has sweet flavor and pleasant aroma and has slightly acidic taste and purple-colored fruit with soft, white, juicy pulp (Jung et al. 2006). It is among the famous fruits in Thailand. Because of its high and instant visual and taste appeal, it is known as "Queen of Tropical Fruits." Mangosteen is a rich source of phenolic compounds such as xanthones, phenolic acids, tannins, and anthocyanins (Fu et al. 2007). Fresh-cut mangosteen is an increasingly valuable commodity with excellent marketability. Processing mangosteen into fresh-cut products is technically challenging, as these processes affect fruit properties like color, odor, flavor, and texture negatively and thus decrease their shelf life.

The effect of 1-methylcyclopropene (1-MCP) and acidified sodium chlorite (ASC) on the quality of stored fresh-cut mangosteen was investigated. In this study, mangosteen fruit before minimal processing was treated with 1-MCP at levels of 0, 20, 40, or 80 ppm for 12 h at 28 + 2 °C. These treated fruits were then cut and packed in PP trays sealed with oriented polypropylene/linear low-density polyethylene (OPP/LLDPE) film and stored at 5 °C for 12 days. Fresh-cut mangosteen without 1-MCP treatment showed rapid softening and continuous weight loss. Ethylene production and respiration rate were lower in 1-MCP-treated fruits and both softening and weight losses were delayed. Therefore, the treatment could provide better quality in packaged fresh-cut mangosteen. The 1-MCP (40 ppm)-treated fruits were cut and dipped in tap water (control) and 500 or 1000 ppm acidified sodium chlorite (ASC) for 1 min before packing in PP trays sealed with OPP/LLDPE film for storage at 5 °C for 12 days. The results indicated that ASC could control browning. The effect of a mangosteen pericarp extract on the physical and chemical properties of fresh-cut mangosteen stored in PP trays at 5 °C with 85%

relative humidity (RH) was investigated. Fresh-cut mangosteen dipped in 0.25 g/l of the pericarp extract retained lightness and hue values better than the control (Ayudhya 2012).

13.3 Exotic Tropical and Subtropical Fruits

13.3.1 Pitahaya

Pitahaya is commonly known as dragon fruit and belongs to genus *Hylocereus*. It is a fruit of the cactus species. Pitahayas are of three types: *Hylocereus undatus* (white-fleshed pitahaya), red-skinned fruit with white flesh; *Hylocereus costaricensis* or *Hylocereus polyrhizus* (red-fleshed pitahaya), red-skinned fruit with red flesh; and *Hylocereus megalanthus* or *Selenicereus megalanthus* (yellow pitahaya), yellow-skinned fruit with white flesh. Minimally processed pitahayas rapidly lose bright white color during storage and develop a brown surface that reduces their acceptability to consumers. Ariffin et al. (2009) reported that after cutting, shelf life of pitahayas gets rapidly reduced due to weight loss and desiccation. Matan et al. (2015) reported that application of green tea extract in combination with atmospheric RF plasma provided protection against the growth of pathogens and also improved the shelf life of fresh-cut dragon fruit. Edible coatings have been used to improve the nutritional quality of fresh-cut fruits. Chien et al. (2007) found that low molecular weight chitosan (LMWC) retarded water loss, inhibited microbial growth, and maintained sensory quality of sliced red pitahayas.

13.3.2 Passion Fruit

Passion (*Passiflora edulis*) fruit is native to Brazil. It belongs to family Passifloraceae. Commercially important species include purple passion fruit and yellow passion fruit.

13.3.3 Carambola

Carambola (*Averrhoa carambola*) belongs to family Oxalidaceae. It is commonly known as star fruit cultivated in tropical and subtropical regions of the world (Narain et al. 2001). Due to increasing demand of fresh-cut fruits, carambola fruits are also minimally processed. The market for fresh-cut carambola is good and its unique star shape after cutting attracts the customers. The operations used during minimal processing such as peeling and slicing result in various physiological and biochemical changes thereby destroying fruit tissue and quality (Watada et al. 1996). The deteriorative changes occur due to endogenous enzymes and microorganisms (Yoo and Lee 1999). Enzymatic browning is the main deteriorative reaction in fresh-cut carambolas which occurs due to oxidation of phenolic compounds by an enzyme known as polyphenol oxidase (PPO) present in fruit tissues (Weller et al. 1995). Therefore, in order to minimize deterioration in carambola, different preservation techniques are used.

The effect of low-oxygen atmospheres in combination with 1% acetic acid on fresh-cut carambolas was studied. The carambola slices treated with ascorbic acid and then stored under low-oxygen atmosphere (0.4% O_2) were seen to have storage life of 12 days without any incidence of browning. Modified atmosphere packaging has also been used to preserve quality of minimally processed carambolas. In a study by Teixeira et al. (2007), the carambola fruit was first washed, given dip treatment in NaClO solution (200 mg L − 1) for 5 min. These dipped fruits were then stored overnight at 10 °C. Then these fruits were cut manually into slices and rinsed with NaClO solution at 20 mg L − 1 and draines. The treated slices were then packaged in polyethylene terephthalate (PET) trays (Neoform® N94), polystyrene trays covered with PVC 0.017 mm (Vitafilm®, Goodyear), and vacuum-sealed polyolefin bags (PLO, Cryovac® PD900). The packages were stored at 6.8 °C and 90% RH for 12 days, with samples taken every 4 days. PET trays and PVC film did not significantly modify the internal atmosphere, and the high water permeability of PVC led to more rapid slice

desiccation. PPO activity was lower when the slices were packaged in PLO vacuum-sealed bags, which reduced degreening and led to better appearance maintenance for up to 12 days.

13.3.4 Litchi

Litchi (*Litchi chinensis*) belongs to family Sapindaceae. It is native to South China. It is a tropical and subtropical fruit. Its white translucent aril and attractive red color have increased its commercial value (Holcroft and Mitcham 1996). There is much demand of litchi in international market. Litchi fruit is often peeled and sliced for consumer convenience and for use in restaurants as ready-to-eat food. These processing methods make litchi susceptible to physiological and biochemical damage, which limits its marketing. Therefore, to avoid these adverse effects, litchi fruit is given various preservation treatments such as the use of edible coating. The effect of combined treatments of antibrowning agents, osmo-vacuum drying, and moderate vacuum packaging on peeled, destoned litchi was investigated. The peeled, destoned litchi fruits were treated with antibrowning agents (4.9 g/kg cysteine, 20 g/kg ascorbic acid, and 0.134 g/kg 4-hexylresorcinol) along with osmo-vacuum dehydration (OVD) and stored at 4 ± 2 °C. The samples were analyzed for physicochemical, sensory, and microbiological qualities during storage. Shelf life of 24 days was observed for the arils given a dip for 10 min at 570 mmHg vacuum followed by packing in polypropylene bags and in package vacuum of −355 mmHg. Packing in moderate vacuum along with osmo-vacuum dehydration was found to be highly effective in preserving the product against microbial proliferation and chemical changes, whereas antibrowning agents effectively controlled browning (Shah and Nath 2008).

In another study, the manually peeled litchi fruits were coated with chitosan and overwrapped with plastic film and then stored at −1 °C. The results of chitosan coating showed retard in weight loss, higher TSS, and ascorbic acid. The coating was seen to suppress activity of PPO and POD, thereby maintaining quality and extending storage life of peeled litchi fruit (Dong et al. 2004).

13.3.5 Rambutan

The rambutan (*Nephelium lappaceum*) belongs to family Sapindaceae and order Sapindales. It is a medium-sized, non-climacteric tropical fruit and is native to the Malayo-Indonesian region. Dehydration and browning of skin and spinterns (long soft hair) limit its shelf life (Ketsa 1985). The darkening of skin and spinterns renders the fruit unmarketable, even though the pulp possesses a good eating quality. O'Hare (1995) reports that under ambient conditions, deterioration of rambutan skin can occur rapidly within 3 days. Various studies have been carried out on shelf life extension of rambutan. Sirichote et al. (2008a) reported that a combination of MAP (20% CO_2, 8% O_2, and 72% N_2) and cold storage (4 °C) maintained the physical, chemical, microbial, and sensory properties of minimally processed rambutan for 21 days of storage. Packaging rambutan in polyethylene bags (70 μm thick) under 5% CO_2, 5% O_2, and 90% N_2 and stored at 10 °C could extend its shelf life for 23 days (Luckanatinvong 2005). Sirichote et al. (2008b) found nylon/LLDPE package and a storage temperature of 4.0 ± 1 °C suitable for extending the storage life of minimally processed rambutans.

13.3.6 Longan

Longan (*Dimocarpus longan*) is a tropical fruit belonging to family Sapindaceae. Longan resembles litchi in structure but is more aromatic in taste. It is a native fruit of Southern Asia. Biew Kiew, Daw, and Chompoo are the commercially important cultivars of longan. Longan fruit is mostly consumed as fresh and dried (vacuum

dried, freeze dried). There is a limited literature available on fresh-cut longan fruit. Zhao and Li (2010) studied the effects of ultra-high pressure on the quality of fresh-cut longans stored at 4 °C for 9 days and found that UHP of 600 MPa resulted in microbial destruction and product stabilization, while maintaining the sensory characteristics. Thus, UHP can be an alternative nonthermal preservation method for preservation of fresh-cut longan fruit.

13.3.7 Durian

Durian (*Durio zibethinus* L.) is a climacteric fruit belonging to family Bombacaceae. It is a tropical fruit and is also called the "king of fruits" in Southeast Asia. It has a unique aroma, taste, and texture. The presence of sharp hexagonal spines on inedible thick husk makes its consumption very difficult. So, minimally processed durian can be an alternative to increase perception of quality among consumers. Minimally processed durian has been studied by a number of researchers. Booncherm and Siriphanich (1991) found that at low temperature, fresh-cut durian fruit can be stored for a longer time than its intact form, because pulp was found to be less susceptible to chilling injury than the husk. At 5 °C, fresh-cut durian was stored up to 8 weeks, with slight chilling injury observed after 4 weeks of storage. Salunkhe and Desai (1984) reported a shelf life of 30 days for durian pulp at 4 °C, with the main problems found being fungal contamination and chilling injury at the seed base. Voon et al. (2006) studied the effect of storage on physicochemical, microbial and sensory qualities of minimally processed durian fruit stored at 28 ºC for 3 days and 4 ºC for 35 days and found that durian stored at 28 ºC showed loss of texture, acidification, increase in fructose and glucose and decrease in sucrose contents. Durian stored at 4 ºC for 35 days maintained fruit firmness, pH and organic acid content, microbial growth also slowed down, however aroma loss and development of off-odours were recorded on day 21, which increased thereafter and rendered the pulp unacceptable on day 28th of storage.

13.4 Tropical and Subtropical Vegetables

13.4.1 Amaranth Leaves and Seeds

Amaranth (*Amaranthus* L.) belongs to family Amaranthaceae. It is commonly known as pigweed. There are about 60 species of amaranth of which three main cultivated species are *Amaranthus caudatus*, *Amaranthus hypochondriacus*, and *Amaranthus cruentus*. Amaranth seeds are used as grains and its leaves are used as vegetable (Mlakar et al. 2010). Grain is used by humans in different forms. It is used in ground form in breads, noodles, cereals, etc. Amaranth leaf is used as steamed vegetable in soups and stews. It is used on large scale because of its high nutritional quality. Amaranth grain contains proteins (12–17%) and it is rich in amino acid lysine that is usually present in lesser quantities in other food grains. Amaranth grain is rich in fiber and unsaturated fatty acids and contains high amount of calcium, iron, potassium, vitamin C, vitamin A, riboflavin, and niacin. The leaves of amaranth are high in protein (15–24%), calcium, and niacin.

To avoid postharvest losses of amaranth vegetable due to poor handling and storage, different techniques have been used to increase its storage life. The effect of packaging material on shelf life of minimally processed amaranth leaves has been investigated. The amaranth leaves with or without stem were packed in polypropylene (100 and 150 gauges), polyethylene (LDPE, HDPE), pouches with or without vent, PET jar, muslin cloth, and brown paper pouches. It was seen that polypropylene 150-gauge package systems extended shelf life of amaranth leaves for up to 6 days with 84.34% retention of moisture, 21.01% physiological loss in weight, and 9.01% decay. PP 100-gauge pouches extended shelf life for up to 4 days with 86.32% moisture retention, 1.27% physiological loss in weight, and 14.52% decay. Thus PP 150 gauge was found to be the best packaging material with maximum edible leaves (Reddy et al. 2013).

Modified atmosphere packaging has also been used to extend shelf life and for nutrient preservation in vegetable amaranth. The freshly harvested amaranth samples were packed in active bags that modified internal atmosphere of packaged sample. These packaged amaranth samples were then stored in cold room. The temperature was maintained between 5–25 °C and RH of 75%. These samples were analyzed after 23 days of storage, and it was seen that loss of ascorbic acid (vitamin C) was lowest compared to control that showed 88% loss of ascorbic acid only after 4 days of storage at room temperature. Thus, it was concluded that MAP at 5 °C

extends shelf life and preserves vitamin C in vegetable amaranth (Nyaura et al. 2014). Other preservation methods to maintain quality of amaranth are sun drying, blanching, etc. For storage of grain, moisture content should be approximately 11%. The seeds are dried by air or heat and then stored in wooden storage containers or a heavy duty paper bags. In one study, the effect of drying on protein content and fraction yield of starch in *Amaranthus* was investigated. The results showed that the most suitable drying temperature to increase starch with minimization of protein content in starch fraction is 40 °C with soaking using 0.05 w/v % of SO_2 (Resio et al. 2010).

13.4.2 Chili Peppers

Chili pepper (*Capsicum annuum*) belongs to nightshade family (Solanaceae) and is native to Central American region and was later extended to other parts of world in the sixteenth and seventeenth centuries by Spanish and Portuguese explorers and is grown as major commercial crop in various parts of the world. The optimum temperature for growth of chili peppers ranges between 20 °C and 32 °C. These peppers are used in foods for their pungent flavor and aroma. The pungent flavor of chili pepper is due to the presence of an alkaloid compound known as capsaicin, which has various health benefits including anticarncinogenic, antidiabetic, antibacterial, and reduced low-density lipoproteins. Chili peppers are rich in vitamin C. Both green and red chili peppers contain approximately 76.4 mg of vitamin C/100 g. These peppers are richest in vitamin A among all spices. These also contain good amount of vitamin E and vitamin K. Minerals such as iron, phosphorus, copper, potassium, magnesium, and zinc are also present in chili peppers and thus have good antioxidant capacity. Chili peppers after harvesting undergo various changes such as shriveling, change in color, wilting, appearance of fungal diseases, and chilling injury. Therefore, proper storage conditions are required to reduce the quality losses. Optimum temperature for proper storage of chili peppers is reported to be between 7 °C and 13 °C for 2–3 weeks (Rico et al. 2002). Chili peppers are also minimally processed for consumer convenience. It has been seen that fresh-cut chili pepper slices can be stored for 12 days at 5 °C using controlled atmosphere of 3% O_2 and 10% CO_2.

To maintain quality of fresh-cut chili peppers, various preservation techniques have been used. One of the preservative treatments is the use of edible coating.

In one study, the fresh-cut green chili peppers were coated with chitosan by dipping, and these coated cut peppers were then stored for 15 days at the temperature of 5 °C. The results showed that chitosan-treated samples of cut chili peppers had good green color compared to control, and also fungal incidence was reduced in these samples. Microbiological analysis showed that total viable cell counts decreased with increasing chitosan coating (Raymond et al. 2012).

Glowacz and Rees (2016) investigated the effect of ozone on red and green chili peppers during storage. The chili peppers were exposed to ozone at 0.45, 0.9, and 2 μmol mol^{-1} continuously during storage at 10 °C. The ozone levels of 0.45 and 0.9 μmol mol^{-1} reduced disease incidence in red peppers. Ozone at 0.9 μmol mol^{-1} extended shelf life of chili peppers.

13.4.3 Cayenne Peppers

Cayenne pepper (*Capsicum annuum*), commonly known as Guinea spice, belongs to nightshade family (S*olanaceae*). Cayenne peppers have originated from a place named cayenne in French Guiana and hence its name. These peppers are mostly grown in tropical and temperate regions. Cayenne pepper is less fiery than other chili pepper varieties and is used widely in European, Creole, Cajun, Mexican, and East Asian cuisines. Cayenne pepper has similar composition as other pepper varieties. It is rich in vitamin C and also contains vitamin A, vitamin B, and vitamin E. It also contains proteins, carbohydrate, and fiber and has various health benefits like antimicrobial, antiseptic, anticarcinogenic, etc. Pungent flavor is due to the presence of active substance known as capsaicin. This capsaicin has various health benefits. Capsaicin is a pain reliever as it reduces substance "P," a substance that carries pain message from nerve endings to the skin to central nervous system.

Postharvest losses cause a lot of damages to farmers. To avoid this postharvest loss in cayenne peppers, various preservation methods have been used. The effect of polypropylene and polyvinyl chloride plastic film on the quality of "Yalova Charleston" during storage was investigated. Yalova Charleston is one of the best cayenne-type peppers. These long peppers were stored in plastic film having various O_2 and CO_2 permeabilities at 7 ± 1 °C and 90 ± 5% relative humidity (RH). The results showed that samples packed in polypropylene showed best physic chemical properties. 35 μ PP gave best results at the end of 30-day storage (Akbudak 2008).

13.4.4 Cassava

Cassava (*Manihot esculenta*) belongs to family Euphorbiaceae. The cassava plant has originated in South America. It is a tropical crop. More than 500 cultivars of cassava are present worldwide. The nutrient composition of cassava varies with the cultivar. Leaves and roots are nutritionally valuable parts of cassava. The leaves of cassava are rich in proteins; vitamins B_1, B_2, and C; and carotenoids. It also contains minerals such as iron, zinc, magnesium, and calcium. Roots are rich in carbohydrates mainly starch. Maintaining postharvest quality in cassava is the major challenge faced. Roots of cassava after harvest are prone to physiological damage such as cut surface browning which results in reduction of shelf life of cassava roots. Refrigeration has been used to preserve fresh-cut cassava root sticks. The fresh-cut cassava root sticks packaged in polypropylene and stored for 12 days at refrigeration temperature of 5 ± 1 °C and relative humidity 90 ± 55 were seen to have greater concentration of carotenoids, total soluble phenolics compounds, greater antioxidant capacity, and increased activity of phenylalanine ammonia lyase (Junqueira et al. 2014). Hot water dip treatment in combination with MAP has been used to reduce browning in peeled cassava roots. The peeled roots were given hot water dip at 57–59 °C for 10 min. This reduced browning and MAP were seen to reduce weight loss in peeled roots (Acedo and Acedo 2013).

13.4.5 Taro

Taro (*Colocasia esculenta*) belongs to family Araceae. It is an important tropical crop in developing countries in Asia, Pacific, Africa, and the Caribbean. Underground stem known as corm is the edible part of taro. Taro contains about 7% protein on dry

weight basis containing essential amino acids. Fresh taro corm contains 13–29% carbohydrate and is also rich in vitamin C. Taro leaf is rich in proteins (23%), calcium, phosphorous, iron, vitamin C, thiamine, riboflavin, and niacin. As the demand of fresh-cut fruits and vegetables is increasing due to consumer awareness about health benefits of fruits and vegetables, taro is also minimally processed to meet consumer demands. The minimal processing techniques like peeling, slicing, cutting, coring, etc., cause various physiological and biochemical changes such as browning and proliferation of microorganisms that in turn decrease its storage life. Different preservation methods have been used to inhibit these deteriorative reactions in minimally processed taro. These techniques include irradiation, modified atmospheric packaging, heat treatment, edible coatings, etc.

The effect of combined treatment of ascorbic acid and chitosan coating to prevent browning in fresh-cut taros has been studied. In this study, the fresh-cut taro slices were treated with 0, 1.0, 5.0, and 10.0 g/L ascorbic acid and coated with 15.0 g/L chitosan. The results showed that the chitosan coating reduced weight loss in taro slices and also inhibited browning process. This treatment was also seen to polyphenol oxidase (PPO), peroxidase (POD), and amylase activity, thereby decreasing browning in fresh-cut taro slices and maintaining their quality (Wei-rong et al. 2011). Hot water dipping treatment has also been used to prevent browning in minimally processed taro. Taro slices were dipped in hot water (55 °C) for 45 seconds, air-dried at room temperature, and packed in polyethylene films or vacuum sealed in nylon/polyethylene films, stored at 4 °C for 12 days. The hot water dip treatment reduced browning of taro and improved its organoleptic properties (Chang and Kim 2015). Onion extract has also been used to prevent browning in taro slices. In this study, the taro slices were cut into 5 mm slices and then immersed in distilled water, fresh onion extract, and heated onion extract. The heated onion extract was prepared at 100 °C for 10 min. After immersion into these, the surfaces of taro slices were coated with 1.0 ml of 0.2 M catechol. This heated onion extract was seen to have higher inhibitory effect on polyphenol oxidase activity of taro than unheated onion extract, and those treated with distilled water showed rapid browning. Thus, onion extract both heated and unheated inhibited browning thereby maintaining quality of taro slices (Lee et al. 2007).

13.4.6 Yam

Yam (*Dioscorea*) is an edible tuber belonging to family Dioscoreaceae. Yam tubers are important dietary sources of carbohydrate in tropical and subtropical regions. Currently in urban population, the demand for minimally processed roots and tubers such as yam and potato has significantly increased (Donega et al. 2013). The operations involved in minimal processing (peeling and cutting) induce some physical changes that reduce the shelf life of minimally processed products (Lunadei et al. 2011). Yam darkens quickly after cutting. The earlier signs of deterioration include the presence of brownish stains on the surface. Tissue dehydration results in deposition of starch on the surface, resulting in a whitish appearance (Donega et al. 2013). There are very few research studies on the preservation of minimally processed yam. Luo et al. (2015) found that nano-$CaCO_3$-based low-density polyethylene (nano-$CaCO_3$-LDPE) package maintain the quality of fresh-cut Chinese yam by inhibiting browning and microbial load. Ascorbic acid and calcium chloride (AACCl) dip in combination with UV-C dosage of 6.84 KJ m^{-2} prevented browning of minimally processed yam slices under storage at 4 ± 1 °C (Teoha et al. 2016). Chun et al. (2013) observed that a combination treatment of aqueous chlorine dioxide (ClO2) and ultraviolet-C (UV-C) maintained the color, retained sensory quality, and reduced the preexisting microbial load of minimally processed yam during storage.

13.4.7 Yam Bean (Jicama)

Jicama (*Pachyrhizus erosus*) is a tropical legume native to Mexico and Central America. Mechanical damage to jicama pieces causes surface browning that is associated with increased phenolic content and activity of phenylalanine ammonia lyase (PAL) enzyme (Aquino-Bolanos et al. 2000). Jicama is processed by osmotic dehydration and freezing and also juice preparation has been reported (Juarez-Goiz and Paredes-Lopez 1994). As jicama is usually consumed as raw, the production of fresh-cut jicama provides an interesting processing alternative. High-quality fresh-cut jicama should be white, crisp, juicy, free from visible defects, and microbiologically safe and possess characteristic odor and flavor. Aquino-Bolanos et al. (2000) reported that controlled atmosphere (CO_2–5 to 10%) and storage temperature of 5 °C were effective in maintaining the quality of fresh-cut jicama by retarding the microbial growth and discoloration. Rangel et al. (2014) studied the effect of modified atmosphere packaging, oxygen permeable films, and storage temperatures on the quality of minimally processed jicama and concluded that fresh-cut jicama

packed and stored at 10 °C showed lower crispiness, visual quality, and higher CO_2 accumulation during 12 days of storage in comparison to those stored at 5 °C, and also its sensory quality was acceptable to less than 8 days.

13.4.8 Chayote

Chayote (*Sechium edule*) is an edible plant belonging to the family Cucurbitaceae. It is known by different names like christophine, cho-cho, vegetable pear, etc. Chayote is native to Mesoamerica. It is a good source of vitamin C. Besides fruit, the root, stem, seeds, and leaves are edible as well. Chayote is consumed mostly in cooked form. As raw, it is added to salads. Chayote is also available as fresh cut. Alves et al. (2010) conducted a study on the effect of storage time on the quality of fresh-cut vegetables including chayote. It was concluded that fresh-cut vegetables packed in low-density polyethylene maintained the quality at 5 °C and RH of 99% during 8 days of storage. The vegetables maintained firmness and color and also showed lower weight loss and respiratory activity up to day 8 of storage. Hernandez et al. (2014) reported that the selective barrier technology (disinfection, heat treatment, edible coating, modified atmosphere, and cooling) was useful in maintaining the overall quality of minimally processed vegetables including chayote (*Sechium edule*) which was maintained during 12 days of storage under refrigeration (4 °C and 95% HR).

13.4.9 Artichoke

The artichoke (*Cynara cardunculus*) is a perennial rosette plant grown for its large, fleshy heads. Due to nutritional benefits and healthy gastronomic properties, the demand for artichoke has increased, so appropriate postharvest technologies are required to make its marketing successful. Minimal processing (washing, removing external leaves, slicing, and packaging) can offer great advantages for artichoke commercialization. However, these operations induce physical damage leading to softening, water loss, microbial contamination, and enzymatic browning that negatively influence its marketability. Control of enzymatic browning can be achieved by combining chemical and physical methods, such as the use of antioxidant agents, modified atmosphere packaging (MAP), and proper temperature control. Gimenez et al. (2003) found that the use of MAP with low O_2 (5–10 kPa) and elevated CO_2 (5–18 kPa) showed little or no effect on visual quality of artichoke heads in comparison to samples stored under normal atmospheric conditions; however, artichoke heads were considered acceptable after 8–10 days of storage at 4–5 °C. Cabezas-Serrano et al. (2013) reported L-cysteine (Cys) and L-cysteine hydrochloridemonohydrate to be the most effective antioxidants for fresh-cut artichoke. However, the antioxidants were not sufficient to improve the shelf life for commercialization. Lattanzio and Linsalata (1989) reported that application of ascorbic acid and citric acid delayed browning and improved quality and shelf life of artichoke heads stored in polyethylene bags at 4 °C. Application of cysteine in combination with soy protein isolate and beeswax (SPI-BW)-edible coating controlled enzymatic browning and extended the storage life of fresh-cut artichokes up to 4 days without producing off-odors (Ghidelli et al. 2015). Del Nobilea et al. (2009) found that sodium alginate-coated fresh-cut artichokes packed in biodegradable film (NVT2) maintained a shelf life of 3 days.

13.4.10 Sweet Potatoes

The sweet potato (*Ipomoea batatas*) is an edible tuberous root belonging to family Convolvulaceae. It is long and tapered, having smooth skin with colors ranging between yellow, orange, red, brown, purple, and beige. Fresh-cut sweet potatoes which are a good source of polysaccharide are marketed on a limited scale. Fresh-cut sweet potatoes suffer from surface browning. Common methods to control

browning involve dipping of fresh cuts in aqueous solution containing antioxidants, such as sulfites, ascorbic acid, and citric acid (Sgroppo et al. 2010). Ojeda et al. (2014) reported that edible coating of cassava starch in combination with ascorbic acid prevented enzymatic browning, retained freshness, and also improved nutritional value of minimally processed sweet potatoes stored at 4 °C for 16 days. McConnell et al. (2005) reported no changes in surface color of minimally processed sweet potatoes under MAP. Degree of browning also depends upon the potato cultivar. Moretti et al. (2002) found vacuum-packed minimally processed sweet potatoes "*brazlandia branca*" and "*brazlandia roxa*" to be less susceptible to browning than "princesa" during storage at 3 °C. Fresh-cut sweet potatoes dipped in 200 ppm chlorine and stored at 1 °C resulted in a reduction of microbial load (Erturk and Picha 2006). Sgroppo et al. (2010) reported that minimally processed sweet potatoes treated with sodium metabisulfite of 2% and citric acid packed in polystyrene trays can be stored for 14 days at 5 °C.

13.4.11 Fresh-Cut Packaged Vegetables

Fresh-cut salads on the market include shredded leafy vegetables and salad mixes. Fresh-cut vegetables for cooking include peeled cut sweet potatoes, cut pak choi, cut pumpkin, baby corn, broccoli and cauliflower florets, cut chayote, cut celery stalks, shredded cabbage, cut asparagus, and stir-fry mixes. Fresh-cut ginger and herbs are also marketed widely.

13.5 Minimal Processing Line for Tropical and Subtropical Fruits and Vegetables

Fresh-cut processing involves peeling, trimming, and deseeding fresh produce and cutting it to specific size (Fig. 13.1). Fresh-cut products must not only look fresh but must have the sensory properties – aroma, taste, texture, and visual appeal – associated with freshly prepared produce. Thus, only fresh produce of good quality must be used as the starting material in fresh-cut processing. Fresh-cut products must also be safe, wholesome, and nutritious.

13.6 Tropical and Subtropical Nuts

Minimal Processing of Nuts and Seeds All phytosanitary tests are properly done**.** Phytosanitary certificate with no treatment is required but must be free from any pest disease and pesticide residues. Raw, organic, whole, shelled nuts and seeds are distributed in consumer packages with a shelf life of 3–6 months. Typical processing of nuts and seeds is given in Fig. 13.2.

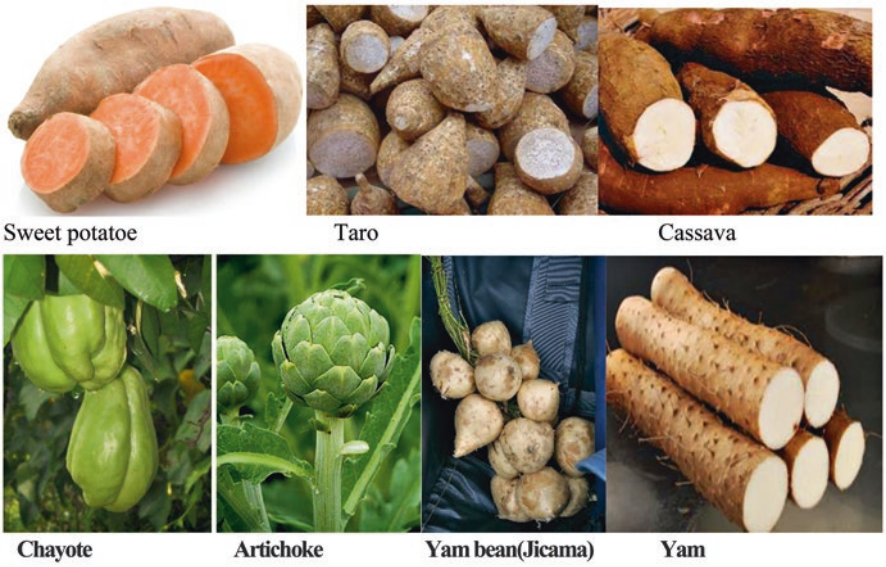

Fig. 13.2 Selected tropical and subtropical vegetables

13.6.1 Almonds

The almond (*Prunus dulcis*) belongs to family Rosaceae and is native to the Middle East, Indian subcontinent, and North Africa. Almonds are utilized as shelled, peeled or unpeeled, raw or roasted, whole or ground kernels. Due to high levels of unsaturated fatty acids, almonds are prone to oxidation. Lipid oxidation is the main cause of off-flavor development in almonds. Mexis and Kontominas (2010) reported a shelf life of 12 months for whole unpeeled almond kernels packed in polyethylene terephthalate/low-density polyethylene (PET/LDPE) and low-density polyethylene/ethylene vinyl alcohol/low-density polyethylene (LDPE/EVOH/LDPE) pouches under N_2 with an oxygen absorber. Mexis et al. (2011) reported a storage life of 12 months for irradiated raw unpeeled almond kernels packaged in PET-SiOx/LDPE pouches under N_2 with an O_2 absorber stored at 20 °C for 12 months. Sanchez-Bela et al. (2008) studied the effects of irradiation on sensory and chemical quality of almonds and found irradiation doses up to 7 kGy to be suitable for postharvest sanitation of almonds. Ziaolhagh

(2013) evaluated different packaging materials and packaging conditions for shelf life extension of almond kernels and found PA-PE-PE-PA laminate as the best packaging material and vacuum packaging conditions as most suitable for the storage of almond kernels. Thomas (1988) reported that almonds irradiated with 0.1 kGy had no adverse flavor and mouthfeel during 6 months of storage.

13.6.2 Macadamia Nut

Macadamia is a genus of four species of trees belonging to family Proteaceae. It is native to northeastern New South Wales and Central and South East Queensland. Macadamia nuts can be eaten as raw, roasted, coated, salted, and flavored. Higher ratio between unsaturated and saturated fatty acids makes macadamia nuts susceptible to oxidation, thereby affecting their quality and shelf life (Frankel 1998). Colzato et al. (2011) suggest that zein/oleic acid edible coatings can be used for large-scale applications to improve the oxidative stability and enhance the shelf life and quality of macadamia nuts. Bowden and Reeves (1983) studied the effect of different packaging materials on the quality of raw Australian macadamia nuts and found that nuts packed in biaxially orientated nylon/aluminum foil/LLD polyethylene showed no deterioration for 18 months. Storage temperature is an important factor governing the quality of macadamia nuts; the lower the storage temperature, the longer the shelf life of the product (Cavaletto 1966). At 1–5 °C, storage life of vacuum-packed raw kernels (2–3% moisture content) was 16 months, but at 37 °C, a rapid deterioration of kernel quality and also storage life of less than 8 months were observed.

13.6.3 Walnuts

Walnut is the nut of the tree of genus *Juglans* and family Juglandaceae. Walnuts are utilized as shelled whole kernels or ground kernels. Walnut kernels contain 65% lipids, out of which 73% include polyunsaturated fatty acid (PUFA) (Crews et al. 2005). High level of PUFAs makes walnuts prone to oxidation. Oxygen concentration, temperature, and relative humidity are some other factors influencing the oxidation potential of walnuts. Lopez et al. (1995) found that at 10 °C temperature and 60% relative humidity, walnut quality was maintained for a period of 12 months. Jensen et al. (2001) did not find any trace of rancid taste in walnut stored in dark at 5 °C during 25 weeks of storage at accelerated storage conditions (50% oxygen). However, significant oxidative changes were observed in walnuts stored at 21 °C in light. In another study, Jensen et al. (2003) obtained a shelf life of 13 months for walnuts with a high-barrier packaging material combined with N_2 flushing. Mexis et al. (2009) studied the effect of temperature, degree of O_2 barrier, and lighting conditions on quality of shelled walnut and reported that the effect of parameters observed followed the order: temperature > degree of O_2 barrier > lighting conditions. Bakkalbas et al. (2012) studied the effects of storage temperature and oxygen permeability of package on oxidative stability of vacuum-packed walnut kernels and concluded that oxidation was sufficiently protected in vacuum-packed walnut kernels in polyamide/polyethylene PA/PE film pouches at 20 °C for 12 months. Chlebowska-Smigiel et al. (2008) found that pullulan coating inhibited hydrolytic rancidity and oxidation of fat in nuts including walnut. It also slowed down weight loss of nuts.

13.6.4 Pecans

Pecans (*Carya illinoinensis)* belong to family Juglandaceae which also includes walnuts (*Juglans* sp.). Pecan is mainly distributed in America, Israel, Australia, South Africa, and China. Pecans are rich in proteins, lipids, tocopherols, and antioxidants. Pecan kernels contain about 65% lipids, out of which 90% are unsaturated fatty acid (UFA). UFA besides having health-promoting properties also makes pecans prone to oxidation. Jigang et al. (2016) reported that fresh pecans exposed to microwaves for 2.5 min duration can be stored at up to 120 days at 2 ± 0.5 °C without any adverse effects on fatty acid composition or tocopherol content. Taipina et al. (2009) found

that pecans irradiated by 1 kGy dose showed no significant changes in aroma, flavor, appearance, and texture. The vitamin E content of irradiated pecans also remained stable. Maness (2004) studied the effect of temperature on storage of pecans and reported that shelled pecans can be stored for 3 months at 22 °C, 9 months at 0 °C, and 18 months at −18 °C.

13.6.5 Pine Nuts

Pine nuts (*Pinus pinea*) are the edible seeds of pines belonging to family Pinaceae. It is widely grown in Mediterranean regions, mainly in Spain, Portugal, Italy, Greece, Albania, and Turkey. Pine nuts are consumed as raw or roasted. Pine nuts contain 5.6% moisture, 31.1% protein, 47.4% fat, 10.7% carbohydrate, and 4.3% ash, and these also contain vitamins (B_1 and B_2) and minerals, especially phosphorus and potassium (Nergiz and Donmez 2004). Due to high content of fats, pine nuts are prone to oxidative and hydrolytic rancidity (Kaijser et al. 2000). Deterioration of pine nuts also depends on storage conditions such as temperature, moisture content, and gas composition. Cai et al. (2013) found that low-moisture conditioning and freezing temperature storage can be a viable alternative for maintaining the postharvest quality and extending the storage life of pine nuts. Mehyar et al. (2012) studied the effect of a combination of edible coatings (pea starch, whey protein isolate, and carnauba wax) on rancidity and sensory properties of pine nuts and concluded that the coating was effective in preventing oxidative and hydrolytic rancidity of pine nuts stored at 25 °C during 12 days of storage. Golge and Ova (2008) studied the effect of gamma irradiation (0.5, 1.0, 3.0, 5.0 kGy) on the chemical, physical, and sensory attributes of pine nuts during 3 months of storage period and found that irradiation had no effect on the quality parameters such as texture and color, fatty acid composition, and sensory attributes.

13.6.6 Cashews

Cashew (*Anacardium occidentale*) belongs to family Anacardiaceae. Cashew nuts have a characteristic odor and taste and are consumed as raw, salted, or unsalted. Cashew nuts are a good source of fats (45%), carbohydrates (23%), and proteins (20%) (Bhattacharjee et al. 2003a). High fat content makes cashews prone to oxidation. Besides, cashew nuts are also susceptible to infestation by molds, insects, and larvae. Among molds, *Aspergillus* species responsible for the production of aflatoxins render cashew nuts unsuitable for consumption. Irradiation can be a viable alternative to control pests in cashew nuts due to its ability to kill insects. Das et al. (2014) reported a shelf life of over 6 months for microwave-treated cashew nuts. The nuts were found to be from infestation and rancidity in comparison to untreated nuts that were heavily infested at the end of 1 month of storage. Mexis and Kontominas (2009a) evaluated the quality parameters of cashew nuts irradiated at 0, 1, 1.5, 3, 5, and 7 kGy doses and reported that cashew nuts remained organoleptically acceptable at doses <3 kGy. Bhattacharjee et al. (2003b) reported that irradiation doses (0.25–1.00 kGy) arrest insect infestation in cashew nuts during 6 months of storage. Sajilata and Singhal (2006) reported a reduction in the antioxidative activity of cashew nuts irradiated by 0.25–1.00 kGy dose.

13.6.7 Pistachios

The pistachio (*Pista ciavera* L.) is a member of the cashew family Anacardiaceae. Pistachio nut is one of the major agricultural products in Iran. It is consumed as raw and roasted. Pistachio is a highly nutritious nut. It is a rich source of unsaturated

fatty acids (89.1%). High lipid content makes it very sensitive to rancidity and mold contamination. Gecgel et al. (2011) studied the effect of γ-irradiation on fatty acid composition of shelled pistachios and reported that free fatty acid and peroxide value of nuts increased as the dose increased. Moreover, the concentration of total saturated fatty acids increased while total monounsaturated and total polyunsaturated fatty acids decreased with the irradiation dose ($p < 0.05$ and $p < 0.01$). Georgiadou et al. (2015) reported that pistachio kernels stored under modified atmosphere packaging (MAP) retained acceptable sensory attributes after 47 days of storage. MAP also prevented oxidative rancidity. Tavakolipour et al. (2011) evaluated the effect of coating based on whey protein concentrate (WPC) and thyme essential oil on aflatoxin production in pistachio kernel and reported that thyme essential oil at a concentration higher than 5000 ppm in WPC edible coating could prevent aflatoxin production in pistachio kernels.

13.6.8 Hazelnuts

Hazelnut is the nut of the tree of genus *Corylus* belonging to family Betulaceae or Corylaceae. It is also known as cobnut or filbert nut. Turkey is the leading producer of hazelnuts. Hazelnuts are available as blanched, roasted, chopped, sliced, and diced, grinded into flour, and made into meal, butter, or paste. Fat is the main constituent of hazelnuts ranging from 56.4% to 64.1% which increases its susceptibility to oxidation. Hazelnuts are also susceptible to infestation by molds, fungi, and insects. Application of zein coating reduced oxidative rancidity of roasted hazelnuts and prevented changes in its color, texture, and moisture content at ambient storage conditions (Yildirim and Mazi 2016). Basaran (2011) evaluated the effects of various acids and surface active compounds to control *Aspergillus* and found benzalkonium chloride, disodium-ethylenediaminetetraacetic acid, and sodium hypochlorite most effective in combating fungal growth. Mexis and Kontominas (2009b) found that hazelnuts retained acceptable sensory quality upon irradiation by a dose of 1.5 kGy. Guler et al. (2017) reported that hazelnuts treated with 0.5 kGy had highest vitamin E and lowest free fatty acid and peroxide values which was also reflected in sensory analysis.

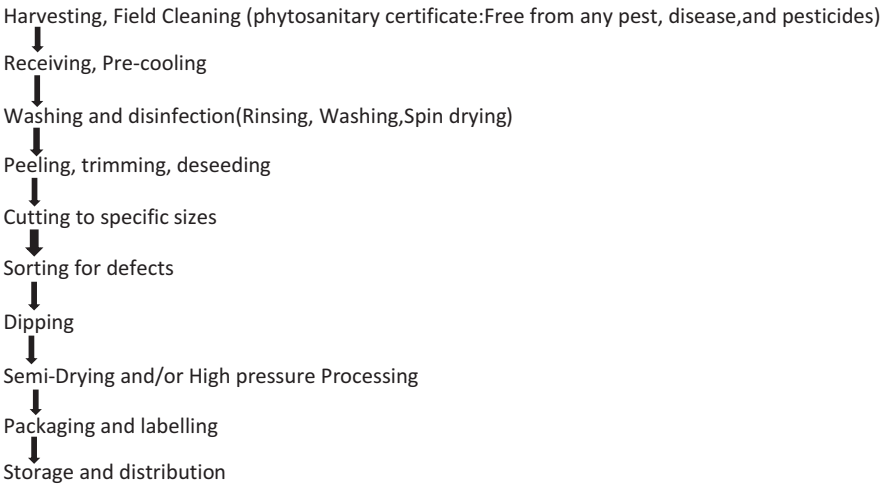

Fig. 13.3 Typical fresh-cut process flowchart for fruits, vegetables, and root crops

13.6.9 Brazil Nuts

The Brazil nuts come from the *Bertholletia excelsa* tree, which is found throughout the Amazon rain forest. It is seeds of the Brazil nut tree. Brazil nuts are commonly eaten raw or blanched and are high in protein, dietary fiber, thiamin, selenium, copper, and magnesium. During the blanching process, if high temperatures (above 50 °C) are not used, it may be called raw blanched Brazil nut; otherwise, it will be called blanched Brazil nuts. Brazil nut production is summarized in Fig. 13.3.

A Brazil nut a day supplies the human body with its daily requirement for selenium, an important trace mineral high in antioxidants. The Recommended Daily Allowance (RDA) for selenium for adults 19 years and up is 55 μg a day. According to the USDA National Nutrient Database for Standard Reference, one Brazil nut delivers 95.8 μg of selenium, well over the daily requirement for the mineral (Thompson Christine et al. 2008). Optimum dietary selenium intake related to optimum glutathione peroxidase activity in serum reduces the risk of coronary heart disease in human (Luoma et al. 1984) (Figs. 13.4 and 13.5).

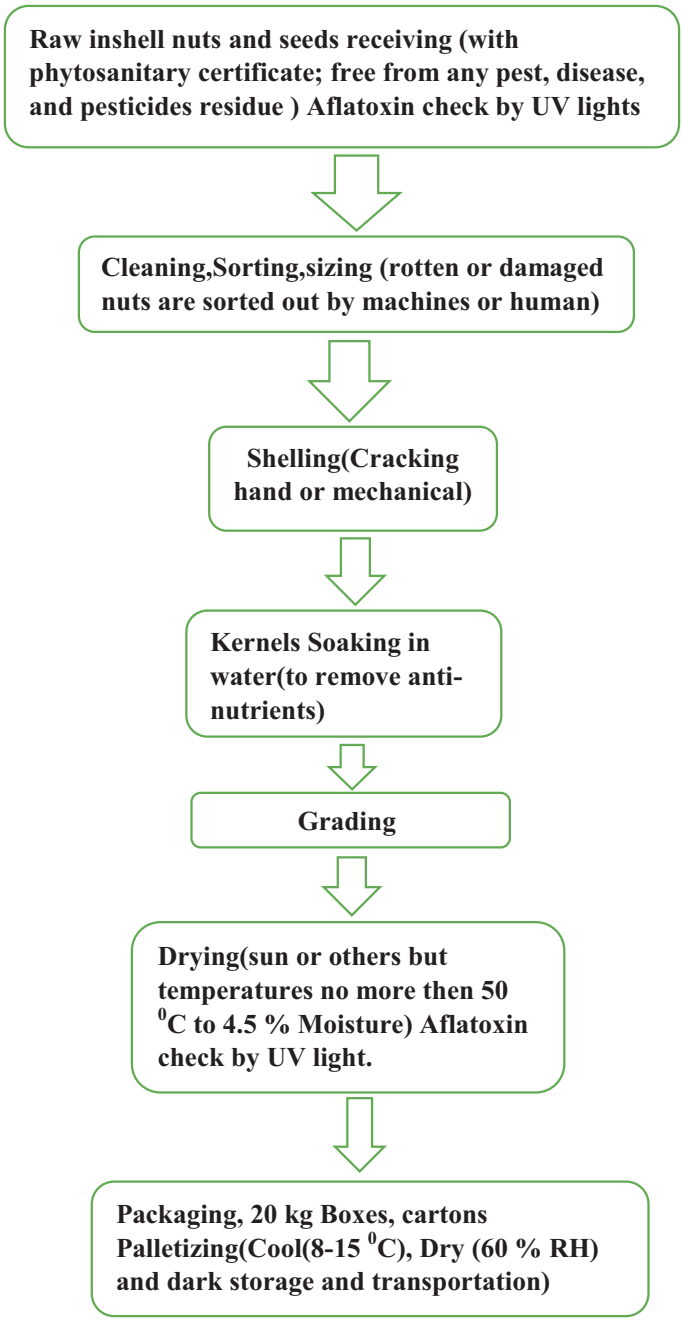

Fig. 13.4 Typical minimally processed flowchart for nuts and seeds

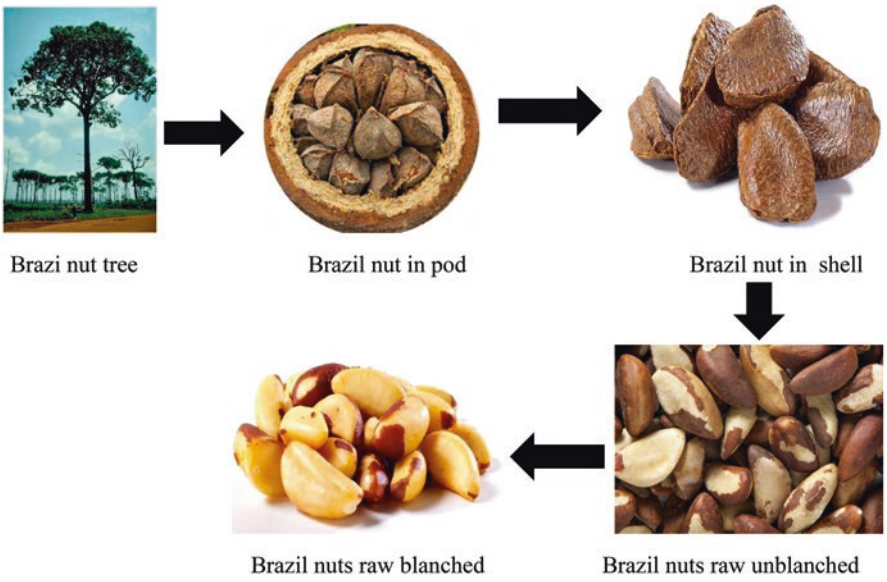

Fig. 13.5 Minimal processing steps of commercial Brazil nuts

13.7 Tropical and Subtropical Seeds

13.7.1 Flaxseeds

Flax (*Linum usitatissimum*) belongs to family Linaceae. It is cultivated in more than 50 countries and Canada is the major producer of flaxseeds. Flaxseed contains good amount of α-linolenic acid (ALA), omega-3 fatty acid, protein, dietary fiber, and lignan, specifically secoisolariciresinol diglucoside (SDG) having antioxidant activity and free oxygen radical-scavenging activity. Flaxseed is grown as either oil crop or a fiber crop with fiber linen derived from the stem of fiber varieties and oil from the seed of linseed varieties (Diederichsen and Richards 2003; Vaisey-Genser and Morris 2003). Flaxseed can be found in brown and gold color. The brown flaxseed is grown in warm and humid climates like Brazil and has the shell a bit tougher than the golden linseed. The texture of flaxseed is crisp and chewy possessing a pleasant nutty taste (Carter 1996).

Various deteriorative changes occur during storage of flaxseeds. These deteriorative changes result from various factors such as high moisture content that increases the rate of deteriorative reactions. Heat produced by respiration also aggravates deteriorative reactions. Different techniques have been used to inhibit these undesirable effects in flaxseeds. In one study, flaxseeds were divided into three lots, of which one lot was treated with ethylene chlorohydrins in concentration of 0.38% on dry weight basis of seed and then was placed in an insulated chamber. The other two lots of flaxseed were untreated, of which one was placed in insulated chamber under conditions similar to those under which treated seed lot was stored. Third lot of untreated flaxseeds was stored in a refrigerator. After 138 days of storage, it was seen that treated samples of flaxseeds had no mold growth, while unrefrigerated untreated samples had mold growth and were in advanced stage of putrefaction. Refrigerated samples showed little evidence of mold growth. The treated samples also had fresh odor of flaxseeds (Altschul et al. 1952).

Flaxseeds are prone to contamination by toxigenic fungi as tropical climates are ideal for their growth. Gamma irradiation has been used to inhibit contamination by aflatoxigenic fungi in flaxseeds. The samples of flaxseeds packed with PVDC film were subjected to the gamma irradiation doses of 2.5, 5.0, 7.5, and 10 KGy and then stored in a cool dry place in laboratory for 6 months. After 6 months, the treated samples were sown in DRBC to check growth of aflatoxigenic fungi. The results showed no growth of aflatoxigenic fungi in irradiated flaxseed samples (Costa et al. 2013).

13.7.2 Hempseeds

Hemp (*Cannabis sativa*) belongs to family Cannabaceae. It is native to Central Asia and cultivated in Asia, Europe, and China. It is grown in tropical, temperate, arctic. Hemp is mainly used for fiber and oil. Seed of cannabis is an important source of nutrition. Hempseed contains high amount of proteins, mainly edestin and albumin, that contain all essential amino acids. Hempseed oil contains high amount of polyunsaturated fatty acids, mostly linoleic acid and alpha linolenic acid. Gamma-linolenic acid is also present in hempseed oil that has vital health benefits in humans. GLA alleviates psoriasis, atopic eczema, and mastalgia and also prevents cardiovascular disorder and also has beneficial effect on psychiatric and immunological diseases. Hempseed also contains small amount of stearidonic acid (Callaway et al. 1996)

and eicosenoic acid (Molleken and Theimer 1997). It contains a group of chemicals known as cannabinoids. Tetrahydrocannabinol (THC) is the major cannabinoid present in hempseed. These cannabinoids are potent lipophilic antioxidants and has been used for various therapeutic purposes from ancient times (Hampson et al. 2000). It is also a rich source of vitamin E.

Hempseed has been used as food by humans generally of lower classes. In the past, whole hempseed was used for the preparation of foods such as peanut butter as these foods had gritty texture due to the presence of hempseed hull. Nowadays, the seeds are dehulled using mechanical hullers. The dehulled seeds produce smooth, white seed meal. Vacuum packaging and canning are used for hempseed storage. In a study by Suriyong et al. (2015), it was concluded that storage of hempseeds at 15 °C or cooler down to 4 and −4 °C maintains seed quality for 1 year.

13.7.3 Sunflower Seeds

Sunflower (*Helianthus annuus*) belongs to family Asteraceae. It has originated in North America. It is an annual flowering plant. It is the leading oilseed crop. Sunflower seeds are nutrient rich that are used to produce edible oil that ranks second after soybean oil (Robertson and burns 1975; Stefansson 2007). Sunflower seeds are rich source of proteins having favorable amino acid distribution. Sunflower seeds contain unsaturated fatty acids mainly oleic and linoleic acid. Sunflower seed is also rich in vitamin E, niacin, pyridoxine, pantothenic acid, and folic acid. Sunflowers seeds are also rich in minerals like calcium, copper, iron, magnesium, manganese, selenium, phosphorous, potassium, sodium, and zinc and also contain good amount of phytosterol that has various health benefits, for example, it reduces risk of cancer due to high antioxidant content, is antifungal and antibacterial, and reduces risk of heart diseases.

After harvesting, the seeds are sorted according to size and color; the larger ones are used as inshell and medium-size sunflower seeds are usually hulled for kernel market. Smaller seeds are used as feed for birds and pet. Seeds of different classes and grades require different packaging system. For storage of seeds for short time, moisture content should be below 12%, and for long storage, it should be below 10%. Abreu et al. (2013) investigated the deterioration of sunflower seeds under different storage conditions. The packaging material used in this study consisted of multiwall kraft paper and plastic packaging with and without vacuum, under cold

chamber and conventional storage conditions. The results showed that the physiological quality of seeds stored under cold chamber conditions packaged in paper bags is more efficient and those packaged in plastic bags under conventional storage had better physiological quality.

13.7.4 Sesame Seed

Sesame (*Sesamum indicum*) belongs to family Pedaliaceae. It is a very ancient oilseed crop grown in tropical and subtropical regions. It is cultivated in 60 countries of world. Asian and African countries are major producers of sesame. The sesame seeds have some potential nutraceutical compounds such as phenolic and tocopherols with antioxidant activity that have significant effect on reducing blood pressure, lipid profile, and degeneration of vessel impact reducing chronic diseases. During storage, sesame seeds are prone to deteriorative changes. To inhibit deteriorative reactions in stored seeds, various preservation methods are used. The sesame seed samples were given irradiation dose of 10 kGy and stored for 1 year at ambient temperature 20–28 °C. At the end of storage period, irradiation dose of 10 kGy greatly reduced the counts of total bacterial count and total fungal count and spore former bacterial to less than 10 cfu/g (Swailam 2009).

13.7.5 Pumpkin Seeds

Pumpkin (*Cucurbita pepo*) belongs to family Cucurbitaceae. It is native to North America. Pumpkin seeds are edible kernels of pumpkin. The seeds of pumpkin are used as food and for extraction of oil. In some parts of central Europe, pumpkin is used as major oilseed crop. Roasted pumpkin seeds are used as snack food. Pumpkin seeds are rich in unsaturated fatty acids mainly oleic 29% and linoleic 47% and also contain vitamin E (Younis et al. 2000). Pumpkin seeds are valuable food supplements as it contains good amount of minerals such as potassium, phosphorus, and magnesium and trace minerals such as calcium, sodium, manganese, iron, and copper (Lazos 1986). Pumpkin seeds are rich source of vitamins A, C, and E and good source of B complex vitamins. Flavonoid compounds such as α- and ß-carotenes, cryptoxanthin, lutein, and zeaxanthin are also present in pumpkin seed. The study on the effect of drying on pumpkin fruit (with and without rind), whole pumpkin seeds, and pumpkin seed kernels showed that pumpkin fruit contains high moisture levels, while seed contains low moisture levels and therefore can be stored for over longer periods and also seeds contain high content of proteins approximately 35–40% compared to fruits that contain low amounts of protein (4–4.9%) (Fedha et al. 2010). Conditioning of pumpkin seeds at 25 °C is suitable for faster germination of seeds under field conditions (Salmasi 2006).

13.7.6 Chia Seeds

Chia (*Salvia hispanica*) belongs to family Lamiaceae. It is an annual herbaceous plant. Chia seeds are good source of omega-3 and omega-6 fatty acids. Average protein content in chia seeds varies from 15% to 23%. The total dietary fiber in chia seed varies from 36 to 40 g per 100 g. Chia seeds contain about 60% omega-3 unsaturated fatty acids that have various health benefits including hyperlipidemia, hyperglycemia, and hypertension. Chia seeds are rich in polyphenols that have antioxidant activity. Chia seeds contain bioactive compound quercetin, myricetin, kaempferol, cholorgenic acid, and 3,4dihydroxyphenyl ethanol-elenolic acid dialdehyde (DHPEA-EDA) that have protective effect against diseases such as cardiovascular diseases, cancers, and diabetes. Chia seed also contains minerals such as zinc, calcium, phosphorus, and magnesium and also contains high amount of niacin.

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Chapter 14
Minimally Processed Fresh Green Beverage Industry (Smoothies, Shakes, Frappes, Pop Ups)

Konstantina Pasvanka, Theodoros Varzakas, and Charalampos Proestos

14.1 Introduction

Food processing is any intentional change in a food that occurs before it's available for us to eat. It can be as simple as freezing or drying of food to preserve nutrients and freshness or as complex as formulating a frozen meal with the right balance between nutrients and ingredients.

The main goal of food technology has been to extend the shelf life of fresh products. Most of the food products classified as fresh are in reality minimally processed foods (MPF). Because of new lifestyles and increasing purchasing power, health-conscious consumers demand foods that are appropriate to prepare and yet maintain a fresh-like quality and contain natural ingredients. The food service industry wants readily available food products for reasons of cost and food safety. As the years go by, the entrepreneurs and retailers are extending this category and consumers are accepting these products enthusiastically. Scientists were involved in these events, which made possible processing of food products of better quality and with an extended shelf life sufficient to make their distribution feasible. As technology is being developed, research and development are conducted on the technique itself, then on the equipment, and then on application to rough material. Depending on the kind of rough material (fruits, vegetables, etc.), the characteristics of MPF are different,

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as well as their preparation for meal. Nowadays, especially in developed countries, consumers demand the so-called minimally processed food products (MPFP). There are several reasons for that as consumers are more educated about nutritional value of food products and more concerned about health (Pilizota 2004).

14.2 Minimally Processed Fruit Juice Products

14.2.1 Terminology and Classification

Consumer demands for suitable but fresh and healthy foods are driving most of the food industries to apply modern and mild preservation techniques, which satisfy the increasing market demands for fewer preservatives, excellent nutritive value, and fresh sensory attributes. Traditional preservation technologies and techniques highly affect the appearance, sensorial characters, and the value of nutrients. Minimal processing techniques (MPT) have emerged to meet this challenge of replacing traditional methods of preservation while retaining nutritional and sensory quality (Ohlsson and Bengtsson 2002). Many terminologies are used for the definition "minimal processing." Minimal processes are those which minimally influence the quality characteristics of a food while, at the same time, giving the food sufficient shelf life during storage and distribution (Huis in't Veld 1996). Minimal processing technologies are techniques that preserve foods but also retain to a greater extent their nutritional quality and sensory characteristics by reducing the reliance on heat as the main preservative action (Fellows 2000). "Minimally processed" is an ambiguous term that is applied to such different types of products as precut, prepackaged fresh products or fresh meat for short refrigerated storage, and mildly cooked or pasteurized foods that can be stored under refrigeration for more than 1 week. The original purpose of minimal processing was to reduce the heat treatment (thermal processing) used by traditional thermal techniques to restrict the quality loss that has been caused by long and high-temperature treatments. The target to minimize the quality loss and to prolong the shelf life of food including fruit and fruit products coerced researchers to develop the nonconventional heat treatment to achieve better balance between preservation and quality and to develop new techniques that extend the shelf life of the products. Nevertheless, minimal processing does not inactivate entirely all microorganisms present in the rough, unprocessed material. So, the microbiological safety during the shelf life of minimally processed foods depends on an appropriate refrigerated storage and distribution, which prevents the growth of hazardous microorganisms and a restriction of "use-by" time period. Moreover, intrinsic hurdles to microbial growth may conduce to the safety of these products either originating from the rough material or introduced during processing (e.g., low pH, reduced water activity, natural antimicrobials, and modified atmosphere packaging (MAP)).

There are many systems to classify the methods of preservation used for minimal processing technology. The traditional classification is based on the methods and

essential effects of the preservation. Ohlsson and Bengtsson (2002) distinguish these different groups:

1. Thermal methods, with subgroups classified according to the heating system of the food: minimal processing by thermal conduction, convection, and radiation, aseptic and semi-aseptic processing, sous-vide processing, infrared heating, electric voltage heating, electric resistance/ohmic heating, high-frequency and radio frequency heating, microwave heating, and inductive electrical heating
2. Nonthermal methods: irradiation, high-pressure processing, pulsed discharge of high capacitor, pulsed white light, ultraviolet light, laser light, pulsed electric field (PEF), oscillating magnetic field, ultrasound, pulse power system, and air ion bombardment
3. MAP
4. Active and intelligent packaging
5. Usage of natural food preservatives

The main effects of using conventional thermal method for fruit preservation are as follows: the loss of fresh appearance, destruction of the respiration pathways, and the irreversible change of some components, such as protein coagulation, starch shriveling, texture softening, formation of aroma compounds, loss of vitamins and minerals, or formation of some thermal reaction components. The desirable or no desirable effects of heat treatments depend upon the exposure time and temperature. Minimal processing utilizes mild heating to keep away from no desirable effects or quality change of the processed food. The bases of nonthermal methods of minimal processing are different, and only few methods are sufficiently efficient in inactivating microorganisms or enzymes that play the main role in the deterioration. Most of them need combination with other preservation methods such as refrigeration and MAP for the prolongation of shelf life and conservation of quality and safety.

As fruits are living products with active respiration even after harvesting, it is possible to categorize the minimal processing technologies used for processing of fruits and fruit products: In minimally processed fruit juices, no acids or sugars are added (Yildiz 1994):

1. Minimal processing technologies with active respiration of fruits and its products
2. Minimal processing technologies for processed fruit products that destroy the respiratory activities of fruits throughout processing

14.3 Manufacturing Fruit Beverages

Fruits have always played an important role in human diet. However, before the twentieth century, drinking fruit juices was the privilege of a few.

Welch was the first to preserve grape juice by heat treatment in America (1869), followed by Muller Thurgan in Switzerland in 1896 (Kardos 1962), and, thus, began

Fig. 14.1 Minimally processed smoothie, shakes, and green juices

the production of preserved fruit juices, which was followed by a prodigious development in the twentieth century. The role of vitamins and minerals in the human body was discovered at that time, which caused substantial changes in eating habits.

Due to the revolutionary development of technical equipment, the appearance of chemicals, and biological substances (enzymes, clarifying and flavoring agents), and the application of new technological procedures, especially the aseptic technique of fruit juice production became widespread. Some of the minimally processed fresh fruit and vegetable juice mixes are given in Fig. 14.1.

14.3.1 Main Fruit Drink Categories

Nowadays, there are countless fruit juice products in the world's markets. They may differ substantially in terms of rough material, composition, quality, nutrient content, sensory traits, and packaging. In some cases, the biggest differentiation is the brand name.

Usually, fruit juice-based drinks are classified according to their fruit content. So, we can declare these categories: juices and fruit musts, fruit nectars, and soft drinks with fruit content (e.g., smoothies, shakes).

14.3.1.1 Juices and Fruit Musts

Juices and fruit musts are obtained by mechanical processes. They preserve the color, taste, and aroma of the original fruit and their composition is identical as well. In the case of certain products, sugar addition and vitamin enrichment are allowed, but these have to be stated on the labeling. Fruit musts and juices are not allowed to contain food industry additives (preservatives, aromas, and coloring agents). For that reason, they are consumed in fresh form soon after production or they are preserved by heat treatment. The permitted ingredients of different fruit beverages are presented in Table 14.1.

Table 14.1 Permitted ingredients of different fruit beverages in Europe

Components	Juice or fruit must	Nectar	Soft drinks
Fruit, min. (%)	100	25–50[a]	Free
Sugar, max. (%)	1.5	20	Free
Acid, max. (g/l)	3[b]	3[b]	Free
Preservatives (mg/l)	–	–	++
Food additives	–	–	++

Note: ++indicates according to the legislation of additives
[a]Depending on fruits
[b]With natural lemon juice

Fig. 14.2 Homemade fresh green fruit and vegetable juices

According to the type of fruit, these products can be separated into two subcategories. They can be filtered to be transparent (grape, apple juice) or they can be cloudy juices containing colloids like all citrus-based juices and green juice. Products belonging to this latter group may include fruit fiber. See Fig. 14.2.

14.3.1.2 Fruit Nectars

Fruit nectars are made of sieved juices or from fruit juices that are dissolved with sugar syrup. They usually contain only one fruit—orange, apple, or peach—but they can also be made from blends of more than one fruit juice or pulp (Szenes 1991). In Europe, the preparation of blends and lowest fruit content is regulated by government standards, industrial specifications, and other voluntary and mandatory requirements. In order to make sure wide international trade, these standards conform to the recommendations of the Codex Alimentarius of the FAO/WHO Food Standard Program (Varkonyi 2000). Nevertheless, many western countries impose their own restrictions on imports and exports.

14.3.1.3 Soft Drinks with Fruit Content (Smoothies, Shakes)

Smoothies are blended beverages containing fruit, fruit juice, ice, yogurt, and milk and are a popular way of drinking fruit (Safefood 2009). These products are purchased freshly prepared from juice bars or as a processed product (mildly pasteurized)

from the chilled section of retail outlets. Despite worsening worldwide economic conditions, smoothies remain a popular and convenient way of consuming fruit. Indeed, the world smoothie market is projected to touch $9 billion by the year 2015 (Global Industry Analysts 2010). This is primarily driven by rising health consciousness among consumers, on-the-go consumption, convenience, and apprehended fresh-like taste offered by smoothies. While an ideal processed product would maintain all the sensory perceptions of a freshly prepared product, the chemical changes induced by processing and postproduction alter the sensory profile of its particular components (Perez-Cacho and Rouseff 2008). Aroma is the key determinant of quality in fruits and this in turn is a function of the volatile profile of a foodstuff. Consequently, in single-component foods, much effort has been directed toward relating level of aroma volatiles to the sensory profile as determined using a trained panel. Sensory profiling is the process during which a panel of trained assessors scores several sensory attributes of a product. It is the technique of choice for relating information of aroma volatiles to sensory perception as it gives detailed insights into panelist's perceptions of a number of flavor notes which can be related to levels of private aroma volatiles. Nevertheless, there is limited information on this relationship for multicomponent foods such as fruit smoothies. This information is of value to processors as it makes an understanding easier of the influence of processing on the levels of key aroma volatiles and ultimately the sensory profile of the food. For instance, thermal processing is undoubtedly the most common and cost-effective method to extend the shelf life by reducing microbial numbers and enzyme activity. However, this type of processing can restrict the concentration of volatiles commonly associated with fresh products and initiate reactions that result in the formation of off-flavors (Bazemore et al. 1999). For that reason, in pursuit of techniques that can both extend shelf life and produce a fresh-like product in terms of flavor, nonthermal pasteurization processing methods are being examined (Gui et al. 2007; Heinz et al. 2003). High hydrostatic pressure (HHP) is one such technique and has been the subject of much investigation (Oey et al. 2008a, b; Oey et al. 2008a, b; Rastogi et al. 2007). Because of the stability of covalent bonds to high pressure (Knorr 1993), HHP should, in theory, have reduced smaller molecules such as volatile compounds associated with the sensory quality (Cheftel 1992). Research has indicated that HHP can both enhance and diminish enzymatic and chemical reactions involved in the formation and degradation of aroma compounds (Oey et al. 2008a, b). Indeed, previous work carried out by this group reported that sensory panelists noted a decrease in aroma and flavor intensity in HHP processed (600 MPa/20 °C/15 min) smoothies after 28 days of storage compared to fresh controls (Gormley et al. 2009).

14.4 Fruit Drink Raw Materials

The most important rough materials of fruit drinks that are available in international trade are citrus, pomaceous fruits, stone fruits, grapes, and berries. Nevertheless, all cultivated and wild-grown fruits are used for drink production. Some of the rough

materials used are suitable for juice production (e.g., apple, orange), since their juices are enjoyable themselves. The juice of other fruits (e.g., sea blackthorn, currant sorts) is delightful only if blended with sugar syrup.

The quality of fruit drinks, made without additives, is basically determined by the quality of rough materials. Usually, acidulous, juicy fruits with high-sugar content and distinctive aromas are suitable for drink production. The ripeness of rough materials is of critical importance because optimally ripe fruits possess the ideal sugar/acid ratio and the most advantageous flavor and aroma components. Prior to the optimal ripeness, the fruit contains minor aromas and sugar. On the other hand, overripe fruits may lose their acids (vitamin C), coloring agents, and consumption value (Steger-Mate et al. 2002).

Due to the development of fruit drink consumption, rough material production and juice consumption were separated both geographically and in time, then versus now. Therefore, fruit pulps and concentrates that are easier to store and transport came to the front in production. Fruit drinks, made of these preserved semifinished products, can only be emulative if their composition and sensory traits are close to those made of fresh fruits. According to different surveys, 70% of fruit drink quality complaints are rooted in the rough materials. All these facts made experts involved in production, quality control, and sales to set up uniform quality requirements for the clarity and origin of fruit drinks. Recommendations of this RSK (Richtwerte und Schwankungsbreiten bestimmter Kennzahlen) system that worked out in Germany for compositional features are generally accepted in the European commercial practice (Bielig et al. 1987). Nevertheless, fruit variety, origin, climate, production, and processing technology can often change the composition, resulting in quality problems. The further growth of this system led to the publication of the European Economic Commission's Association for Juice and Nectar Production (AIJN), called "Code of Practice."

Besides consumption value, quality, genuineness, rough material, and technological deficiencies, these criteria comprise factors concerning the environmental pollution of the production land such as arsenic and heavy metal level. Technological deficiencies usually result in high concentration of biogenic acids, hydroxymethylfurfural (HMF), ethyl alcohol, and patulin; thus their maximum levels are under regulation. The values determined by the RSK system and the AIJN Code of Practice are primarily used in Europe, but significant deviations may put the competitiveness of products in danger in the world market.

14.5 Production of Filtered and Cloudy Fruit Drinks

Filtered and cloudy fruit drinks are made of mechanically pressed and cleaned juice either directly or from the dilution of concentrated semifinished products. As it can be seen below, production technology comprises five main operations (Fig. 14.3).

Juice extraction is a basic technological step of fruit juice production. The fruit has to be prepared afore to juice extraction, which is then followed by juice clarification and drink completion. Then, the finished drink is packed and preserved.

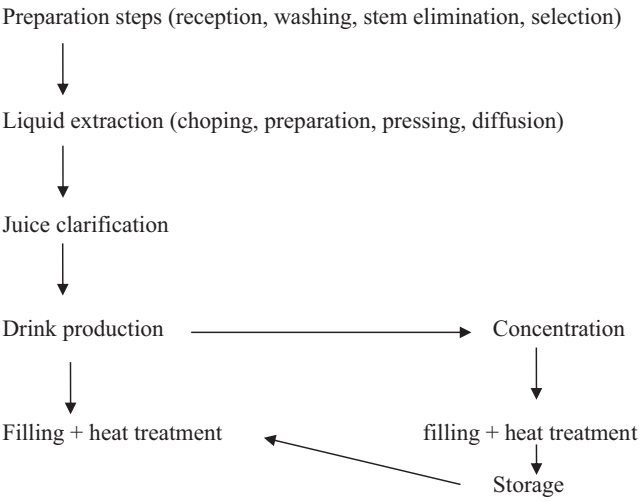

Fig. 14.3 The flowchart of fruit juice production

14.5.1 Preparation Steps

14.5.1.1 Rough Material Reception

Only those rough materials are allowed for fruit drink production that meet the following criteria: appropriate ripeness and flavor, no signs of deterioration, and free from extraneous ingredients, pathogenic organisms, and their effect. Furthermore, rough materials have to comply with the regulations and standards in force. During reception, prodigious attention has to be paid to the cleanliness of berries in which washing may cause substantial damages (Szenes 1991). The conformity of each batch has to tally to the methods and examinations of the relevant descriptions. Then, it has to be labeled for further establishment and traceability. What is more, conformity to the production technology requirements also has to be checked (crop spraying records).

14.5.1.2 Washing

The aim of this step is to remove every contamination from the surface of the fruit so as to increase physical, chemical, and microbiological cleanliness (Fellows 2000).

The surface of rough materials is strongly contaminated by microorganisms (it can attain 10^5–10^9 microorganisms per gram). Even with efficient washing, it can be decreased only by 3–5 orders of magnitude. Therefore, washing effectively has a significant impact on the heat treatment necessary for preservation.

Physical and chemical surface contaminations are eliminated by water soaking, since these substances are water soluble or their adhesion properties decrease in

aqueous solution. The efficiency of the dissolving process can be increased with higher water flow. This can be achieved by streaming, by air injection, and by mechanical means. Due to the water flow, close contacts between fruit particles increase washing capacity but potentially lead to damages to the fruits. Thus, the texture of the rough material always has to be taken into consideration when choosing washing equipment. Washing usually is made up of three main steps. The first phase is soaking, which breaks up surface contamination and deducts soil particles. In the case of fruits covered with wax layer, warm water (50–60 °C) is applied. Warm water soaking or even a long soaking period may result in substantial loss of valuable fruit components (Barta and Kormendy 1990).

The active phase of washing is meant to remove every contamination, and it is always followed by a clean water rinse. Washing means water flows all around the fruit; meanwhile, rinsing means water spraying in order to take away washing water residues from the fruit's surface.

14.5.1.3 Stem Elimination

To prepare fruits for juice extraction, in the case of certain fruit species (e.g., cherry, sour cherry, plum), long green peduncle parts have to be deducted. In a different state, they will spoil the color and other quality traits of the juice. Mechanized stem elimination can only be carried out in rough materials of homogenous size that do not tend to damage and burst. The most regularly used equipment is the belt-based solution, but in Eastern Europe, roller-based machines are still widely used.

14.5.1.4 Selection

This step, which usually follows washing, separates everything from the rough material that is unsuitable for processing. These can be strange substances, stem and leaf particles, or moldy, deteriorated fruits. This activity is performed manually and requires huge attention. Therefore, the necessary job environments (proper lighting and reasonably positioned waste containers) must be provided for the workers. The selection table on which this operation is done should be able to roll the fruits, enabling the workers to notice the entire fruit surface. Both the roller-based and belt-based machines conform to this requirement. In order to achieve better efficiency, proper adjustments must be made for single-layer fruit flow and optimal belt speed (Parker 2003).

14.5.2 Juice Extraction

This operation can be divided into two steps: fruit chopping and preparation and the separation from solid fruit particles.

14.5.2.1 Chopping

The purpose of this step is to smash, cut the fruit, increase its surface, and launch cell-fluid elimination. Nevertheless, this can lead to enzymatic reactions damaging valuable components. For that reason, the fruit has to be processed immediately after chopping. If this step is done suitably, the fruit is not pulpy but consists of homogenous, irregular-shaped, few-millimeter-sized particles, which tend to form channels to drain the liquid when pressed.

14.5.2.2 Chopped Fruit Preparation

Procedures designed to prepare chopped fruits are to augment juice yield and prevent undesirable changes (chemical, biological, mechanical, etc.) to achieve better aroma, flavor, and color properties. The type of preparation will depend on the type of fruit and production technology. There are a lot of methods for this operation, such as mechanical, freezing, enzymatic, vibration, ultrasonic, electro-plasmolytic, ion-radiation procedures, and heat treatment (Szenes 1991). In practice, mechanical operations, heat treatments, and enzymatic solutions are extensively used.

Mechanical preparation is used to chop fruit flesh, smash the tissues, and increase the surface when stiff rough materials (apple) are usually crushed; meanwhile, soft ones (red currant) are only cracked. Crushing opens up the tissues, damaging some of the cells and the draining of cell fluid begins.

The degree of chopping is designated by the method of juice extraction. If pressing is applied, the chopped fruit releases the juice under a relatively minor amount of pressure. Appropriately prepared chopped fruits contain particles of nearly similar size, enabling channels to form for the liquid to drain.

If the fruit is chopped into very fine pieces, it spreads easily, expands under pressure, and does not intend to form channels to drain the juice.

Diffusion-based liquid extraction needs chopping to minimize the thickness of the slices and strips. Furthermore, the size of these pieces should form channels to ensure the flow of the extraction liquid. There are many devices for the crushing of fruits. These can be specialized for a given fruit (apple crusher) or generally used as hammer and roller-based machines. Their mutual feature is the rotating system and the pressing, shearing, pulling, and striking forces applied.

Preparation with heat treatment is mainly used previous to the pressing of berries, since it can increase juice yield by 5–10%. In addition, this procedure contributes to a better color. Within the framework of this procedure, the crushed–cracked fruit is rapidly heated to 80–85 °C and then rapidly cooled back. This short heat treatment enables various physical, chemical, and microbiological processes to take place. The denaturation of proteins and the hydrolysis of the protopectin lead to the deactivation of enzymes, making the cell walls permeable, thus accelerating the diffusion of water-soluble substances. In the case of technological failures, tissues become too soft and damaged, then fruit will be difficult to press, and juice taste changes as well (Szenes 1991). There are different heat exchanger devices for this

process. Enzymatic treatments are also frequently used before pressing, to make the procedure easier and to increase the yield. Fruit rough materials possess different amounts and types of pectin, depending on the species and the variety. Pectin can be found between the cell wall layers connecting the solid shells that include cellulose and hemicellulose. Pectin can also be found in diluted form in the tissues, increasing their density and sticking properties. High pectin content influences the following juice-producing steps negatively. For that reason, the level and the composition of pectin have to be decreased or modified according to the quality criteria of the finished product or the production technology. The most general solution is the enzymatic treatment of the cracked–crushed fruit with pectin-decomposing enzymes such as pectin transeliminase and polygalacturonase (Aehle 2004). The usage of these enzymes leads to the decomposition of glycoside bounds, rapidly decreasing the viscosity of the mash (Reising 1990). The enzyme products added also contain cellulose and hemicellulase enzymes so as to decompose the cell wall and improve the permeability. Enzyme treatment can also be implemented under cold and warm circumstances.

Cold treatment at 20–25 °C takes more hours, which puts in danger the juice quality (Schmitt 1990). Warm treatment takes place in 0.5–1 h, at 50–55 °C. Because enzymes are protein-based molecules, these are heat sensitive and are only active at certain pH values. If the temperature and pH conditions of the mash are not optimal, successful pectin decomposition needs longer time or higher enzyme concentration (Dietrich 1998). The pressing waste of high-pectin fruits (apple) is usually used for pectin production. In these cases, enzyme treatment should not be practiced.

14.5.3 Liquid Extraction

Through this process, the liquid phase of fruits is detached from solid particles. There are many methods for this separation: pressing, diffusion, centrifugal procedures, and reverse osmosis (Fellows 2000). The type of equipment applied hinges on the fruit species, production line, and economical background. The most known used solution is pressing.

Pressing separates a food system into two phases. In this process, fruit tissues mean the solid phase, while the liquid between the particles is the liquid phase.

Pressing requires outside forces to create tension in the system, drain liquid, resulting in shape modification. The equipment hinders the disposition of the solid phase and the liquid gathers in a vessel. The remaining material, with low level of liquid content, is called marc. The most important parameter of pressing is the liquid yield, which means the percentage of juice extracted, compared to the rough material at the beginning of the process. Juice yield is basically determined by the type of the pressing device and the quality and preparation of the rough material (Lengyel 1995).

Fruit processing industry applies continuous and intermittent pressing machines. In addition, decanters are based on centrifugal forces (Nagel 1992).

The juice of fruits can also be isolated with extraction. It means that semipermeable cell walls are made permeable following a heat treatment and the cell fluid is then diluted with water. This process is featured by the degree of extraction, expressing the amount of extracted valuable substances, contrast to the total valuable matter content of the fruit. The amount of substances diffused is in direct analogy with the diffusion coefficient, the active surface, and the concentration gradient (Patkai and Beszedics 1987).

In order to increase the diffusion factor and the permeability of the cell walls, diffusion fluid extraction is performed at 50–70 °C. Active surface can be increased by suitable chopping. The concentration gradient is determined by the stream situations and the solvent–cell fluid ratio. Nevertheless, the amount of solvent applied is limited by the concentration decrease of the liquid extracted. Diffusion juice extraction is often carried out in double-screw extractor devices.

14.5.4 Juice Clarification

Extracted fruit juices are often turbid, due to the plant particles that are water-insoluble (fibers, cellulose, hemicellulose, protopectin, starch, and lipids) and colloid macromolecules: pectin, proteins, soluble starch fractions, certain polyphenols, and their oxidized or condensed derivatives. Depending on the finished product, these substances must be partially or completely eliminated to avoid further turbidity and precipitation and to improve sensory attributes (taste, smell, and color). Juice clarification can be performed by physical and chemical methods, mechanical procedures, and their combinations.

A physical and chemical clarification is applied when eliminating all substances causing turbidity. Clarifying agents and enzymes are added during this process. The effect of mineral-clarifying agents is based upon their surface activity and electric charge. Bentonite and solid silicic acid are used for the clarification of fruit juices. Bentonite is of volcanic origin that belongs to the group of montmorillonites. It possesses huge surface and good thickening properties; its negatively charged particles strongly adsorb positively charged proteins. Solid silicic acid is a negatively charged colloid resolution. It is often combined with other clarifying agents or with enzyme treatment. Its clarifying effect is good, with a short clarification period. In the case of enzymatic pectin decomposition, solid silicic acid is added to the juice in tandem with the enzyme. Gelatin is a protein-based clarifying agent that deposits negatively charged particles (polyphenols, decomposed pectin). It is usually completed with tannin, which reacts with protein molecules. Polyvinylpolypyrrolidon is a water-insoluble powder that adsorbs and precipitates polyphenols. During juice clarification, the so-called protecting colloids need to be decomposed, since they hinder aggregate formation and the settling of floating substances. This procedure can be accomplished with the use of enzymes. Pectin decomposition is often completed with starch and protein decomposition; thus enzyme products marketed contain other enzyme components as well (Dietrich 1998; Grassin 1990).

Mechanical clarification targets the deletion of suspended fibers and precipitation. This procedure is often carried out in centrifuges and filtration devices. In this stage, the first filtration phase is performed in settling centrifuges; meanwhile, decanters are used to eliminate fibers from cloudy juices (Welter et al. 1991; Nagel 1992). Filtration is a significant step of fruit juice production. Traditional filtration is implemented in slurry layer-based devices. First filtration additives are added to the liquid to be filtered (Szenes 1991). The equipment can be based on frame, column, and vacuum. For the clarification of filtered juices, membrane and ultrafiltration techniques are extensively applied (Szabo 1995; Galambos 2003; Capannelli et al. 1994). These devices enable clarification and filtration in one step. To increase the active period of the membrane, enzyme treatment is often performed prior to the filtration (Kinna 1990). Clarified, filtered, or cloudy juices are prepared for consumption. These can be further processed to preserved products in two ways. They are packaged right after production or concentrated to semifinished products.

14.5.5 Concentrate Production

The aim of concentration is to increase the dry matter content and decrease the water content of juices, so as to extend shelf life and to improve transportation and storage properties. This operation has to be enacted with minimal loss of valuable ingredients and minimal damage to sensory traits (Braddock 1999).

14.5.5.1 Evaporation

This is the most regularly used concentration solution. This means water evaporation by means of boiling. This operation is materialized in evaporators, and steam ensures the energy necessary for boiling. Many of the solution's water content evaporate during boiling. The vapor formed is then driven out from the device and condensed. As the valuable juice ingredients are heat sensitive, short-time, low-temperature condensation is desired. In order to ensure low boiling point, the procedure is performed under vacuum. Often more evaporators are applied in sequence to minimize the energy costs. Such systems of three to four elements are commonly used (Fabry 1995; Fellows 2000). Chemical, rheological, and thermal juice properties play a major role in the condensation process. As these features depend on the rough material, operation parameters may diversify with the use of different fruit species. Evaporators should be selected according to the juice properties. The most commonly used devices are the film, pipe, plate, and centrifuge-based ones (Szenes 1991). Evaporator systems are often combined with aroma recovery units. These are connected to the first part of the evaporator and condense the most volatile aroma compounds. Aromas thus condensed are usually remixed into the concentrate to improve its smell and flavor. In a different situation, these can be concentrated and applied as natural aroma extracts for other fruit products.

14.5.5.2 Concentration by Freezing

This method is utilized for the concentration of valuable, heat-sensitive fruit juices. During this procedure, the water content of the juice is frozen with ice crystal formation. These crystals include clean water; thus solvent loss occurs in the solution. As the process goes on, the fluid gets more and more concentrated and contains more and more crystals. Then the two phases can be districted mechanically (Fellows 2000).

This type of concentration is a very gentle procedure, as there is no aroma, color, and vitamin loss due to the low temperatures. Concentrates thus prepared contain every valuable ingredients of the original juice. Its disadvantages are high energy consumption and lower concentration efficacy compared with the heat treatment process (Varszegi 2002).

14.5.5.3 Reverse Osmosis

This membrane-based fruit concentrate-producing technology is becoming more significant. This means that some of the water is filtered out of the solution.

Due to the rapid increase of osmotic pressure, concentrates up to 30 Brix can be generated. It is often used as a preconcentration step prior to the aforementioned freezing devices in order to increase capacity (Beaudry and Lampi 1990; Hribar and Sulc 1990).

14.5.5.4 Fruit Concentrate Storage

The method of storage depends on the properties of the rough material and the characteristics of the concentrate (Stéger-Máté et al. 2006). From the filtered, clarified juice of less valuable fruit, concentrates containing 70% water-soluble dry matter can be prepared. These are microbiologically stable enough to be stored in cooled stainless steel containers until further usage.

Nevertheless, colored fruits and berries can only be concentrated up to 45–55 Brix percent depending on the fruit species. Furthermore, there are concentrates with special composition and ones made by freeze concentration that hardly achieve 40–45 Brix percent. These can only be stored in frozen shape or with aseptic technology.

14.5.6 Drink Production

Clarified, filtered, and cloudy juices are packed in their original composition. Meanwhile, there are juices to which sugars or vitamins are put together.

Juices are usually made of concentrates by dilution. In this procedure the finished drink composition should be similar to that of the original juice. The water applied for dissolution is condensed or softened; flavors and aromas are adjusted with the aroma compounds condensed during concentration. As the sensory value of the product thus made is not as good as the one made with the direct procedure, labeling must contain the information that it was dissolved from the concentrates.

Fruit nectars can be made of juices and concentrates. These are made of the suitable amount of juice or concentrate and heated sugar syrup. Their acid content can be regulated with lemon juice. As far as their rough material is concerned, they can be made of one or more fruits. Their filling and preservation are similar to that for the juices.

14.5.6.1 Packaging and Preservation of Fruit Juices and Nectars

Prepared fruit drinks are filled into glass or plastic bottles of alternative shape and size, but they can also be packed into combined boxes. Fruit juices are maintained with heat treatment. According to the traditional method, the juice is heated up to 82–85 °C, then filled at that temperature, closed, and then pasteurized. Pasteurization is executed at 84–88 °C for 15–45 min depending on the size of the packaging.

Coming after heat treatment, products are cooled back to room temperature. Nevertheless, the aseptic procedure preserves juice quality much better. This means that the juice is pasteurized when flowing in a closed system, and then cooled under conditions where no infection may occur, and finally filled into sterilized containers (Buchner 1990). This procedure applies heat treatment of 100–110 °C for 0.5–1.5 min.

14.6 Production of Fruit Nectars with Fruit Flesh Content

These products are made up of fruit pulps. They are obtained by passing the rough materials through sieves, thus fruit flesh can be separated from seed and skin particles. Before performing this operation, fruits have to subsist preparation steps. Fruit nectars containing fruit flesh can be made of fruit pulp after production or later from the concentrated pulp (Fig. 14.2).

14.6.1 Preparation Steps

This above operation includes steps such as rough material reception, washing, stem elimination, and selection, which are identical with the previously introduced technology. Nevertheless, the last steps are different: coarse chopping and preheating.

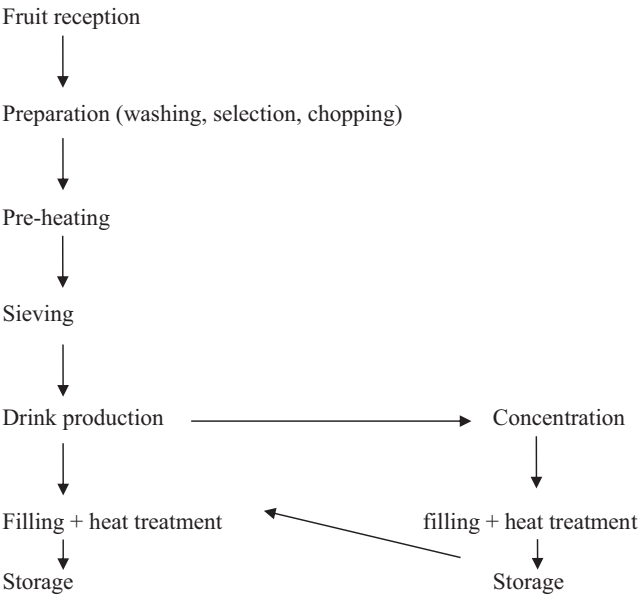

Fig. 14.4 The flowchart of fruit drink production

14.6.2 Fruit Pulp Processing and Storing

Fruit pulp is a valuable rough material for drink production but possesses no refreshing effect itself. For that reason, it is used for fruit nectar production. Fruit nectar can be made after pulp production or later at another point in time. Fruit pulp prepared is ready to be stored and transported. For storage it is usually concentrated to 26–32 Brix percent.

Fruit pulps can safely be stored under aseptic circumstances, heat treatment, and storage in sterile containers. Aseptic systems are mechanically closed under slight pressure to exclude from microbiological infections (David et al. 1996).

Heat treatment in aseptic technology is often performed above 100 °C (90–120 °C) for a relatively short period (1–2 min). After pasteurization, the pulp is cooled (30 °C) to stop further chemical reactions (Ott 1990).

Filling takes place in specific aseptic machines, which also sterilize the packaging material. Fruit pulps are stored in large containers that can range from bag in a barrel (120 L) to stainless steel tanks of more than a 100 m^3. The premier advantage of bag in a barrel packaging is the easy transportation and the compatibility with international carriage system (Szenes 1991) (Fig. 14.4).

14.6.3 Preparation of Fruit Nectars Containing Fruit Flesh

These nectars contain not only the juice but also the fruit flesh in filtered and homogenized form. Besides the necessary fruit content, sugar syrup is also added to nectars. These products can be flavored with lemon juice or condensed and enriched with vitamins. Carbohydrates or sweeteners might also be added. Nectars can be made of fruits. As far as the rough materials are concerned, fruit pulps and concentrated pulps can be used, combined with filtered juices or concentrates.

First, the water and sugar are blended, the syrup is boiled, and then the fruit pulp is added. Subsequently, it is supplemented with lemon juice and vitamin solutions.

Refining is a method that determines the nectar quality. It is performed in colloid mills, which are ground on hydrodynamic shearing forces. Homogenization chops fibers to fine particles and generates a dispersal system. The fluid thus prepared is stable, even if stored. This procedure substantially decreases visible viscosity and results in a tender smooth texture and flavor (Hidegkuti and Kormendy 1990).

14.7 Irradiation of Fresh and Minimally Processed Fruits, Vegetables, and Juices

Vegetable products, including fresh produce, precut salad vegetables, and fresh juices, receive only minimal processing. Often eaten rough or only lightly cooked, these commodities play an important dietary role in many societies.

While concerns related to the presence of pathogenic bacteria on food products have historically been associated with animal products such as meat, milk, and eggs, vegetable products have come under international investigation as sources of foodborne illness. This increased predominance is due to changes in farm technology, processing and shipping practices, and changing consumption patterns associated with minimally processed fruits, vegetables, and juices (Hedberg et al. 1994; Tauxe et al. 1997; NACMCF 1999). Beuchat (1996) discussed the incidence of human pathogens on fresh produce, citing contact with contaminated soil, water, compost, harvesting or processing equipment, or human handlers as means by which produce might become contaminated.

Application of conventional preservation and antimicrobial tools, such as heat or chemical wash treatments, to the specific needs of produce is being widely investigated (Hotchkiss and Banco 1992; Beuchat 1998); among the alternative antimicrobial technologies is the use of ionizing radiation. Irradiation as a food treatment is endorsed by a diversity of professional and governmental organizations such as the US Food and Drug Administration, US General Accounting Office, American Dietetic Association (Wood and Bruhn 1999), and the UN World Health Organization (WHO 1999).

The majority of food irradiation research efforts in recent years have been devoted to treatment of meat products, and irradiation as a terminal control step is becoming more widespread for a diversity of meat products in the United States. Low-dose irradiation may be implemented during post packaging as a terminal control step and is therefore of concernment to producers of nonthermally pasteurized (NTP) juices, fresh sprouts, precut vegetables, prepared salad mixes, fruit salads, and other minimally processed vegetable products (NFPA 2000), but applications are as yet limited.

Vegetables were the first experimental subjects in studies of physiological response to irradiation (Guilleminot 1908; Miege and Coupe 1914). Produce, fruit, and juices were all subjects of investigation to extend shelf life with irradiation (Proctor et al. 1955; Thayer et al. 1996). Primal studies with irradiated produce typically used relatively high doses and were intended to achieve pasteurization-level reductions in spoilage bacteria and fungi (Diehl 1995). Though this research helped to define the effective doses to eliminate spoilage organisms, the doses employed often exceeded the maximum radiation tolerances of vegetable commodities tested, resulting in loss of quality (Howard and Buescher 1989; Prakash et al. 2000b). Therefore, irradiation was regarded as unsuitable for application to produce (Maxie and Abdel-Kader 1966; Yu et al. 1996; Osterholm and Potter 1997). Nevertheless, more recent research with lower radiation doses has suggested a role for irradiation as one of several "hurdles" in fruit and vegetable processing (Tauxe et al. 1997; Thayer and Rajkowski 1999).

Maintenance of fresh produce is limited to refrigeration and judicious use of modified atmosphere packaging (Sumner and Peters 1997). Unlike meat, fresh produce is living tissue, able to conduct respiration, preserve water relations with its environment, and continue synthesis of defense compounds and secondary metabolites (Salisbury and Ross 1984). Irradiation can modify these processes, leading to changes in firmness, aroma, color, or taste (Yu et al. 1996; Prakash et al. 2000b). Fresh and lightly processed vegetable products, as well as enzymatically active NTP juices, are sensitive to storage conditions of temperature, humidity, light levels, and packaging atmosphere. The upshots of irradiation on plant physiology and surface-associated microbial ecology are expressed during refrigerated handling and storage (Howard and Buescher 1989; Al-Kahtani et al. 2000; Prakash et al. 2000a).

14.8 Microflora of Minimally Processed Vegetable Products

Plant sections such as leaves, stems, fruits, and roots typically support 10^3–10^6 colony-forming units (CFUs) per gram of plant tissue (Sumner and Peters 1997; Mercier and Lindow 2000). The majority of these microorganisms consist of nonpathogenic bacteria that interact with the plant and with each other, often forming biofilms as part of the phytoplane ecology (Carmichael et al. 1999; Fett 2000). These organisms come from the environment in which the plants are nurtured, including the soil, water, air, and manure and compost, as well as from postharvest

handling, processing, and shipping (Beuchat 1996). Formation of bacterial biofilms on the inert surfaces of processing equipment such as steel, glass, rubber, and plastics is a well-known phenomenon (Costerton et al. 1995), but the widespread nature of the biofilm habitat on leaf and root surfaces has been illustrated only in current years (Fett 2000).

The biofilm habitat is a complex community of many bacterial species bound to the plant surface in a resistant exopolysaccharide matrix. Most of the details regarding this communal existence are still being defined, but it is known that the sheltered matrix environment allows for interspecies exchange of nutrients and metabolites and protects the resident bacteria from desiccation and exposure to antimicrobial chemical rinses (Morris et al. 1997; Fett 2000). The microbial populations on and in fruits and vegetables are carried into juices or fresh-cut products during processing (Parish and Higgins 1988; Sizer and Balasubramaniam 1999). Fresh-cut produce cannot be heat pasteurized without damage of quality.

Even if the majority of fruit and vegetable juices sold in the United States are pasteurized and therefore present little risk of food-borne pathogens, fresh NTP juices command a premium price for flavor and aroma. NTP juices also have a relatively short shelf life due to their microbial load (Buchanan et al. 1998).

The increasing significance of human pathogens on fresh fruits, vegetables, and juices has been recognized in current years (Beuchat 1996; Tauxe et al. 1997; Thayer and Rajkowski 1999). Like nonpathogens, pathogenic organisms come from the environments in which the plants are nurtured as well as from postharvest handling, processing, and shipping (Beuchat 1996). Due to the repeated international outbreaks of salmonellosis and enterohemorrhagic *Escherichia coli* O157:H7 infections associated with raw sprouts (Taormina et al. 1999), in 1999 the US Food and Drug Administration warned the public against consumption of this commodity. Salmonella and *E. coli* O157:H7 contamination of NTP juices (Cook et al. 1998; Buchanan et al. 1998) and contamination of consumed products such as lettuce, tomatoes, cabbage, and melons have led to an increased effort to address the problem of bacterial contamination of minimally processed fruits, vegetables, and juices.

14.9 The Case for Irradiation Processing

Increased consumption of fresh produce in the United States and increased globalization of the fresh produce market has come increasing worry over produce-associated food-borne illness (Beuchat 1996; Tauxe et al. 1997; Taormina et al. 1999; Thayer and Rajkowski 1999). Conventional antimicrobial procedures such as washing, chemical sanitization, thermal treatment, and modified atmosphere packaging have historically been developed and refined to suppress spoilage organisms efficiently (Luh 1997). Much research is dedicated to improving their efficiency against pathogenic bacteria such as *L. monocytogenes*, Salmonella, and *E. coli* (Beuchat 1998; Jacxsens et al. 1999; Liao and Sapers 2000).

It was long believed that pathogenic bacteria were not likely to be present in the internal of fruits and vegetables and that antimicrobial measures could reasonably be limited to surface disinfection. Nevertheless, it is increasingly recognized that leaves, fruits, and seeds provide bacteria with numerous mechanisms to avoid these antimicrobial measures. As more current research has shown, bacteria not only are likely to enter fruits and vegetables through natural openings (stomata, calyx, stem, stem scar, etc.), abiotic wounds, or phytopathogenic penetrations, but they also can survive within the produce for days or weeks (Buchanan et al. 1999; Fisher and Dolden 1998; Takeuchi and Frank 2000). The ramifications of internalization are reduction in the effectiveness of chemical treatments such as hot water, trisodium phosphate, chlorine, chlorine dioxide, and peroxyacetic acid (Zhuang and Beuchat 1996; Beuchat 1998; Pao and Davis 1999; Fett 2000).

The protective nature of bacterial biofilms also reduces the efficiency of antimicrobial measures (Morris et al. 1997; Fett 2000). Biofilms can vary in size and species composition, depending on the host plant species, the position of the leaf within the plant canopy, or their location on an individual leaf due to variations in substrate availability or microclimate suitability (Mercier and Lindow 2000; Fett 2000). Human pathogens such as *E. coli* and Salmonella are known to form resistant biofilms on industrial surfaces (Dewanti and Wong 1995; Korber et al. 1997). The participation of enteric bacteria, as well as the psychrotrophs *L. monocytogenes* and *Y. enterocolitica*, in pre-existing phytoplane biofilms formed by nonpathogenic bacteria is not acquainted (Fett 2000; Liao and Sapers 2000). By penetrating sheltered areas in the surface, subsurface, or interior of fruits and vegetables, ionizing radiation inactivates bacteria all over the leaf.

14.10 Irradiation of Juices

Conventional heat pasteurization is used for the overwhelming majority of juices sold in the United States; this pasteurization procedure results in the loss of essential oils and other juice components, changing the flavor of the resulting juice. Even if fresh, NTP juices are prized for premium flavor and aroma, these products have also been responsible for outbreaks of salmonellosis, enterohemorrhagic *E. coli* infection, hemolytic uremic syndrome, and other illnesses that have led to numerous hospitalizations and deaths. In response to these food-borne illnesses, the US Food and Drug Administration has implemented a policy requiring processors of NTP juices to proceed control plans and technologies that will provide 5-log (99.999%) reductions in human pathogen load. These regulations will take effect for all processors by 2004; within this time frame, nonthermal antimicrobial measures must be brought to commercial standards of reliability and cost efficacy if NTP juices are to be sold in the United States. A variety of nonthermal means of reducing the microbial load of NTP juices was recently discussed (Sizer and Balasubramaniam 1999). These comprise pulsed electric fields, minimal thermal processing, high-pressure processing, and ultraviolet radiation. Regulatory approval for the use of ionizing

radiation is recently being sought (NFPA 2000). These technologies are being researched to determine the most applicable means of reducing the risk of pathogen contamination of NTP juices while preserving the flavor and aroma characteristics that allow these products to command lower prices.

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Chapter 15
Fermented Vegetables

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15.1 Introduction

Fermentation of food and beverages is one of the oldest ways of food processing. A variety of foods and alcoholic beverages are produced naturally, i.e. by indigenous microorganisms or by using starter cultures. Every community in the world has its typical fermented food products. There are approximately 5000 varieties of major and minor unlisted fermented foods and beverages in the world. Traditionally, the largest varieties of ethnic fermented foods and beverages can be found in the Chinese, Indian and African population. Estimations suggest that about 20% of the total food consumed across the world is fermented food.

Fermented fruits and vegetables have an important role in feeding the world's population on every continent today (Panda et al. 2005). They play an important role in preservation and production of wholesome nutritious foods in a wide variety of flavours, aromas and textures which enrich the human diet and remove anti-nutritional factors to make the food safe to eat. Fermentation serves many benefits,

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which include food security, improved nutrition and better social well-being of the people living in marginalized and vulnerable society (Montet et al. 2006).

The definition of food fermentation varies according to different interpretations of food. Some examples are given below:

- Fermented foods are foods that are fermented till at least one of the constituents has been subjected to the action of microorganisms for a period so that the final products have often undergone considerable changes in chemical composition and other aspects due to microbial and enzymatic changes (van Veen 1967).
- Indigenous fermented foods are foods where microorganisms bring about some biochemical changes in the substrates during fermentation, such as the enrichment of the human diet through the development of a wide variety of flavours, aromas and textures; the preservation of foods through lactic acid, alcoholic, alkaline or acetic acid fermentation; the enrichment of food substrates biologically with proteins, essential fatty acids, vitamins and essential amino acids; and the decrease in cooking times and fuel requirements (Steinkraus 1994).
- Fermented foods are those foods that have been subjected to the action of microorganisms or enzymes so that desirable biochemical changes cause significant modification of the food and the direct consumption of fungal fruit bodies and mushrooms (Campbell-Platt 1987).
- Fermented foods are palatable and wholesome foods prepared from raw or heated raw materials by microbial fermentation (Holzapfel 1989).
- Ethnic, fermented foods are foods produced by ethnic people using their native knowledge from locally available raw materials of plant or animal sources either naturally or by adding starter cultures containing functional microorganisms that modify substrates biochemically and organoleptically into edible products that are culturally and socially acceptable to the consumers (Tamang 2015).

Fermentation of foods requires several essential elements: composition of the food, microorganisms and water. Fermentation itself is part of a larger manufacturing process. Unit operations in the fermentation process include:

- Physical operations, e.g. transport, grading, sorting, cleaning, washing, separations, size reductions and mixing
- Bioprocessing operations carried out by the microorganisms and the enzymes, e.g. uptake and removal of substrates, production and degradation of metabolites, synthesis of complex substances, biomass production and enzymatic modification of food components
- Thermal operations, e.g. cooling, heating and freezing

Among the microorganisms involved in the food fermentations, all groups of bacteria, yeast and moulds are encountered (Rhee et al. 2011). Among the bacteria, lactic acid bacteria are the most widely used at home scale and industrial processing of fermented dairy, meat, vegetable and cereal food products. The most important yeast used is *Saccharomyces cerevisiae*, mainly involved in alcoholic beverages and bread leavening. The moulds used in fermented foods are mainly *Aspergillus* and *Penicillium* sp., and some *Mucor* sp. are involved in the production of wine, cheese and soybean-derived foods.

Lactic acid bacteria (LAB) are a group of bacteria with similar morphological, metabolic and functional characteristics. The bacteria in this group can be divided in about 20 different genera, based on their morphology, mode of glucose fermentation, configuration of the formed lactic acid, growth at different temperatures, salt concentrations and alkaline or acid tolerance. The general description of the bacteria in the group is gram-positive, non-sporing, non-respirating cocci of rods, with lactic acid as the major end product formed during the fermentation of carbohydrates. They can grow anaerobically, but most of LAB can grow in the presence of oxygen (aerotolerant). As they obtain energy mainly from their metabolism of sugars, their growth is restricted to environments where sugars are present. They have a complex nutritional requirement since they cannot synthesize themselves many amino acids, vitamins, purines and pyrimidines. Therefore, LAB are generally associated with habitats rich in nutrients, e.g. dairy products, meat products, beverages and vegetables.

Only the genera *Lactobacillus* and *Carnobacterium* are rods, while the other genera are all cocci. The genus *Weissella* can contain both rods and cocci.

Within the lactic acid bacteria, one can distinguish between two main sugar fermentation pathways: homolactic versus heterolactic fermentation. The main aim of LAB metabolism is the efficient fermentation of carbohydrates coupled to substrate-level phosphorylation (ATP production). The ATP generated is used for biosynthetic purposes. LAB are characterized by their enormous capacity to degrade different carbohydrates. Lactic acid is the predominant end product of carbohydrate fermentation. However, other end products can be formed.

Homofermentative bacteria produce only one end product and that is lactate. Heterofermentative bacteria produce lactic acid, ethanol, acetic acid and CO_2.

Leuconostoc cells are spherical to lenticular, appear in pairs or chains and are non-motile, non-sporulating cells. They are facultative anaerobic. *Leu. mesenteroides* spp. *cremoris* and *Leu. lactis* are used in dairy and vegetable fermentations.

Moulds are in general multicellular, filamentous fungi. The filaments or hyphae contain nuclei. In food production, beneficial effects are known from species of *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus*. It is important that these strains do not produce mycotoxins while used in food fermentations, especially when selected as starter. Typically strains used are *Aspergillus oryzae* in oriental fermented foods, e.g. sake, soy sauce and miso.

Rhizopus oligosporus is used in fermentation of tempe, a fermented soybean product based in Indonesia. It is highly nutritious (high protein content, 30–40% protein on dry matter) and easily digestible aiming for a meat replacer.

The most common fermented vegetables in Europe and in the USA are sauerkraut, cucumbers and olives. In Korea, the most traditional fermented vegetable food is kimchi. But also other vegetables are fermented in Asia, e.g. dhamuoi in Vietnam, dakguadong in Thailand and burong mustasa in the Philippines. We will examine in this chapter fermented vegetables both from Europe and Asia.

In general, fermented vegetables are produced by placing vegetables in a solution of sodium chloride. Normally fermentation of vegetables does not use starter cultures; however, it is the result of the presence of naturally occurring

microorganisms at the appropriate environmental conditions (pH, temperature, salt concentration). During fermentation, lactic acid is produced as the major fermentation product. The salt allows the lactic acid bacteria to grow by extracting liquid from the vegetables that is used as a substrate for growth, while on the other hand the growth of undesirable spoilage microorganisms is inhibited by the salt.

15.2 Soybean Tempe and Other Soybean Paste Products

Tempe is an Indonesian fermented soybean food product produced by the growth of *Rhizopus oligosporus* (Nout and Rombouts 1990). Tempe involves two distinct fermentations. The first which takes place is bacterial and results in acidification which prevents growth of *Bacillus cereus*.

The second is fungal and results in growth on the bean cotyledons with *R. oligosporus* mycelium (Varzakas 1998).

The purpose of the fermentation is not as much to enhance preservation, but rather the modification of ingredients and an increase of the nutritional properties because of enzymatic activity (Nout and Kiers 2005).

Tempe, a *Rhizopus* ssp.-fermented soybean food product, was investigated for bacteriostatic and/or bactericidal effects against cells and spores of the food-borne pathogen *Bacillus cereus*.

Tempe extract showed a high antibacterial activity against *B. cereus* ATCC 14579 based on optical density and viable count measurements.

This growth inhibition was manifested by a 4 log (CFU ml^{-1}) reduction, within the first 15 min of exposure. Tempe extracts also rapidly inactivated *B. cereus* spores upon germination. Viability and membrane permeability assessments using fluorescence probes showed rapid inactivation and permeabilization of the cytoplasmic membrane confirming the bactericidal mode of action. Cooked beans and *Rhizopus* grown on different media did not show antibacterial activity, indicating the unique association of the antibacterial activity with tempe. Subsequent characterization of the antibacterial activity revealed that heat treatment and protease addition nullified the bactericidal effect, indicating the proteinaceous nature of the bioactive compound (Roubos-van den Hil et al. 2010).

Doenjang

Doenjang is a Korean traditional soybean paste popularly consumed as a condiment for vegetables, fish and meats or used as a seasoning ingredient in authentic Korean cuisine. The paste has received considerable attention because of numerous reported beneficial human health effects, including antioxidant, fibrinolytic, antimutagenic and anticancer properties (Kim 2004; Yun 2005; Jung et al. 2006; Park et al. 2008; Namgung et al. 2009; Kwon et al. 2010; Tamang et al. 2016).

Doenjang samples were prepared in triplicate, and their microbial abundance, bacterial communities and metabolites throughout fermentation were analysed to investigate the functional properties of microorganisms in *doenjang*. Viable bacterial

cells were approximately three orders of magnitude higher than fungal cells, suggesting that bacteria are more responsible for *doenjang* fermentation. Pyrosequencing and proton nuclear magnetic resonance spectroscopy were applied for the analysis of bacterial communities and metabolites, respectively. Bacterial community analysis based on 16S rRNA gene sequences revealed that *doenjang* samples included *Bacillus*, *Enterococcus*, *Lactobacillus*, *Clostridium*, *Staphylococcus*, *Corynebacterium*, *Oceanobacillus* and *Tetragenococcus*. These genera were found either in *doenjang-meju* or solar salts, but not in both, suggesting two separate sources of bacteria. *Bacillus* and *Enterococcus* were dominant genera during the fermentation, but their abundances were not associated with metabolite changes, suggesting that they may not be major players in *doenjang* fermentation. *Tetragenococcus* was dominant in 108-day *doenjang* samples, when lactate, acetate, putrescine and tyramine increased quickly as glucose and fructose decreased, indicating that *Tetragenococcus* might be primarily responsible for organic acid and biogenic amine production. *Lactobacillus* was identified as a dominant group from the 179-day samples, associated with the increase of gamma-aminobutyric acid (GABA) and the decrease of galactose, indicating a potential role for this genus as a major GABA producer during fermentation. The results of this study clarified the functional properties of major bacterial communities in the *doenjang* fermentation process, contributing to the production of safe and high-quality *doenjang* (Jung et al. 2016).

In Korea, traditional *doenjang* is typically made by further fermentation of the solid parts from a fermented mixture of *doenjang-meju* (fermented soybean bricks) and brine. The additional fermenting procedure also suggests that the microbial community and indigenous enzymes in *doenjang-meju* are likely important in determining the microbial community and metabolite change during *doenjang* fermentation. However, no research exists on how *doenjang* microbial communities alter when *doenjang-meju* with known microbial community composition is used.

Salt is an essential seasoning used for making traditional Korean fermented foods such as kimchi (fermented vegetables), doenjang (fermented soybean paste), ganjang (a soy sauce made with fermented soybean) and jeotgal (fermented seafood). Salt inhibits growth of spoilage microorganisms, allows selective growth of salt-tolerant organisms and contributes to the development of flavours and extension of shelf life.

Samples of doenjang (a fermented soybean paste) were prepared with different types of salts: purified salt (PS), 3-year-aged solar salt (SS3), 1-year-aged solar salt (SS1) and bamboo salt (BS, third processing product). For starter doenjang samples, selected starters comprising two bacilli, one yeast and one fungus were inoculated, whereas for non-starter doenjang samples, microorganisms present in rice straw were inoculated after enrichment. The doenjang samples were fermented for 13 weeks at 25 °C. During the fermentation period, SS and BS doenjang samples showed higher bacilli counts as well as much lower yeast counts than PS doenjang. At 13 weeks, yeast counts of starter doenjang samples were 7.75, 5.69, 6.08 and 4.74 log CFU/g for PS, SS3, SS1 and BS doenjang, respectively. For non-starter doenjang samples, counts were 7.17, 5.05, 5.92 and 4.54 log CFU/g for PS, SS3, SS1 and BS doenjang, respectively. SS and BS promoted growth of bacilli but

inhibited growth of yeasts compared with PS. *Debaryomyces hansenii* was the dominant yeast in PS doenjang, whereas *Candida guilliermondii* and *Pichia sorbitophila* were dominant in SS and BS doenjang. In the sensory evaluation, SS and BS doenjang scored better than PS doenjang (Shim et al. 2016).

Doenjang, a traditional Korean fermented soybean paste, is made by mixing and ripening meju with a high-salt brine. Meju, a naturally fermented soybean product, is prepared by soaking, steaming, crushing and moulding the soybean into blocks and allowing it to ripen for 1–2 months. The ripened meju is a source of naturally occurring microorganisms and enzymes that degrade macromolecules in the soybean block. Meju supplies nutrients, flavours, enzymes and microorganisms not only in the production of doenjang but also in other traditional Korean fermented seasonings including ganjang (soy sauce) and gochujang (hot pepper paste).

To select starters for the production of meju and doenjang, traditional Korean fermented soybean foods, Jeong et al. (2016) assessed the safety and technological properties of their predominant isolates, *Staphylococcus saprophyticus*, *Staphylococcus succinus* and *Staphylococcus xylosus*. Phenotypic antibiotic resistance, haemolysis and biofilm formation were strain specific. None of the *S. succinus* isolates exhibited antibiotic resistance or haemolytic activities.

Thirty-three selected strains, identified through safety assessments of 81 coagulase-negative staphylococci (CNS) isolates, produced cadaverine, putrescine and tyramine, but not histamine, in the laboratory setting. The production of these three biogenic amines may, however, be insignificant considering the high levels of tyramine produced by the control, *Enterococcus faecalis*. The 33 CNS strains could grow on tryptic soy agar containing 21% NaCl (w/v), exhibited acid-producing activity at 15% NaCl and expressed strain-specific protease and lipase activities.

S. succinus 14BME1, the selected starter candidate, produced significant amounts of benzeneacetic acid, 2,3-butanediol, trimethylpyrazine and tetramethylpyrazine through soybean fermentation.

Meju is a fermented starter for the production of Korean traditional fermented soybean products (doenjang, ganjang and gochujang). Meju is often contaminated with OTA because of the growth of ochratoxigenic fungi. In the present study by Cho et al. (2016), two strains of OTA-biodegrading fungi were isolated from traditional Korean meju samples. The fungal strains were identified and examined for their OTA-biodegradation activities.

A total of 130 fungal isolates obtained from 65 traditional Korean meju (a fermented starter for fermentation of soybeans) samples were examined for OTA-biodegradation activity using thin-layer chromatography.

Both *A. tubingensis* strains degraded OTA by more than 95.0% after 14 days, and the HPLC analysis showed that the OTA biodegradation by the *A. tubingensis* strains led to the production of ochratoxin α, which is much less toxic than OTA. Moreover, crude enzymes from the cultures of *A. tubingensis* M036 and M074 led to OTA biodegradation of 97.5% and 91.3% at pH 5 and 80.3% and 75.3% at pH 7, respectively, in a buffer solution containing OTA (40 ng/ml) after 24 h. In addition, the OTA-biodegrading fungi did not exhibit OTA production activity. Our data suggest that *A. tubingensis* isolates and their enzymes have the potential for practical application to reduce levels of OTA in food and feed.

Kanjang

Kanjang (Korean fermented soy sauce) is an important fermented soy food and is widely consumed in Korea as a condiment, dip sauce and flavouring for many types of foods. Although kanjang is considered a traditional food, there have been only a few reports related to its chemical constituents and pharmacological properties (Song et al. 2014; Thom and Wollan 1997). Kanjang was shown to exert anti-colitic effects partially by decreasing the serum levels of pro-inflammatory cytokines and inhibiting the mRNA expression of these factors in the colon tissue of mice treated with 2% dextran sulphate sodium (Song et al. 2014).

Kanjang (Korean soy sauce) is a byproduct of the production of the Korean fermented soybean. In the present study, seven indole alkaloid derivatives were isolated from methanol extract of kanjang. Their structures were identified as 1-propyl-1,2,3,4-tetrahydro-b-carboline-3-carboxylic acid (1), 1-methyl-1,2,3,4-tetrahydro-b-carboline-3-carboxylic acid (2), 1-methyl-1,2,3,4-tetrahydro-b-carboline-1-carboxylic acid (3), 3-indoleacetic acid (4), Nb-acetyltryptamine (5), 1-methyl-3,4-dihydro-b-carboline (6) and flazine (7) by NMR and MS analyses. Preliminary screening for anti-neuroinflammatory effects of isolated indole alkaloids in lipopolysaccharide (LPS)-stimulated BV2 cells revealed that these compounds inhibited the production of nitric oxide and prostaglandin E2. For the subsequent investigation of anti-neuroinflammatory action of these metabolites, compounds 4 and 7 were selected, and the results revealed that these inhibitory effects correlated with the suppressive effect of 4 and 7 on inducible nitric oxide synthase and cyclooxygenase-2 expression in LPS-stimulated BV2 cells. In regard to the mechanism of the anti-inflammatory effect, 4 and 7 significantly inhibited the nuclear factor kappa B pathway (Kim et al. 2016).

Kinema

Kinema, an ethnic fermented, non-salted and sticky soybean food is consumed in the eastern part of India. The stickiness is one of the best qualities of good *kinema* preferred by consumers, which is due to the production of poly-g-glutamic acid (PGA). Average load of *Bacillus* in *kinema* was 107 cfu/g and of lactic acid bacteria was 103 cfu/g. *Bacillus* spp. were screened for PGA production, and isolates of lactic acid bacteria were also tested for degradation of PGA. Only *Bacillus* produced PGA; none of lactic acid bacteria produced PGA. PGA-producing *Bacillus* spp. were identified by phenotypic characterization and also by 16S rRNA gene sequencing as *Bacillus subtilis*, *B. licheniformis* and *B. sonorensis* (Chettri et al. 2016).

Ethnic people of Northeast India consume spontaneously fermented soybean foods as side dish in meals, which include *kinema*, *tungrymbai*, *hawaijar*, *bekang*, *aakhone* and *peruyaan* (Tamang 2015). *Kinema* is a naturally fermented, sticky, mild ammoniacal flavour and non-salted soybean food of Sikkim and Darjeeling in India, east Nepal and west Bhutan. It is similar to *natto* of Japan and *chungkokjang* of Korea. PGA is produced by *Bacillus* spp. in many Asian fermented soybean products giving the characteristic of a sticky texture to the product (Nishito et al. 2010) such as *natto* of Japan (Nagai 2012; Kada et al. 2013), *chungkokjang* of Korea (Lee et al. 2010), *tungrymbai* and *bekang* of India (Chettri and Tamang 2014) and *thaunao* of Thailand (Chunhachart et al. 2006).

15.2.1 Soybean Meal

Soybean meal (SBM), a commonly used protein source for animal feed, contains anti-nutritional factors such as trypsin inhibitor, phytate and oligosaccharides, among others, which limit its utilization.

Microbial fermentation using bacteria or fungi has the capability to improve nutritional value of SBM by altering the native composition. Both submerged and solid state fermentation processes can be used for this purpose. Bacterial and fungal fermentations result in degradation of various anti-nutritional factors, an increase in amount of small-sized peptides and improved content of both essential and non-essential amino acids. However, the resulting fermented products vary in levels of nutritional components as the two species used for fermentation differ in their metabolic activities.

Compared to SBM, feeding non-ruminants with fermented SBM has several beneficial effects including increased average daily gain, improved growth performance, better protein digestibility, decreased immunological reactivity and undesirable morphological changes like the absence of granulated pinocytotic vacuoles (Mukherjee et al. 2016).

15.2.2 Miso

Miso is fermented soybean paste, a traditional Japanese seasoning produced from steamed soybean, salt and koji malt (cooked cereal or soybean malted with *Aspergillus oryzae*) and is ripened for 3–24 months (Fukushima et al. 1987). During the ripening process, diverse microorganisms such as moulds, yeasts and lactic acid bacteria hydrolyse and ferment the soybean components. The characteristic flavour of miso is derived not only from the fermentation but also the several reactions that occur among the components during ripening, principally the Maillard reaction. Miso is classified into several types according to the type of koji malt used and the period of ripening.

Thin-coloured salty rice miso (white miso; popular in eastern Japan) and red salty rice miso (red miso; popular in Northern Japan) are made using rice koji, and their ripening periods are typically 3–6 and 6–12 months, respectively. In contrast, soy miso (all soybean, no cereal; Mame miso; popular in the middle region of Japan), which is exemplified by Hatcho miso, is prepared using soybean koji and is ripened over 2 years (Nikkuni and Ebine 1999).

Because the aroma of miso determines its quality, the volatile compounds present in miso have been extensively studied. Sugawara and Yonekura (1998) analysed the aroma concentrates of several raw miso types using a method involving a porous polymer column and reported that the amounts of 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone (HEMF), methionol and 4-ethylguaiacol differed among miso types made using distinct types of koji (Sugawara and Yonekura 1998).

Inoue et al. (2016) identified the key odorants of cooked soy miso and their influence on umami aftertaste perception. Volatile compounds of soy miso and two rice misos were prepared using simultaneous distillation-extraction, and the key odorants were identified by using the gas chromatography-olfactometry/aroma extract dilution assay approach, and soy miso was compared with rice misos. Forty-one aroma-active compounds were detected in cooked soy miso, and malty, green, roasty and sulphury aromas were identified as the characteristic aromas. Finally, sensory evaluation was conducted to assess the contribution to the umami aftertaste of six key compounds with the highest flavour dilution factor. Results revealed that dimethyl trisulfide, which was newly identified in cooked miso, contributes to the umami aftertaste and palatability of cooked soy miso.

Soymilk and miso, a fermented soybean product originated from Japan, are traditional soy foods consumed by Asian people. They contain several isoflavones, including daidzin, genistin, daidzein and genistein, which have shown a variety of beneficial pharmacological activities, such as chemoprevention (Romagnolo and Selmin 2012; Zhao and Mu 2011), estrogenicity (Choi et al. 2008; Vitale et al. 2012) and anti-inflammation (Blay et al. 2010; Khan et al. 2012).

P-glycoprotein (P-gp), one of the ATP-binding cassette (ABC) drug transporters, is expressed on the apical membrane of several tissues, including the intestines, liver, kidney and brains; it mediates the inside-out efflux of a number of different endogenous compounds and xenobiotics (König et al. 2013).

P-glycoprotein (P-gp) and CYP3A4 both play very important roles in drug bioavailability, resistance and interactions. In vitro studies indicated that P-gp function was activated by many isoflavones. This study by Yu et al. (2014) investigated the in vivo effects of soymilk and miso, isoflavone-rich soy foods, on P-gp and CYP3A by tracing the pharmacokinetics of cyclosporine (CSP), a probe drug of P-gp. Rats were orally administered CSP with and without soymilk or miso. A specific monoclonal fluorescence polarization immunoassay was used to determine the blood concentration of CSP. The results showed that soymilk and miso significantly decreased the Cmax of CSP by 64.5% and 78.3% and reduced the AUC0–540 by 64.9% and 78.3%, respectively. Mechanism studies revealed that the activities of P-gp and CYP3A4 were induced by soymilk and miso. In conclusion, ingestion of soymilk and miso significantly activated the functions of P-gp and CYP3A.

15.3 Sauerkraut

LA fermentation of cabbage to produce sauerkraut has been widely studied for many years (Pederson and Albury 1969; Stamer et al. 1971; Yildiz and Westhoff 1981).

Sauerkraut means sour cabbage. In sauerkraut fermentation, fresh cabbage is shredded and mixed with 2.3–3.0% salt before allowing for natural fermentation. Sauerkraut production typically relies on a sequential microbial process that involves heterofermentative and homofermentative LAB, generally involving *Leuconostoc* spp. in the initial phase and *Lactobacillus* spp. and *Pediococcus* spp. in

the subsequent phases (Lee et al. 2005). The pH of final product varies from 3.5 to 3.8 (Gardner et al. 2001). At this pH, the cabbage or other vegetables will be preserved for a long period of time (Steinkraus 1997).

Sauerkraut brine is an important byproduct of the cabbage fermentation industry and can be used as a substance for the production of carotenoids by *Rhodotorula rubra* or for β-glucosidase production by *Candida wickerhamii* for commercial applications (Yoon et al. 2006).

The production of sauerkraut is the result of a natural lactic acid fermentation of fresh shredded cabbage, mixed with dry salt placed in a crock or wooden tub. The tub is covered with a heavy lid, and fermentation takes place at temperatures below 15 °C for 1 month. The heads of the cabbages are washed, and outer and defective leaves are removed, as well as the core is removed. The leaves are shredded to ensure a larger total surface area and to allow extraction of juice. Dry salt is added to the shredded cabbage at a concentration of 2.25–2.5%. The salt, together with the packing of the leaves, extracts the liquid from the leaves, which is the substrate for the growth of lactic acid bacteria. The conditions should be as anaerobic as possible. In this way, outgrow of spoilage microorganisms is prevented. When the cabbage is properly shredded and salted, fermentation takes place which is a sequence of lactic acid bacteria resulting in the distinctive flavour of sauerkraut. The fermentation starts with 10^6 CFU/g aerobic microorganisms, 10^6 CFU/g *Enterobacteriaceae* and less than 10^2 CFU/g yeasts and moulds. During the first days of fermentation, less acid-tolerant lactic acid bacteria dominate, while later the acid-tolerant lactic acid bacteria predominate. Each of these populations reaches concentrations of 10^8–10^9 CFU/g. In the early fermentation, *Leuconostoc mesenteroides* is the major species present, producing lactic acid, acetic acid and CO_2. In this stage, the balance between lactic and acetic acid is important, and the production of flavour volatiles is dictated. Due to the organic acid production, pH drops rapidly, limiting the outgrowth of undesirable microorganisms and inhibiting activities of some enzymes. Due to the CO_2 production, residual oxygen is flushed out, making the environment more anaerobic and thus stimulating the growth of the lactic acid bacteria. Then *Lactobacillus brevis* and *Pediococcus cerevisiae* grow, resulting in a further acid production. Finally, *Lactobacillus plantarum* is the major bacterium involved in the final fermentation stage, allowing the pH to drop below 4 (Fig. 15.1).

After fermentation, the brine is separated from the cabbage, boiled and poured hot over the cabbage shreds in jars. Or when packed in cans, the cans are filled with hot juice, exhausted and processed till the temperature of the centre in the can reaches 82 °C. In this way the cabbage can be stored in anaerobic conditions for a long time.

The sauerkraut flavour is affected by the acidity, salt concentration and the volatile organic compounds. A good-quality sauerkraut has an optimal titratable acidity of 1–1.5% (expressed as lactic acid).

Several spoilage problems are known to affect sauerkraut, such as discoloration, loss of acidity, off-flavour and odours, slime, softened texture and pink coloured. All these defects are due to the aerobic growth of moulds and yeasts. Slimy or ropy sauerkraut is due to the dextran formation by *Leuconostoc mesenteroides* (Pederson 1979; Yildiz and Westhoff 1981).

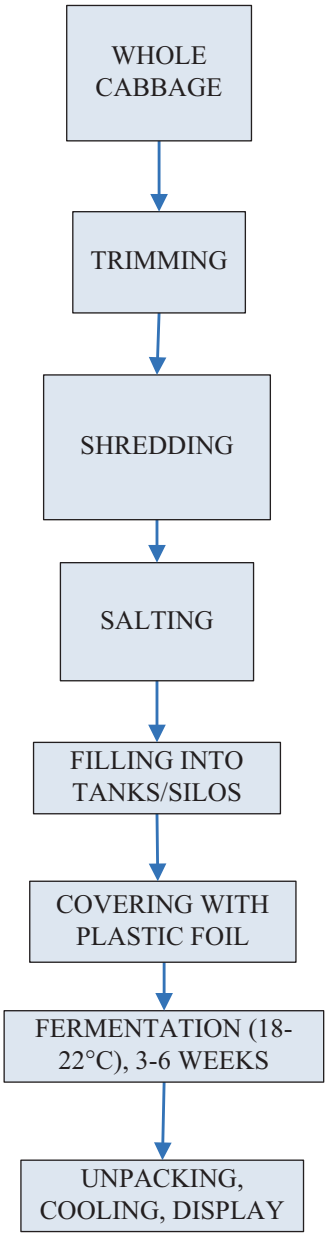

Fig. 15.1 Flow diagram of sauerkraut fermentation

Spontaneous fermentations, such as the one occurring during sauerkraut production, rely on autochthonous lactic acid bacteria (LAB) present on the raw substrate. Characterization and control of these autochthonous microorganisms are essential for the sensory quality and the safety of the spontaneously fermented sauerkraut. The fermentation processes involve mixed cultures of LAB, yeast and

fungi, and as such traditional fermented foods are a plentiful source of starter microorganisms, with some of them exerting even probiotic characteristics. Still, the research of these vegetables, especially sauerkraut as source for probiotic microorganisms, is rather scarce compared with their dairy counterpart (Tamminen et al. 2004; Sánchez et al. 2005; Madrau et al. 2006; Kos et al. 2008; Lebos Pavunc et al. 2011, 2012).

Spontaneous sauerkraut fermentation was performed at industrial scale in "Prehrana Inc.", Varazdin in order to select autochthonous lactic acid bacteria (LAB) which were evaluated according probiotic criteria and tested for their capacity as probiotic starter cultures. At the end of the spontaneous sauerkraut fermentation, total LAB counts reached 9.0×10^5 CFU/ml. This underlines that the need for addition of the well-characterized probiotic cultures, in appropriate viable cell counts, would be valuable in probiotic sauerkraut production. Phenotypic characterization through API 50 CHL and SDS-PAGE of cell protein patterns revealed that *Lactobacillus plantarum* is predominant LAB strain in homofermentative phase of fermentation. Autochthonous LAB isolates SF1, SF2, SF4, SF9 and SF15 were selected based on the survival in in vitro gastrointestinal tract conditions. RAPD fingerprints indicated that the selected autochthonous LAB were distinct from one another. All of the strains efficiently inhibited the growth of indicator strains and satisfied technological properties such as acidification rate, tolerance to NaCl and viability during freeze-drying. Strains *Lb. paraplantarum* SF9 and Lb. brevis SF15, identified by AFLP DNA fingerprints, have shown the best properties to be applied as probiotic starter cultures, because of their highest adhesion to Caco-2 cells and expression of specific, protective S-layer proteins of 45 kDa in size. With addition of these strains, probiotic attribute of the sauerkraut will be achieved, including health-promoting, nutritional, technological and economic advantages in large-scale industrial sauerkraut production (Beganovic et al. 2014).

It was reported that *Leu. mesenteroides*, a heterofermentative LAB, was a starter in spontaneous fermentation of sauerkraut, and *L. plantarum*, a homofermentative LAB, terminated the fermentation of sauerkraut (Fleming et al. 1985; Plengvidhya et al. 2004).

The characteristics of Chinese sauerkraut fermented by *Leuconostoc mesenteroides* NCU1426, *Lactobacillus plantarum* NCU1121 and binary coculture (*Leu. mesenteroides* NCU1426-*L. plantarum* NCU1121) were studied by Xiong et al. (2014). The mixture of materials was sterilized firstly and then was fermented for 7 days in the inoculation with lactic acid bacteria. The pH value and number of viable cells of lactic acid bacteria in the brine were monitored during the fermentation. The changes in the concentrations of substrates and products in the three fermentations were analysed by high-performance liquid chromatography. The study has determined the characteristics of Chinese sauerkraut fermentation in pure culture fermentation with either *Leu. mesenteroides* NCU1426 or *L. plantarum* NUC1121 and by binary coculture as well as the interaction among the two lactic acid bacteria used.

Feng et al. (2015) selected strains of lactic acid bacteria (LAB) by their in vitro adhesive and immunomodulatory properties for potential use as probiotics.

In this study, 16 randomly selected LAB strains from fermented vegetables (sauerkraut, bean and cabbage) were first screened for their tolerance to acid, bile salts, pepsin and pancreatin, bacterial inhibitory activities and abilities to adherence to Caco-2 cells. Then, four strains with the highest adhesion abilities were selected for further studies of their immunomodulatory properties and inhibitory effects against *Salmonella* adhesion and invasion to Caco-2 cells in vitro. The results showed that these 16 LAB strains effectively survived in simulated gastrointestinal condition and inhibited growth of six tested pathogens. *Lactobacillus rhamnosus* P1, *Lactobacillus plantarum* P2, *Lactobacillus rhamnosus* P3 and *Lactobacillus casei* P4 had the highest abilities to adhere to Caco-2 cells. Furthermore, *L. plantarum* P2 strain showed higher abilities to induce expression of tumour necrosis factor-a and interleukin 12 by splenic monocytes and strongly inhibited the adhesion and invasion of S. enteritidis ATCC13076 to Caco-2 cells.

These results suggest that *Lactobacillus* strains P2 could be used as a probiotic candidate in food against Salmonella infection.

Chinese sauerkraut (also known as "PaoCai"), a kind of sound fermented vegetable product, is widely consumed in China. Changes of lactic acid bacteria flora throughout spontaneous fermentation of Chinese sauerkraut were analysed in this study. Results have shown that *Enterococcus faecalis*, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus zeae* dominated the fermentation. *E. faecalis* and *L. mesenteroides* subsp. *mesenteroides* were present in the brine as soon as the vegetable was pickled, while other four species were isolated successively during fermentation. *E. faecalis* and *L. lactis* subsp. *lactis* were mainly present in the early stage of fermentation and died at the later stage; *L. mesenteroides* subsp. *mesenteroides* did not die until the fifth day; *L. zeae* existed in the middle stage and disappeared at the fifth and a half day. *L. plantarum* and *L. casei* dominated the final stage of fermentation. In summary, the fermentation process was initiated by *L. mesenteroides* subsp. *mesenteroides*, followed by *E. faecalis*, *L. lactis* and *L. zeae* and finally succeeded by *L. plantarum* and *L. casei* (Xiong et al. 2012).

Probiotic strain *Lactobacillus plantarum* L4 and strain *Leuconostoc mesenteroides* LMG 7954 were applied for the controlled fermentation of cabbage heads. The parameters of the controlled and spontaneous fermentations, including antimicrobial effect of cabbage brines obtained at the end of both fermentations, were monitored. To check out the influence of starter culture strains, ten randomly chosen lactic acid bacteria, isolated at the end of controlled cabbage heads fermentation, were identified by API 50CHL test, and the presence of the probiotic culture was confirmed by pulsed-field gel electrophoresis. The starter cultures applied for cabbage heads fermentation allowed lowering of NaCl concentrations from 4.0% to 2.5% (w/v), considerably accelerated fermentation process by 14 days and improved the product quality. The produced sauerkraut heads are considered probiotic product as viable probiotic cells count in final product was higher than 10^6 colony-forming units (CFU) per gram of product (Beganovic et al. 2011).

White cabbage (*Brassica oleracea* L. var. capitata cv. Bronco) was fermented, at 0.5% and 1.5% NaCl, using *Lactobacillus plantarum* or *Leuconostoc mesenteroides*

as starter cultures, and, subsequently, sauerkraut was stored at 4 °C for 3 months. Microbial populations and six biogenic amines (putrescine, cadaverine, histamine, tyramine, spermine and spermidine) were investigated by Penas et al. (Peñas et al. 2010). Fermentation and storage increased aerobic mesophilic bacteria and LAB populations in sauerkrauts, and this was accompanied by a rise in biogenic amine content. *L. plantarum* sauerkrauts produced with 0.5% NaCl had the highest microbial counts, while no differences between salt contents were found with *L. mesenteroides*. Total biogenic amine amount was lower at 0.5% NaCl than at 1.5% in both induced fermentations, and *L. mesenteroides* produced a lower content than did *L. plantarum*. Spermidine was the major contributor to the total biogenic amine content, followed by putrescine, while histamine was present at the lowest level. The individual and total biogenic amine levels in the experimental sauerkrauts stored at 4 °C for 3 months were below the upper limits reported in the literature for fermented products, indicating good quality and safety of the sauerkrauts. *L. mesenteroides* starter and 0.5% NaCl were the optimal fermentation conditions for producing sauerkrauts with the lowest biogenic amine contents.

The contents of free amino acids and biogenic amines in spontaneously fermented sauerkraut, inoculated or not with specific lactic acid bacterium strains, were monitored throughout 45 days of storage. The strains tested were *Lactobacillus plantarum* 2142, *Lactobacillus casei* subsp. *casei* 2763 and *Lactobacillus curvatus* 2771. In both the control and the experiments, the total amino acid contents increased with time – and the predominant ones were aspartic acid, arginine and glutamic acid. However, upon inoculation with either strains, the total biogenic amine contents remained considerably lower than those of the control (especially in the cases of *L. casei* subsp. *casei* and *L. curvatus*); every single biogenic amine was actually below the 100 ppm threshold. The dominant biogenic amines in the control were putrescine – and tyramine and histamine, to a lesser extent; the putrescine content was tenfold lower if inoculation had taken place with either *Lactobacillus* tested; and histamine and tyramine were essentially absent during storage, whereas they ranked above 200 ppm in the control by 45 days. Hence, an efficient food-grade biological tool was made available that constrains build-up of dangerous biogenic amines in fermented vegetables during storage (Rabie et al. 2011).

15.4 Fermented Olives

Table olives are the fermented fruit of the *Olea europaea* L., which is traditionally cultivated in the Mediterranean countries (Spain, Italy, Greece and Turkey, mainly) and, more recently, in America, Australia and the Middle East (IOC 2012). Table olives are an important component of the Mediterranean diet with potential beneficial effects on human health due to all of the above-described antioxidant properties of phenolic compounds (Corsetti et al. 2012). Immediately after harvesting, olives are inedible due to their high amount of oleuropein, a phenolic compound with a bitter taste (Garcia et al. 2004). Several traditional protocols are used for the

treatment of table olives, and these include the Spanish- and Greek-type protocols, which are universally adapted to the production of table olives. Based on the Spanish-type protocol, olives are treated with 2–3% NaOH aqueous solution to reduce their bitterness by the hydrolysis of polyphenol compounds. After washing in water, the olives are brined with an initial concentration of 8–12% NaCl and allowed to ferment for 30–60 days at room temperature (Garcia et al. 2004). Based on the Greek-type protocol, olives are directly brined and allowed to ferment for 8–12 months until they lose their bitterness. Overall, the fermentation period depends on the type of cultivar, NaCl content and temperature (Tassou et al. 2002; Arroyo-Lopez et al. 2008; Panagou et al. 2008). The flow diagram of fermented fresh black olives in brine is shown in Fig. 15.2.

In general, the fermentation process of table olives is carried out by endogenous lactic acid bacteria and yeasts without the addition of starters (Heperkan 2013; Corsetti et al. 2012).

The olive is the fruit of the olive tree, characterized by its bitter taste (due to the presence of oleuropein), low sugar content and high oil content. Due to these characteristics, olives cannot be consumed without a treatment. There are many methods to process the olives; however, only three are of importance from an economical point of view: the Spanish style (most important one for industrial preparations), the Californian style (black oxidized olives) and the Greek style (naturally black olives). In all cases, the olives undergo fermentation in a brine solution. In the Spanish method, first a lye treatment is performed to hydrolyse the bitter glucoside component oleuropein. This is followed by a washing step to remove the excess of alkaline solution. After washing the olives twice, brine (8–10% salt) is added and fermentation can start. Fermentation is due to the growth of gram-negative bacteria, lactic acid bacteria and yeasts. The fermentation of olives is comparable to that of sauerkraut; however, it is slower. The main lactic acid bacteria controlling the fermentation process are *Lactobacillus plantarum*, *Lactobacillus pentosus* and *Lactobacillus casei.* In the first stage of the fermentation (2–3 days), gram-negative rods, *Enterobacteriaceae* and *Bacillus* spp., dominate. As a result the pH drops from an initial value of 8 or higher to 6 or below. Gas is formed in this stage by *Enterobacteriaceae* and *Aeromonas* spp. When fermentation progresses, *Pediococcus* and *Leuconostoc* appear, and the second stage of the fermentation starts. In this stage, mainly *Lactobacillus plantarum* and, to a lesser extent, *Lactobacillus delbrueckii* are responsible for the fermentation. During this second stage of the fermentation (12–15 days), pH drops to below 4. The third stage of the fermentation, which lasts until all substrates are exhausted, *Lactobacillus* species, mainly *Lactobacillus plantarum*, dominate in coexistence with yeast. The yeast metabolites, alcohol, acetaldehyde and ethyl acetate contributes to the specific flavour of the product (Fernández-Díez et al. 1985).

De Angelis et al. (2015) aimed to utilize an "omics" approach to evaluate the ability of selected *Lactobacilli* and yeasts to improve the fermentation process of Bella di Cerignola table olives. Four types of fermentations were performed at the pilot-plant scale: un-started fermented olives used as a control (Ctrl); olives started with a commercial *Lactobacillus plantarum* strain (S); commercial *L. plantarum*

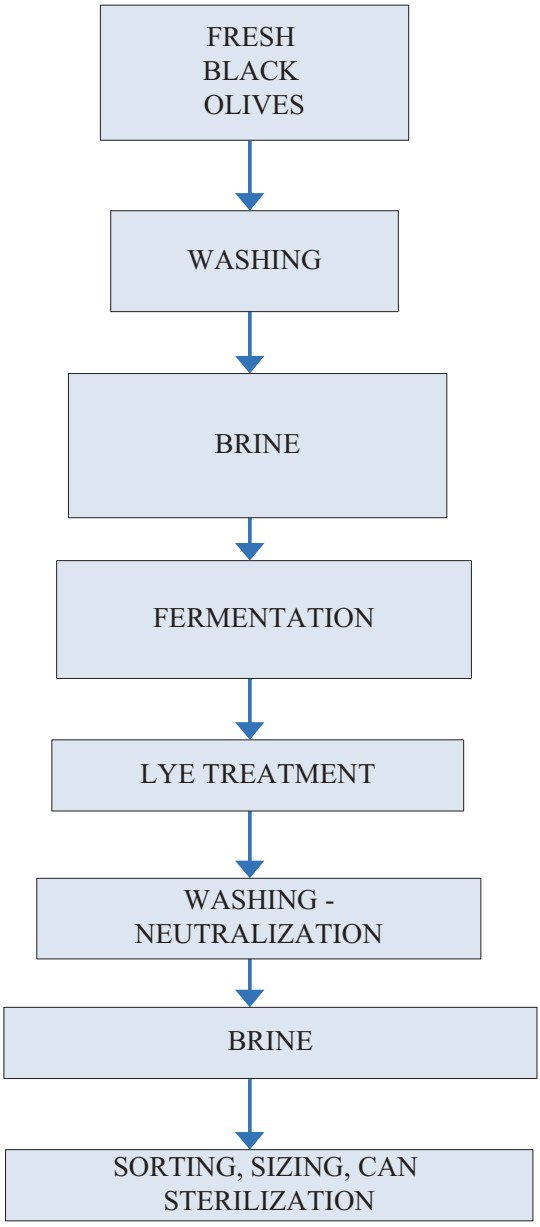

Fig. 15.2 Flow diagram of fermented fresh black olives in brine

strain and autochthonous yeast *Wickerhamomyces anomalus* DiSSPA73 (SY); and *L. plantarum*, *W. anomalus* DiSSPA73, autochthonous *L. plantarum* DiSSPA1A7 and *Lactobacillus pentosus* DiSSPA7 (SYL). Compared to Ctrl, S and SY, SYL showed a higher acidification ($P < 0.05$) of the brine, which reached a pH value of 4.49 after 1 day of fermentation.

The microbiota of unfermented olives and brine after 1 day of fermentation was primarily composed of enterobacteria that belonged to *Hafnia alvei* and *Methylobacterium*. However, *L. plantarum* and *L. pentosus* dominated the total and metabolically active microbiota of the Ctrl brines and olives at the end of the fermentation. The use of *Lactobacilli* and *W. anomalus* DiSSPA73 as a starter culture markedly affected the microbiota of the brines after 1 day of fermentation. The number of species (OTU) and the results of an alpha diversity analysis indicated that the microbial diversity of the brines was markedly simplified by the S, SY and, in particular, SYL fermentations. According to the lowest biodiversity, S, SY and SYL samples showed the lowest abundance of *Proteobacteria*, including *Enterobacteriaceae*, *Lactococcus lactis*, *Propionibacterium acidipropionici* and *Clostridium*. The *Lactobacilli* and *W. anomalus* DiSSPA73 used in this study markedly affected the amounts of free amino acids and phenolic and volatile organic compounds. Both a texture profile analysis and a sensory evaluation showed the highest appreciation for all of the started table olives. As shown through microbiological, biochemical and sensory analyses, an accelerated fermentation of Bella di Cerignola table olives was achieved using the selected *Lactobacilli* and yeast strains.

Probiotics from different vegetables foods such as cabbage and table olives, among others (Yoon et al. 2006; Abriouel et al. 2012; Patel et al. 2012, 2014; Peres et al. 2012), have a promising future. In this sense, LAB isolated from naturally fermented Aloreña green table olives (Abriouel et al. 2012) were mainly represented by *L. pentosus* and were screened in depth in the following study for their probiotic potential.

A collection of 31 *Lactobacillus pentosus* strains isolated from naturally fermented Aloreña green table olives were screened by Perez-Montoro et al. (Pérez Montoro et al. 2016) in depth for their probiotic potential. Several strains could be considered promising probiotic candidates since they showed good growth capacity and survival under simulated gastrointestinal conditions (acidic pH of 1.5, up to 4% of bile salts and 5 mM of nitrate), good ability to auto-aggregate which may facilitate their adhesion to host cells as multiple aggregates and the subsequent displacement of pathogens. Moreover, co-aggregation of *Lactobacilli* with pathogenic bacteria was shown with *Listeria innocua*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella enteritidis* as good defence strategy against gut and food pathogens. Furthermore, they exhibited adherence to intestinal and vaginal cell lines, such property could be reinforced by their capacity of biofilm formation which is also important in food matrices such as the olive surface. Their antagonistic activity against pathogenic bacteria by means of acids and plantaricins, and also their different functional properties, may determine their efficacy not only in the gastrointestinal tract but also in food matrices. Besides their ability to ferment several prebiotics, the new evidence in the present study was their capacity to ferment lactose which reinforces their use in different food matrices including dairy as a dietary adjunct to improve lactose digestibility. *Lactobacillus pentosus* CF2-10 N was selected to have the best probiotic profile being of great interest in further studies. In conclusion, spontaneous fermented Aloreña table olives are considered a natural source of potential probiotic *L. pentosus* to be included as adjunct functional cultures in different fermented foods.

The most technological procedures employed in the industrial production of table olives are (i) the Spanish system (green olives), (ii) the Californian system (black oxidized olives) and (iii) the Greek system (black olives in brine) (Garrido-Fernández 1997). In particular, the last one is used for the production of Conservolea, Kalamàta, Leccino and Cellina di Nardò table olives cultivars. Conservolea and Kalamàta represent the most economically important cultivars in Greece for domestic and foreign market (Garrido-Fernández et al. 1997), whereas, among Italian table olive cultivars, Leccino is the most important olive variety used for dual purpose in the world (Vossen 2007), and Cellina di Nardò is a very promising table olive cultivar in Salento (Apulia, Southern Italy), highly appreciated for its peculiar organoleptic and sensorial features (Bleve et al. 2015). Bleve et al. (2014, 2015) studied the evolution of several compounds (sugars, organic acids, alcohols, mono- and polyphenols and volatiles) associated with yeasts and bacteria fermentative metabolism of during the spontaneous fermentation process of these Italian and Greek olive cultivars. By this approach, chemical compounds deriving by microbiological activities during the fermentation process were proposed as chemical descriptors to monitor the fermentation process.

During this study by Tufariello et al. (2015), a new protocol for the production of black table olives belonging to two Italian (Cellina di Nardò and Leccino) and two Greek (Kalamàta and Conservolea) cultivars has been developed: for each table olive cultivar, starter-driven fermentations were performed inoculating, firstly, one selected autochthonous yeast starter and, subsequently, one selected autochthonous LAB starter. All starter formulations were able to dominate fermentation process. The olive fermentation was monitored using specific chemical descriptors able to identify a first stage (30 days) mainly characterized by aldehydes; a second period (60 days) mainly characterized by higher alcohols, styrene and terpenes; and a third fermentation stage represented by acetate esters, esters and acids. A significant decrease of fermentation time (from 8 to 12 months to a maximum of 3 months) and a significant improvement in organoleptic characteristics of the final product were obtained. This study, for the first time, describes the employment of selected autochthonous microbial resources optimized to mimic the microbial evolution already recorded during spontaneous fermentations.

Moreover, LAB produce small amounts of ethanol and other volatile compounds that make a significant contribution to the final flavour of table olives (Hurtado et al. 2012).

A total of 145 lactic acid bacteria (LAB) isolates have been recovered from fermented table olives and brine and characterized at strain level with molecular tools by Doulgeraki et al. (2013). Pulsed-field gel electrophoresis (PFGE) of ApaI macrorestriction fragments was applied for strain differentiation. Species differentiation was based either on PCR-denaturing gradient gel electrophoresis (PCR-DGGE) (black olives) or on restriction analysis of the amplified 16S rRNA gene (PCR-ARDRA) (brine and green olives). Species identification was based on sequence analysis of 16S rRNA gene. When the data were insufficient to resolve the species level of the isolates, specific multiplex PCR assays targeting the recA or tuf genes were employed. From 145 LAB isolates, 71 different strains were recovered from fermented olive and brine samples; 17 strains were assigned to *Leuconostoc*

mesenteroides, 51 were grouped in *Lactobacillus plantarum* group (including 13 *L. plantarum*, 37 *Lactobacillus pentosus*, 1 *Lactobacillus paraplantarum*), 2 in *Lactobacillus paracasei* subsp. *paracasei* and 1 in *Leuconostoc pseudomesenteroides*. *L. plantarum* was recovered mainly from green olive fermentation, whereas in black olives the main species identified were *L. pentosus* and *Ln. mesenteroides*.

These observations reveal that olives are a highly diverse ecosystem regarding the presence of LAB, which may affect the quality of the final fermented product.

In another work (Argyri et al. 2015), green olives, subjected to inoculated Spanish-style fermentation with probiotic LAB strains (*L. pentosus* B281 and *Lactobacillus plantarum* B282), were packed in polyethylene pouches under modified atmospheres (70% N2e30% CO_2) and stored at 4 and 20 °C for 12 months. The authors reported that both strains presented high survival rates during storage with *L. pentosus* B281 exhibiting higher survival rates (94.1%) after 6 months of storage.

The difference between treated and natural olive processes are that treated olives have to undergo an alkaline treatment before they are placed in brine to start their fermentation. Traditionally, table olives have been classified according to the colour of the fruit and the processing method, and the models used to classify the various existing processes are the most important industrial preparations (Fernández-Díez et al. 1985; Garrido-Fernández et al. 1997; Panagou et al. 2008). The present Trade Standard (IOOC 2004) goes further and classifies table olives exclusively by the processing method: (a) treated olives, (b) natural olives, (c) olives darkened by oxidation, (d) dehydrated and/or shrivelled olives and (e) specialities.

Only treated and natural olives have to be fermented. It has been generally established that lactic acid bacteria are responsible for the fermentation of treated olives. However, lactic acid bacteria and yeasts compete for the fermentation of natural olives, and in some cases yeasts can be exclusively responsible for fermentation (Garrido-Fernández et al. 1997; Aponte et al. 2010).

Independent of their colour – green, turning colour or black – all olives can be processed as treated or natural. The fermentation process will depend on the cultivar itself and on industrial and agricultural practices. Although some cultivars can be harvested at different stages of maturity and processed by both the treated and natural methods, normal practice is to prepare each cultivar using a single procedure and well-established local practices (Garrido-Fernández et al. 1997).

The aim of this work by Bautista-Gallego et al. (2013) was to study the potential probiotic properties of *Lactobacilli* associated with table olives. From a total of 111 isolates from spontaneously fermented green olive brines, 109 were identified at species level by multiplex PCR amplifications of the recA gene. One hundred and seven of these were identified as *Lactobacillus pentosus*, one as *Lactobacillus plantarum* and another as *Lactobacillus paraplantarum*.

Repetitive bacterial DNA element fingerprinting (rep-PCR) with GTG5 primer revealed a higher variability within the *L. pentosus* isolates, and nine different clusters were obtained. Most of them showed high auto-aggregation ability, low hydrophobicity properties and lower survival to gastric than to pancreatic digestion; however, no isolate showed bacteriocin, haemolytic or bile salt hydrolase activities. A multivariate analysis based on results from phenotypic tests led to the segregation

of some *L. pentosus* isolates with promising potential probiotic characteristics, which are even better than probiotic reference strains. Due to the autochthonous origin of the strains, their use as starter cultures may contribute to improving natural fermentation and the nutritional characteristics of table olives.

Olive fermentation is fundamental for the production of high-quality product. Principally, microorganisms metabolize the sugars contained in the olive mesocarp producing lactic acid. The low pH of the brine, together with the production of antimicrobial substances, minimizes undesirable microorganism population and increases safety of product (Rubia-Soria et al. 2006). Generally, a spontaneous fermentation is performed by the indigenous microbiota present in both the raw material and the processing environment (Campaniello et al. 2005).

Technological properties of two strains of *Lactobacillus plantarum* (B3 and B11) and one of *Lactobacillus pentosus* (B4), previously isolated from natural fermented green olives, have been studied in vitro by Iorizzo et al. (2016). Acidifying ability, salt, temperature and pH tolerances of all strains were found in the range reported for similar strains produced in Italy, and optimal growth conditions were found to be 6.0–8.0 pH, 15–30 °C temperature and less than 6% NaCl. Moreover, all strains showed very good tolerance to common olive phenol content (0.3% total phenol) and high oleuropein-degrading capability. It was found that medium composition affected the bacterial oleuropein degradation. B11 strain grown in a nutrient-rich medium showed a lower oleuropein-degrading action than when it was cultivated in nutrient-poor medium. Furthermore, enzymatic activity assays revealed that oleuropein depletion did not correspond to an increase of hydroxytyrosol, evidencing that bacterial strains could efficiently degrade oleuropein via a mechanism different from hydrolysis.

This article by Lopez-Lopez et al. (López-López et al. 2016b) contains processed data related to the research published in "Fermentation in nutrient salt mixtures affects green Spanish-style Manzanilla table olives" (López-López et al. 2016a). It displays information on the salt substitution by other nutrient salts (potassium chloride and calcium chloride) during fermentation of green Spanish-style Manzanilla table olives to produce healthier products. Particularly, it studies the relationship between the different colour parameters (L*, a*, b* and Ci), firmness and sensory attributes (saltiness, bitterness, hardness and fibrousness) and the composition of the initial brine in NaCl, KCl and $CaCl_2$. The composition of the brines affected the characteristics of the product. In general, the higher the proportion of $CaCl_2$ in the initial brines, the better was the colour. Also, the presence of this salt mitigated the saltiness perception but increment those of bitterness, hardness, fibrousness and crunchiness. Besides, most of the sensory attribute scores could successfully be predicted as a function of the Na, K and Ca concentrations in the fermented olive flesh. The work allows the production of table olives with specific characteristics and predetermined mineral nutrient composition.

The bitter taste of olives is mainly caused by the phenolic compound named oleuropein, and the mechanism of its hydrolysis during the processing of natural green olives was studied by Ramírez et al. (2016). First, a rapid chemical hydrolysis of oleuropein takes place at a high temperature of 40 °C and at a low pH value of

2.8, but the chemical hydrolysis of the bitter compound is slow at the common range of pH for these olives (3.8–4.2).

However, decarboxymethyl elenolic acid linked to hydroxytyrosol and hydroxytyrosol have been found in a high concentration during the elaboration of natural green olives. When olives were heated at 90 °C for 10 min before brining, these compounds are not formed. Hence, the debittering process in natural green olives is due to the activity of b-glucosidase and esterase during the first months of storage and then a slow chemical hydrolysis of oleuropein happens throughout storage time.

The technology of "pied de cuve" (PdC), largely used for wine production (Clavijo et al. 2011; Li et al. 2012), limits the reduction of the microbial complexity of the driven processes. This method promotes the growth of the desirable microbial strains in a small volume of grape must which act as a starter inoculums for higher volumes but does not exclude the risk of development of unwanted microorganisms. Usually, the ratio PdC/bulk for wine application is 1:10 (Li et al. 2012).

The technology of "pied de cuve" (PdC) is applied in food process only to produce wines with an enriched community of pro-technological yeasts. PdC promotes the growth of the desirable microbial strains in a small volume of grape must acting as a starter inoculums for higher volumes. The aim of the present work by Martorana et al. (2015) was to investigate the use of partially fermented brines, a technology known as PdC, developed with lactic acid bacteria (LAB) on the microbiological, chemical and sensory characteristics of green fermented table olives during two consecutive campaigns. The experimental plan included two trials based on different PdCs: trial A, PdC obtained with *Lactobacillus pentosus* OM13, and trial B, PdC obtained through a spontaneous fermentation. Two control additional trials without PdC were included for comparison: trial C, spontaneous fermentation, and trial D, direct inoculation of *L. pentosus* OM13. The use of PdCs favoured the rapid increase of LAB concentrations in both trials A and B. These trials showed levels of LAB higher than trial C and almost superimposable to that of trial D. Trial B was characterized by a certain diversity of *L. pentosus* strains and some of them dominated the manufacturing process. These results indicated PdC as a valuable method to favour the growth of autochthonous *L. pentosus* strains.

Hierarchical cluster analysis (HCA) and principal component analysis (PCA) visibly discriminated olive processes fermented with the two experimental PdCs. Interestingly, on the basis of microbial and pH variables, both approaches showed that the olives produced with PdC technology are closely related to those of trial D, with the advantages of reducing the amount of starter to inoculate (trial A) and a higher LAB biodiversity (trial B). Volatile organic compound (VOC) composition and sensory analysis showed trials A and B different from the trials with no PdC added, in both years. Furthermore, the trial B showed the highest scores of green olive aroma and taste complexity. Spoilage microorganisms were estimated at very low levels in all trials. Undesired off-odours and off-flavours were not revealed at the end of the process.

Mateus et al. (2016) aimed at studying the effect of the partial replacement of NaCl with KCl and $CaCl_2$ of the fermenting brines on the microbiological quality of natural cracked green Maçanilha Algarvia table olives. Olives were fermented

in different salt combinations (Brine 1, 8% NaCl; Brine 2, 4% NaCl 4% KCl; Brine 3, 4% NaCl 4% $CaCl_2$; Brine 4, 4% KCl 4% $CaCl_2$; and Brine 5, 2.7% NaCl 2.7% KCl 2.7% CaCl2), and the abundance of yeasts and enterobacteria was determined. At the end of fermentation, the main microbial safety parameters were evaluated.

Samples were analysed according to standard methodologies and using ChromoCult Agar (coliforms and *Escherichia coli*). The yeasts collected were grouped by restriction analysis of the ITS-5.8S rRNA gene and identified by partial sequencing of the 26S rRNA. Throughout the study, a decrease of the enterobacteria population was observed in all the fermentations, which was greater and faster in brines containing potassium and calcium. The main yeasts identified were *Pichia membranifaciens*, *Candida boidinii*, *Zygosaccharomyces mrakii*, *Priceomyces carsonii*, *Saccharomyces cerevisiae*, *Wickerhamomyces anomalus* and the yeast-like fungus *Galactomyces geotrichum*. The highest yeast diversity was found in olives produced in Brines 1, 2 and 3 and the lowest in Brines 4 and 5, where only the species *P. membranifaciens*, *C. boidinii* and *G. geotrichum* were identified. No *Pseudomonas*, *E. coli*, *Salmonella*, *Staphylococcus aureus* and *Listeria monocytogenes* were found in the table olives produced.

The main purpose of using starter cultures during table olive manufacturing is to drive the fermentation and inhibit the development of spoilage microorganisms, such as pseudomonads, *Enterobacteriaceae* and staphylococci. In the absence of consistent levels of LAB, undesired microbial groups can rapidly increase and negatively affect the final product. Starter cultures may stabilize the manufacturing process in terms of chemico-physical, microbiological and sensory quality of table olives. The technological characteristics of starter cultures for table olive production include mainly the ability to grow at 15 °C, in the presence of different concentrations of salt and phenolic compounds. Therefore, high levels of viability and efficiency of starter cultures are required to create the rapid drop of brine pH during the production of hydrogen sulphide, the b-glucosidase, lipolytic and proteolytic activities (Rodríguez-Gomez et al. 2010). Furthermore, the use of starter cultures may improve the food shelf life by inhibition of spoilage microorganisms based on nutrient competition.

The main objective by Martorana et al. (2017) was to set up a methodology to improve the high volume production of green table olives, cv. Nocellara del Belice. *Lactobacillus pentosus* OM13 was applied during three different industrial processes of table olives as follows: Trial one (IOP1) was subjected to an addition of lactic acid until a brine level of pH 7.0 was reached; trial two (IOP2) subjected to same addition of lactic acid as in trial one plus nutrient adjuvant; and trial three (IOP3) subjected to same addition as trial two, but with the strain *L. pentosus* OM13 acclimatized in brine for 12 h before inoculation. These trials were compared against two untreated controls (spontaneously fermented and addition of *L. pentosus* OM13 only).

Within the third day of fermentation, the pH of the brines decreased significantly, reaching pH 4.85 for trial three, pH 5.15 for trial two and pH 5.92 for trial one. The pH of both controls decreased more slowly and had values below pH 5.0 only after

the 15th day of fermentation (control one) and the 65 day of fermentation (control two). Trial three reached the highest lactic acid bacteria (LAB) concentration on the third day of fermentation. After 6 days of fermentation, all trials showed similar values of LAB counts that were significantly higher compared to control number one. The result from genotypic identification showed that *L. pentosus* OM13 was the most frequently isolated in the inoculated trials.

Lactobacillus plantarum, *Lactobacillus coryniformis* and *Pediococcus pentosaceus* were also detected at very low concentrations. Homoguaiacol, 2-butanol, 4-ethylphenol, phenylethyl alcohol and 4-ethylphenol were the volatile organic compounds detected at the highest levels in all experimental trials. Trial three showed a higher concentration of squalene that was not detected in other trials. The highest sensory scores of green olive aroma and overall satisfaction were found for all experimental olives, especially for those of trial one and trial two, that differed significantly from the untreated controls.

This study provides evidence that the addition of lactic acid and nutrient adjuvants and, most importantly, the acclimatization of LAB cells significantly shorten the acidification process of olive brine and improve safety and sensory quality. Shorter acidification processes result in a more rapid transformation of table olives, with reduced commodity loss and lower costs of production compared to conventional manufacturing protocols.

15.5 Salting Procedures

Dry Salted Fermented Vegetables

The application of dry salting involves treatment of vegetables with dry salt. The salt extracts the juice from the vegetables, and in this way brine is formed. The vegetable is washed in potable cold water and drained. To 100 kg of vegetables, 3 kg salt is added. The vegetables are placed in layers (2.5 cm thick) in a container. The salt is sprinkled over the vegetables. Then another layer of vegetables and salt is added and repeated until the fermenter is filled up to three quarters of its weight. A cloth is then placed above the vegetables as well as a weight added to compress the vegetables and to help the brine formation. As soon as the brine is formed, the fermentation starts and is initiated as CO_2 bubbles appear. Fermentation lasts between 1 and 4 weeks, depending on the temperature. When the fermentation is complete, no gas bubbles appear.

Brine Salted Fermented Vegetables

A brine solution of 10–20% is made. Fermentation takes place in this brine. The duration of brining is important for the overall keeping qualities. The vegetable is immersed in the brine and allowed to ferment. The strong brine solution draws sugars and water out of the vegetable, resulting in a decrease in salt concentration. The salt concentration needs to be kept higher than 12%; otherwise, no fermentation will take place. Therefore, salt can be added periodically.

Brine Salted Fermentation of Vegetables: Pickles
The process is quiet similar compared to the sauerkraut production, but instead of dried salt, a brine is used.

15.6 Fermented Cucumber

Cucumber fermentation and storage in bulk tanks is a method for the preparation of pickle products that allows the preservation of cucumber fruits for extended periods of time and results in a unique food product. Occasionally, tanks of commercially fermented cucumbers spoil after the primary fermentation. The unpredictable nature of this spoilage has resulted in increased production costs for the pickling industry, mainly in the form of increased monitoring of fermentation tanks.

Cucumbers are preserved commercially by natural fermentations in 5–8% sodium chloride (NaCl) brines.

Occasionally, fermented cucumbers spoil after the primary fermentation is complete. This spoilage has been characterized by decreases in lactic acid and a rise in brine pH caused by microbial instability. Objectives of this study by Johanningsmeier et al. (2012) were to determine the combined effects of NaCl and pH on fermented cucumber spoilage (FCS) and to determine the ability of lactic acid bacteria (LAB) spoilage isolates to initiate lactic acid degradation in fermented cucumbers. Cucumbers fermented with 0%, 2%, 4% and 6% NaCl were blended into slurries (FCS) and adjusted to pH 3.2, 3.8, 4.3 and 5.0 prior to centrifugation, sterile filtration and inoculation with spoilage organisms. Organic acids and pH were measured initially and after 3 weeks and 2, 6, 12 and 18 months anaerobic incubation at 25 °C. Anaerobic lactic acid degradation occurred in FCS at pH 3.8, 4.3 and 5.0 regardless of NaCl concentration. At pH 3.2, reduced NaCl concentrations resulted in increased susceptibility to spoilage, indicating that the pH limit for lactic acid utilization in reduced NaCl fermented cucumbers is 3.2 or lower. Over 18 months incubation, only cucumbers fermented with 6% NaCl to pH 3.2 prevented anaerobic lactic acid degradation by spoilage bacteria. Among several LAB species isolated from fermented cucumber spoilage, *Lactobacillus buchneri* was unique in its ability to metabolize lactic acid in FCS with concurrent increases in acetic acid and 1,2-propanediol. Therefore, *L. buchneri* may be one of the multiple organisms that contribute to the development of fermented cucumber spoilage.

Cucumbers are fermented in a brine with an initial salt concentration of 5–8% at ambient temperatures. Fermentation is finished after 2–3 weeks, reaching a final pH of 3.3–3.5 and a lactic acid concentration of 1.1%. About 1 kg of salt is added to every 20 kg of small cucumbers and to every 15 kg of large cucumbers. The brine should be formed within 24 h by osmosis. The formed brine needs to cover all the cucumbers. Two days after the tank is filled and closed, the brine needs to be stirred to ensure an equilibrium of the salt throughout the cucumber mass. When the brine is formed, fermentation starts immediately. This is observed by the appearance of gas bubbles. When no CO_2 bubbles appear anymore, the fermentation is finished.

At the start of the fermentation, a rapid growth is observed of gram-positive and gram-negative bacteria, as well as of yeasts. During fermentation, the following lactic acid bacteria are present: *Leuconostoc mesenteroides*, *Pediococcus pentosaceus* and *Lactobacillus plantarum*. Normally the latter is predominant at the end of the fermentation but is present in all phases of the fermentation process. If the brine is not covered, a film of yeast growth will develop on the surface.

Fresh cucumbers are fermented in 6440–37,860 L open-top tanks using a cover brine solution that may contain 5–10% sodium chloride (NaCl) during the active fermentation period. The salt concentration may be increased up to 18% NaCl post-fermentation in northern regions where the winter temperatures are sufficient to freeze the fermented cucumbers in the tanks during long-term storage. The flow diagram of cucumber fermentation is shown in Fig. 15.3.

Commercially, fermented cucumbers in high-salt brines are desalted once or twice by addition of fresh water to bring the NaCl levels down to edible concentrations (2%) in the finished pickle products. The processing of cucumbers in US tank yards is estimated to generate up to 45 million litres of effluent waste waters annually, containing a minimum of 3.4% NaCl. Additional processing activities to manufacture acidified and fermented cucumbers in the USA are estimated to generate 1.3 billion litres of treated waste waters with a minimum average discharge of 2800 ppm chlorides and 12,000 tons of salty sludge containing a minimum of 15% solids that are deposited in landfills every year.

McFeeters and Perez-Dıaz (2010) proposed a low salt fermentation using calcium chloride ($CaCl_2$) as the only salt to maintain firmness and a starter culture to induce a fast fermentation.

Laboratory scale testing of cucumber fermentations brined with $CaCl_2$ indicated that a complete conversion of sugars primarily to lactic acid and a decrease in pH proceeds over a range of $CaCl_2$ concentrations and cucumber sizes (McFeeters and Perez-Dıaz 2010). In closed containers, cucumbers were microbiologically stable after the primary lactic acid fermentation was completed. Firmness retention of cucumbers fermented in 100 mM CaCl2 alone was equivalent to that obtained in cucumbers fermented with 1.03 M (6%) NaCl and 40 mM $CaCl_2$ (McFeeters and Perez-Dıaz 2010).

The objective of this research by Perez-Diaz et al. (Perez-Dıaz et al. 2015) was to translate cucumber fermentation brined with calcium chloride (CaCl2) instead of NaCl to commercial scale production.

Although $CaCl_2$ brined cucumber fermentations were stable in laboratory experiments, commercial scale trials using 6440 L open-top tanks rapidly underwent secondary cucumber fermentation. It was understood that a limited air purging routine, the use of a starter culture and addition of preservatives to the cover brine aid in achieving the desired complete cucumber fermentation. The modified process was used for subsequent commercial trials using 12,490 and 28,400 L open-top tanks packed with variable size cucumbers and from multiple lots and cover brines containing $CaCl_2$ and potassium sorbate to equilibrated concentrations of 100 and 6 mM, respectively. *Lactobacillus plantarum* LA0045 was inoculated to 10^6 CFU/mL, and air purging was applied for two 2–3 h periods per day for the first 10 days

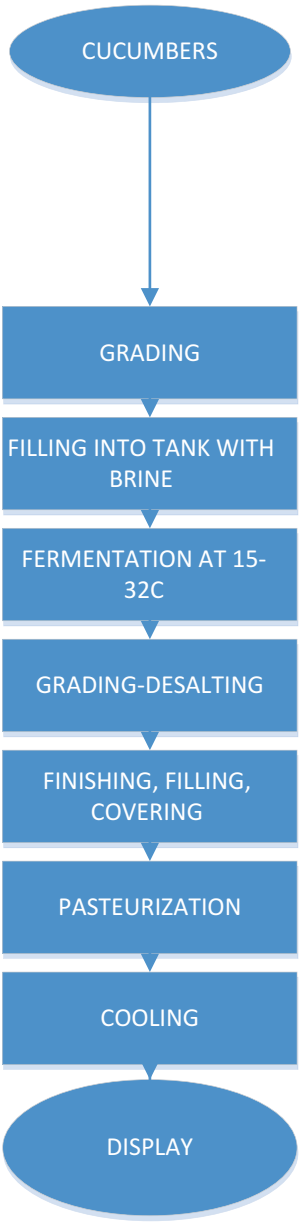

Fig. 15.3 Flow diagram of cucumber fermentation

of fermentation and one 2–3 h period per day between days 11 and 14. All fermentations were completed, as evidenced by the full conversion of sugars to lactic acid, decrease in pH to 3.0 and presented microbiological stability for a minimum of 21 days. This $CaCl_2$ process may be used to produce fermented cucumbers intended to be stored short term in a manner that reduces pollution and waste removal costs (Perez-Dıaz et al. 2015).

Fermentation of cucumbers in calcium chloride ($CaCl_2$) brine has been proposed as an alternative process to reduce the environmental impact of traditional, high-salt fermentations. The objective of this research by Wilson et al. (2015) was to determine whether consumer acceptability of pickle products would be impacted by fermentation and storage of cucumbers in $CaCl_2$ brine. Cucumbers were fermented and stored with 0.1 M CaCl2 or 1 M sodium chloride (NaCl) in open-air, 3000 gal tanks at a commercial facility and processed into hamburger dill chips containing 0.38 M NaCl. Cucumbers fermented in CaCl2 required additional desalting to reduce CaCl2 concentrations to that of current products. Consumers (n = 101) showed no significant preference for pickles from different fermentation treatments, whether stored for 2 months ($P = 0.75$) or 8 months ($P = 0.68$) prior to processing. In contrast, NaCl fermented pickles were preferred over $CaCl_2$ fermented pickles stored for 10 months and desalted only once ($P < 0.01$). A series of preference tests indicated that the taste of $CaCl_2$ was not the factor affecting consumer preference, and the 50% detection threshold of $CaCl_2$ in dill pickle chips was found to be 61.8 ± 7.6 mM, indicating that processors could potentially use $CaCl_2$ fermentations with a single desalting step.

Consumer liking of flavour ($n = 73$) was not influenced by fermentation in CaCl2 or by 23 or 35 mM CaCl2 in finished products ($P > 0.05$), but variability in texture decreased consumer liking ($P < 0.05$). Although promising, individual fermentation variability and texture quality of $CaCl_2$ fermented products should be further evaluated prior to broad implementation of this process.

Koozh is a traditional south Indian porridge made from millet and consumed in fermented form in rural and urban Indian households. Koozh is prepared from kezhvaragu (finger millet: *Eleusine coracana*), cumbu (pearl millet: *Pennisetum glaucum*) and noyee (broken rice) in clay pots. Gherkins/cucumbers (*Cucumis sativus*) are pickled by lacto-fermentation or immersion in acidic solution which renders a tart flavour.

This study sought to evaluate the probiotic potential of lactic acid bacteria (LAB) isolated from traditionally fermented south Indian koozh and gherkin (cucumber). A total of 51 LAB strains were isolated, among which four were identified as *Lactobacillus* spp. and three as *Weissella* spp. The strains were screened for their probiotic potential. All isolated *Lactobacillus* and *Weissella* strains were capable of surviving under low pH and bile salt conditions. GI9 and FKI21 were able to survive at pH 2.0 and 0.50% bile salt for 3 h without losing their viability. All LAB strains exhibited inhibitory activity against tested pathogens and were able to deconjugate bile salt. Higher deconjugation was observed in the presence of sodium glycocholate ($P < 0.05$). Strain FKI21 showed maximum auto-aggregation (79%) and co-aggregation with *Escherichia coli* MTCC 1089 (68%). Exopolysaccharide production of LAB strains ranged from 68.39 to 127.12 mg/L ($P < 0.05$). Moreover, GI9 (58.08 mg/ml) and FKI21 (56.25 mg/ml) exhibited maximum cholesterol reduction with bile salts. 16S rRNA sequencing confirmed GI9 and FKI21 as *Lactobacillus crispatus* and *Weissella koreensis*, respectively. This is the first study to report isolation of *W. koreensis* FKI21 from fermented koozh and demonstrates its cholesterol-reducing potential (Anandharaj et al. 2015).

Jiang-gua (fermented cucumbers) is a widely used traditional food in Taiwan and can be served as a side dish or a seasoning. *Jiang-gua* is sold in almost every market in Taiwan, both traditional markets and supermarkets.

Harvested cucumbers (*Cucumis sativus* L.) are washed, cut and mixed with salt (NaCl), layered in a bucket and then sealed with heavy stones on the cover. This process usually continues for 4–5 h, but some producers maintain a longer processing time. After the exuded water has been drained off, the cucumbers are mixed with sugar and vinegar. In addition, soy sauce is added optionally depending on the recipe. Fermentation usually continues for at least 1 day at low temperature (6–10 °C), but some producers maintain a fermentation time of 3 days or even longer. *Jiang-gua* can be used as a seasoning for pork, fish, chicken and various other foods. Although the product is very popular, it has not been studied in detail.

Lactic acid bacteria (LAB) are distributed in various Taiwanese fermented foods such as *yan-jiang* (fermented ginger), *jiangsun* (fermented bamboo shoots), *suan-tsai* (fermented mustard), *dochi* (fermented black beans), *yan-dong-gua* (fermented wax gourd) and *pobuzihi* (fermented cummingcordia) (Chen et al. 2006a, b, 2010a, b; Chang et al. 2011; Lan et al. 2009).

Jiang-gua (fermented cucumbers) is a popular traditional fermented food in Taiwan. The microflora of lactic acid bacteria (LAB) in *jiang-gua* have not been investigated in detail. In this study by Chen et al. (2012), LAB from *jiang-gua* were isolated, characterized and identified.

A total of 103 LAB were isolated; 70 cultures were isolated from *jiang-gua* samples, and 33 cultures were isolated from its raw substrate, cucumber. These isolates were mainly characterized phenotypically and then divided into seven groups (A–G) by restriction fragment length polymorphism analysis and sequencing of 16S ribosomal DNA. The isolates were identified as *Enterococcus casseliflavus*, *Leuconostoc lactis*, *Leuconostoc mesenteroides*, *Lactobacillus pentosus*, *Lactobacillus plantarum*, *Lactobacillus paraplantarum*, *Lactococcus lactis* subsp. *lactis*, *Weissella cibaria* and *Weissella hellenica*. The antibacterial activities of the isolates were determined, and 11 *Lc. lactis* subsp. *lactis* strains showed inhibitory activity against the indicator strain *Lactobacillus sakei* JCM 1157T.

Heterofermentative *W. cibaria* and *Leu. lactis* were the major LAB found in *jiang-gua* samples without soy sauce. In soy sauce-added samples, homofermentative *L. pentosus* and *L. plantarum* were the most abundant LAB. In addition, the results also suggested that *Hha*I and *Rsa*I restriction enzymes could be applied to distinguish *W. hellenica* and *Weissella paramesenteroides*.

Recent evidence suggests that *Lactobacillus buchneri* may play an important role in spoilage-associated secondary fermentation of cucumbers. Lactic acid degradation during fermented cucumber spoilage is influenced by sodium chloride (NaCl) concentration, pH and the presence of oxygen. Objectives were to evaluate these factors on lactic acid utilization by *L. buchneri* and to compare the biochemical changes to those which occur during fermented cucumber spoilage. Effects of NaCl (0, 2, 4 and 6% w/w), pH (3.8 vs 5.0) and aerobic environment were investigated using fermented cucumber media (FC) inoculated with spoilage microorganisms. At pH 3.8, *L. buchneri* degraded lactic acid in all NaCl

concentrations. The highest rate of lactic acid utilization occurred in FC with 2% NaCl ($P < 0.05$). Lactic acid utilization was nearly identical under aerobic and anaerobic conditions, indicating that oxygen does not influence lactate metabolism by *L. buchneri*. Lactic acid utilization was accompanied by increases in acetic acid and 1,2-propanediol, and *Lactobacillus rapi* was able to convert 1,2-propanediol to propionic acid and propanol.

L. buchneri initiated spoilage in a wide range of environmental conditions that may be present in commercial cucumber fermentations, and *L. rapi* may act syntrophically with *L. buchneri* to produce the commonly observed spoilage metabolites (Johanningsmeier and McFeeters 2013).

Lactobacillus buchneri has recently been associated with anaerobic spoilage of fermented cucumbers due to its ability to metabolize lactic acid into acetic acid and 1,2-propanediol. However, there is limited knowledge of other chemical components in fermented cucumber that may be related to spoilage and the unique metabolic capabilities of *L. buchneri*. Comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry metabolite profiling methods were applied for nontargeted detection of volatile and nonvolatile compounds to determine changes that occurred during anaerobic fermented cucumber spoilage by *L. buchneri* LA1147 and during reproduction of spoilage with natural microbiota. Univariate analysis of variance combined with hierarchical clustering analysis revealed 92 metabolites that changed during spoilage ($P < 0.01$). Decreases were observed in mono- and disaccharides, amino acids, nucleosides, long-chain fatty acids, aldehydes and ketones, and increases were observed in several alcohols and butanoic and pentanoic acids. Most of the metabolite changes preceded lactic acid utilization, indicating that lactic acid is not a preferred substrate for anaerobic spoilage organisms in fermented cucumbers. The ability to detect biochemical changes that preceded lactate utilization revealed citrulline, trehalose and cellobiose as compounds that may signify metabolic activity of *L. buchneri* spoilage strains prior to any significant product degradation (Johanningsmeier and McFeeters 2015).

Most important during probiotic selection are gastric acid and bile tolerance, the adhesion to the luminal epithelium to colonize the lower gastrointestinal tract of a human and safety for human consumption. The aim of this study by Zielinska et al. (2015) was to evaluate the selected probiotic in vitro properties of *Lactobacillus* spp. strains isolated from traditional fermented food (pickled cucumbers and cabbage). A total of 38 strains were isolated from the pickled samples, and 14 were identified as *Lactobacillus* spp. The survival of almost all strains after incubation at pH 2.5 did not change markedly and remained at above 90% (10^9 CFU/mL). The strains also exhibited a high survival rate at pH 3.5 (90%), whereas at pH 1.5 all died. Just four strains could survive 90 min at pH 1.5 (<39%). The incubation with 0.2% bile salt solution resulted in a survival rate of 81–94% after 24 h, whereas after incubation in 2% and 4% bile salt solution, it was 59–94%. All tested strains showed very good and good resistance to 0.4% phenol addition, however only Lb. johnsonii K4 was able to multiply. The hydrophobic nature of the cell surface of the tested strains was moderated recording hydrophobicity of Lb. johnsonii K4 and Lb.

rhamnosus K3 above 60%. Safety evaluation excluded four of tested strains as candidate probiotics, according to antibiotic resistance patterns and certain metabolic activities. On the basis on the results 10 of the selected Lactobacillus strains are safe and can survive under gastrointestinal conditions, which requires them to future in vitro and in vivo probiotic studies (Zielinska et al. 2015).

The characterization of NaCl cucumber fermentation spoilage bacteria using culture-dependent and culture-independent techniques, and an enrichment step revealed that *Propionibacterium* and *Pectinatus* species play a role in converting lactic acid to propionic acid at pH above 4.2 (Breidt and others 2013).

Fermented cucumber spoilage (FCS) characterized by rising pH and the appearance of manure- and cheese-like aromas is a challenge of significant economical impact for the pickling industry. Previous culture-based studies identified the yeasts *Pichia manshurica* and *Issatchenkia occidentalis*; four gram-positive bacteria, *Lactobacillus buchneri*, *Lactobacillus parrafaraginis*, *Clostridium* sp. and *Propionibacterium*; and one gram-negative genus, *Pectinatus*, as relevant in various stages of FCS given their ability to metabolize lactic acid. It was the objective of this study by Medina et al. (2016) to augment the current knowledge of FCS using culture-independent methods to microbiologically characterize commercial spoilage samples. Ion Torrent data and 16S *rRNA* cloning library analyses of samples collected from commercial fermentation tanks confirmed the presence of *L. rapi* and *L. buchneri* and revealed the presence of additional species involved in the development of FCS such as *Lactobacillus namurensis*, *Lactobacillus acetotolerans*, *Lactobacillus panis*, *Acetobacter peroxydans*, *Acetobacter aceti* and *Acetobacter pasteurianus* at pH below 3.4. The culture-independent analyses also revealed the presence of species of *Veillonella* and *Dialister* in spoilage samples with pH above 4.0 and confirmed the presence of *Pectinatus* spp. during lactic acid degradation at the higher pH. *Acetobacter* spp. were successfully isolated from commercial samples collected from tanks subjected to air purging by plating on Mannitol Yeast Peptone agar. In contrast, *Lactobacillus* spp. were primarily identified in samples of FCS collected from tanks not subjected to air purging for more than 4 months. Thus, it is speculated that oxygen availability may be a determining factor in the initiation of spoilage and the leading microbiota (Medina et al. 2016).

Selected yeast and bacteria, isolated from cucumber secondary fermentations, were inoculated as single and mixed cultures in a cucumber juice model system. Results confirmed that during storage of fermented cucumbers and in the presence of oxygen, spoilage yeasts are able to grow and utilize the lactic and acetic acids present in the medium, which results in increased brine pH and the chemical reduction in the environment.

These conditions favour opportunistic bacteria that continue the degradation of lactic acid. *Lactobacillus buchneri*, *Clostridium bifermentans* and *Enterobacter cloacae* were able to produce acetic, butyric and propionic acids, respectively, when inoculated in the experimental medium at pH 4.6. Yeast and bacteria interactions favoured the survival of *Cl. bifermentans* and *E. cloacae* at the acidic pH typical of fermented cucumbers (3.2), but only *E. cloacae* was able to produce a secondary product.

The methodology used in this study confirmed that a complex microbiota is responsible for the changes observed during fermented cucumber secondary fermentation and that certain microbial interactions may be essential for the production of propionic and butyric acids (Franco and Perez-Dıaz 2012).

The effects of different packing conditions and storage times on the stability of monosodium glutamate (MSG) added to two different fermented vegetables (Spanish-type green table olives and pickled cucumbers) were studied. Factors such as packaging material (glass bottle versus plastic pouch), heat treatment (pasteurization versus non-pasteurization) and the presence or not of a preservative compound (potassium sorbate) were considered. The MSG content of pickled cucumbers was stable for up to 1 year of storage in all packing conditions studied. The MSG content also remained stable in pasteurized green table olives. On the contrary, MSG was extensively degraded (>75% degradation) after 54 weeks of storage in unpasteurized green olives with a higher degradation rate in glass bottles compared with plastic pouches. In the presence of potassium sorbate, MSG was also considerably degraded in olives packed in plastic pouches (>50% degradation), but hardly degraded in glass bottles. The results indicate that MSG degradation in olives is due to the action of both lactic acid bacteria and yeasts, with the formation of γ-aminobutyric acid as the major end product (de Castro et al. 2014).

15.7 Kimchi

Kimchi is a Korean traditional fermented vegetable made from Chinese cabbage (beachu), radish, green onion, red pepper powder, garlic, ginger and fermented seafood (jeotgal), which is traditionally made at home and served as a side dish at meals (Kim and Chun 2005; Park and Jeong 2015). Kimchi is a generic term indicating a group of traditional LA fermented vegetables in Korea (Lee et al. 2005). The major raw materials (oriental cabbage or radish) are salted after prebrining, blended with various spices (red pepper, garlic, green onion, ginger, etc.) and other minor ingredients (seasonings, salted seafood, fruits and vegetables, cereals, fish, meats, etc.) and then fermented at low temperature (2–5∘C). Kimchi fermentation is a temperature-dependent process. It ripens in 1 week at 15 °C and takes 3 days at 25 °C. But low temperature is preferred in kimchi fermentation to prevent production of strong acid, over ripening and extended period of optimum taste (Nam et al. 2009).

Kimchi is characterized particularly by its sour, sweet and carbonated taste and differs in flavour from sauerkraut and pickles that are popular fermented vegetables (Hong et al. 1999). The classical identification of bacterial isolates from kimchi revealed that *Leuconostoc mesenteroides* and *Lactobacillus plantarum* were the predominant species (Kim and Chun 2005). Several results suggested that LAB contributing to kimchi fermentation include *L. mesenteroides*, *L. citreum*, *L. gasicomitatum*, *Lactobacillus brevis*, *L. curvatus*, *L. plantarum*, *L. sakei*, *L. lactis*, *P.*

pentosaceus, *W. confusa* and *W. koreensis* (Cho et al. 2006). Some important species thought to be responsible for kimchi fermentation are *Leuconostoc mesenteroides*, *L. pseudomesenteroides* and *L. lactis*, as the pH gradually falls to 4.0 (Kim and Chun 2005; Lee et al. 2005).

Kimchi contains various health-promoting components, including β-carotene, chlorophyll, vitamin C and dietary fibre (Nam et al. 2009). In addition, antimutagen (Oh et al. 2005), antioxidation and angiotensin-converting enzyme inhibition activities of kimchi are thought to protect against disease (Yoo et al. 2004).

Production of kimchi in Korea is used to preserve the fresh and crispy texture of vegetables during winter when the fresh vegetables are not available. Almost all kinds of vegetables can be used to produce kimchi: cabbage, cucumber, radish, Welsh onion leaves and mustard leaves are the most popular ingredients. Based on the main ingredient, kimchi has a particular name. Minor ingredients used in the production are ginger, green onion, red pepper and garlic, as well as salt is added. In general, kimchi has a sour, sweet, carbonated taste and is usually served cold.

The production process of kimchi is as follows: fresh-cut or shredded cabbage is soaked in brine with 10% salt overnight or in brine with 15% salt for 5–10 h. Also the temperature of brining determines the time. Salting is a very important step for taste, texture, fermentation and preservation. Brining at low temperatures results in kimchi with a better flavour compared to brined at higher temperatures. A salt concentration of 3% in the end product is aimed. A salt concentration lower than 2.2% in the final product results in a too fast fermentation, leading to quick acidification and soft cabbage tissues. As a result of the brining, the total aerobic bacteria, yeasts and mould counts are reduced, while the lactic acid bacteria are increased. After salting, the cabbage is washed and drained to regulate the salt content. Now other minor ingredients can be added. It is important that also these ingredients have a good quality as they can influence substantially the final quality of the kimchi. Traditionally, a large quantity of cabbage heads were used to make kimchi, which were further stored in underground potteries (onggi or doks) for winter. This practice is called kimjang. Normally this is done between mid-November and early December, depending on the climate of that particular year. This kimchi is then consumed the following spring. The freshly prepared unripened kimchi is tightly stacked in earthenware crocks, covered with the remaining leaves of the brined cabbage. Stones are used to weigh down the potteries and thus to guarantee the facultative anaerobic conditions. To maintain a constant temperature, crocks are buried underground for 80–90% of the container's depth. They then convened by thick rice straw mats to protect the kimchi from direct sunlight and cold. The flow diagram of kimchi fermentation is shown in Fig. 15.4.

The fermented product has an optimal taste at a pH of 4–4.5 and an acidity of 0.5–0.6 (expressed as % lactic acid equivalents.

Fermentation occurs mainly because of the endogenous microorganisms present on the raw material. Various microorganisms can initiate the fermentation process; however, the lactic acid bacteria ferment the sugars present in the cabbage and other subingredients. They gradually dominate the other anaerobic microorganisms by acid formation. Factors that influence the kimchi fermentation are the kind of microorganisms present, salt concentration, fermentable carbohydrates, the presence of

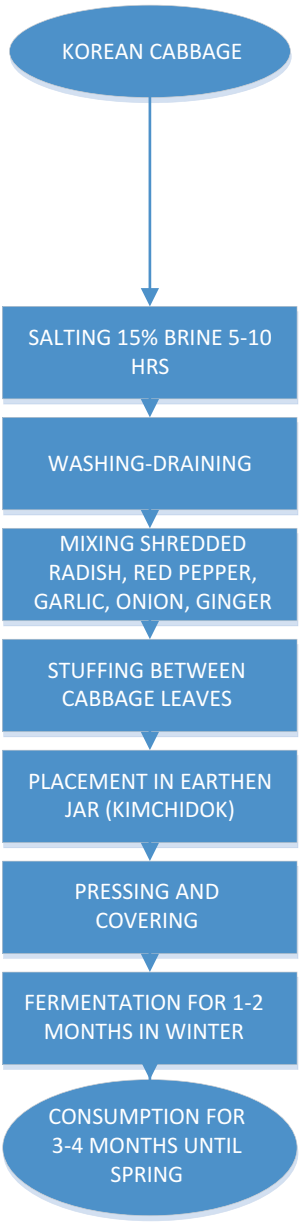

Fig. 15.4 Flow diagram for Kimchi fermentation

inhibitory compounds, oxygen, pH and temperature. *Leuconostoc mesenteroides* predominates during the first hours of the fermentation process. The pH starts to decrease. When the pH falls to 4, *Lactobacillus plantarum* becomes predominant.

Kimchi (kimjang) is the main fermented food consumed in South Korea. The kimchi preparation process is deeply rooted in the Korean culture and family life.

This tradition can be traced back to 1500 years ago (Surh et al. 2008; Oh et al. 2014). In 2015, the kimchi-making was added on UNESCO's list of the intangible cultural heritage of humanity. Each year after a harvest in November, Korean families and various communities collectively make and share large quantities of kimchi which will be consumed over the winter. As such, the kimchi-making can be considered as a cultural ritual that is strongly embedded in the Korean tradition.

South Korea has a harsh winter climate, and therefore preparation of kimchi accounts for a main food preservation technique that uses lactic acid fermentation. Despite the modern way of life in South Korea and available wide range of food products, kimchi remains to be consumed as a main side dish. It is estimated that adult Koreans consume 124, 3 g of kimchi per day (Surh et al. 2008).

Kimchi contains four types of ingredients: raw vegetables, seasonings, spices and other additional materials. There are up to 30 different types of vegetables that might be used for kimchi preparation. These include Chinese cabbage (*Brassica rapa L.* spp. *pekinensis*) and other vegetables such as radish, onion, red pepper, leek, carrot, watercress, cucumber and mushrooms. Frequently used seasonings are salt, sesame seeds, soybean sauce and corn syrup. The spices include black pepper, cinnamon, ginger, onion and mustard seed. Additional materials may include barley, rice, peer and apple. Kimchi also contains animal-derived proteins added such as shrimps, anchovy paste, octopus, oyster, pork and beef. Depending on the ingredients, methods of preparation, geographic locations and special occasions, such as weddings, different types of kimchi are prepared (Kim et al. 2014b; Patra et al. 2016).

The kimchi-making process is an anaerobic fermentation performed by various microorganisms at low temperatures (Oh et al. 2014; Kim et al. 2012). This characteristic allows Koreans to store prepared kimchi in traditional stone jars outside under the ground or in modern apartments inside the fridges while fermentation is still ongoing. Fermentation at low temperatures allows for a slow breakdown of different organic substances due to the enzymatic action of various microorganisms, this resulting in the digestion of carbohydrates to organic acids or alcohols. As such, kimchi preparation involves several stages including washing and cutting raw vegetables, brining (sodium chloride treatment) and blending vegetables with various spices, seasonings and other ingredients such as animal proteins. At the end, slow fermentation process is conducted at 4–5 °C (Cheigh and Park 1994; Kim et al. 2012; Battcock and Azam-Ali 1998). The microorganisms present in kimchi come from plant material, soil, water and in general local environment. Firstly, kimchi fermentation is initiated by a large variety of microorganisms present in raw materials; this process becomes later gradually dominated by lactic acid bacteria. The latter decreases the pH of kimchi to 4.5, eliminating other bacterial species and possible pathogenic once creating suitable conditions for food preservation. Typically, in order to complete the fermentation process, 30 days are needed (Jung et al. 2011). The texture of vegetables changes with time; kimchi gains acidity and softness accompanied by the accumulation of sugars and ascorbic acid. Nutritionally, fermented kimchi becomes a source of macro- and micronutrients, flavonoid antioxidants, dietary fibre, vitamins and other nutrients (Patra et al. 2016).

The consumption of fermented kimchi allows for colonization of the lower intestine by symbiotic bacteria. These become a part of the resident microbiome. The latter is estimated to harbour trillions of microbes, encompassing two broad phylogenetic groups *Firmicutes* and *Bacteroidetes*. Based on the sequencing of 16S rRNA genes extracted from human faecal samples, it was concluded that the human gut contains approximately 500–1000 distinct bacterial species with the dominance of *Bacteroidetes* and *Firmicutes* accounting for 90% of all present phylotypes (Krajmalnik-Brown et al. 2012; Jung et al. 2011; Jiang et al. 2016). It is postulated that gut microbes have coevolved with the human taking over some of the vital biochemical functions that were lost by the human body. As such, the gut microbiome is involved in different biological reactions such as digestion of plant-derived dietary fibre, expenditure of energy obtained from food, micronutrient synthesis and drug metabolism (D'Argenio and Salvatore 2015). Lactic acid bacteria present in kimchi were found to belong to the phylum *Firmicutes*, and as such ingestion of kimchi may contribute to the overall composition of the human microbiome (Jiang et al. 2016; Jung et al. 2011).

In relation to a dietary fibre, kimchi contains non-digestible carbohydrates that pass through the large intestine. These carbohydrates include fibres such as cellulose, pectins, hemicellulose, polyfructoses and lignins that constitute cell wall of plants. They are the source of energy for the resident microbiome species, and as such kimchi can be considered as having prebiotic properties stimulating growth and activity of beneficial bacteria in the gut. The latter have an ability to salvage energy from the plant material that is non-digestible for the human. As such, they digest fibre into molecules called short-chain fatty acids (SCFA) and produce a wide range of metabolites in the gut (Ríos-Covián et al. 2016). These metabolites are used by microbes as a source of energy for their own growth as well as they contribute to the metabolic cross feeding of other resident microbiota that have reduced fermentability potential. Taken together, kimchi-derived prebiotic characteristics aid in the maintenance of a wide spectrum of intestinal microbes (Park et al. 2015).

Moreover, the SCFA resulted from kimchi fibre breakdown such as acetate, propionate, butyrate and lactate can be utilized also by the human body. It was demonstrated that human colonic epithelium is able to metabolize butyrate, and the liver can utilize propionate and muscle acetate. As such after absorption, SCFA are used in human gluconeogenesis and lipid biosynthesis. For example, propionate can serve as precursor of gluconeogenesis and at the same time was shown to attenuate lipogenesis by inhibition of fatty acid synthase (FAS) expression (Ríos-Covián et al. 2016; Canfora et al. 2015). Taken together, the SCFA were shown to influence the physiology of the large intestine; they can be used as nutrient and energy sources by host cells and microbiota. Moreover, SCFA were also shown to be responsible for lowering of the intestinal pH, enhancing the absorption of various nutrients, causing an increase in mucin production, enhancing gut integrity through tight junction formation and impacting on bacterial cell adhesion (Ríos-Covián et al. 2016).

Lactic acid bacteria identified in kimchi may play a role in the synthesis of vitamins group B and K (Patel et al. 2013; Capozzi et al. 2012). Vitamins are known to be essential micronutrients being cofactors of various enzymes involved in vital

biochemical processes ongoing in every living cell. Humans are incapable of synthesizing most vitamins, and they have to rely on the external dietary sources. Lactic acid bacteria are able to de novo conduct synthesis of various vitamins such as biotin, pantothenic acid, folate and riboflavin, cobalamin and vitamin K (Thakur et al. 2016; Patel et al. 2013). The large intestine is able to absorb some of the vitamins, and as such the microbe-derived vitamins and vitamins present in kimchi-derived vegetables contribute to the total body nutrition and local nutrient requirements of colonocytes. For example, different lactic acid bacteria such as *Lactobacillus* species in various fermented foods are known to produce folate (Rossi et al. 2011). As such these bacteria present in kimchi might contribute to the vitamin biosynthesis. However, the mechanisms and uptake efficiency remains to be further clarified. Another species reported to be present in kimchi *Bacillus subtilis* could contribute to the overall synthesis of riboflavin (Lee et al. 2013). This vitamin is a precursor of coenzymes such as flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). Both molecules are important hydrogen carriers in various cellular redox reactions (Hustad et al. 2002).

The complexity of bacterial species present in kimchi was studied using first culture-dependent methods and recently taking a culture-independent metagenomic approaches. First studies used 16S rRNA genes as a phylogenetic marker (Kim and Chun 2005; Park et al. 2010). These resulted in identification of various bacterial species belonging to lactic acid bacteria such as *Leuconostoc mesenteroides*, *Le. kimchi*, *Le. citreum*, *Le. gasicomitatum* and *Le. gelidum*. In addition, microbes present in kimchi contained various species of *Lactobacillus* such as *L. brevis*, *L. curvatus*, *L. plantarum*, *L. sakei* as well as *Lactococcus lactis*, *Pediococcus pentosaceus*, *Weissella confusa*, *W. kimchi and W. koreensis* (Bae et al. 2005; Park et al. 2010; Kim et al. 2000a; Lee et al. 2005; Shin et al. 2008). Culture-independent metagenomic approaches based on the random sequencing of collected sample can provide a large number of data eliminating at the same time PCR bias that can be introduced while using primers derived from 16S rRNA genes. The usage of 16S rRNA sequences might also be insufficient to find a good correlation with phenotypic and genotypic diversity (Wilmes et al. 2009). Therefore, metagenomic approach based on next-generation sequencing offers a possibility to analyse DNA that is randomly fragmented in order to obtain a mean fragment size of 680 bp for sequencing (Jung et al. 2011). Recent metagenomics analysis of kimchi microbiome using pyrosequencing method revealed that kimchi microbiome was dominated by three genera: *Leuconostoc*, *Lactobacillus and Weissella* confirming previous studies. In particular mapping of metagenomics reads onto the available database of completed genomes indicated that *Leuconostoc mesenteroides* subsp. and *Lactobacillus sakei* subsp. were highly represented in fermented kimchi (Jung et al. 2011).

Interestingly metagenomics analysis revealed also a high abundance of phage DNA pointing out that bacterial species present in kimchi might be infected with bacteriophages. Further detail analysis revealed the absence of *Archaeal* sequences in kimchi preparations (Jung et al. 2011). In addition, this study indicated that kimchi fermentation is a dynamic process leading to a subsequent appearance and disappearance of various bacterial species depending on an increasing pH. While analysis

of early fermentation stages indicated a presence of many unclassified *Deferribacterales* and other unclassified bacteria, late fermentation process led to gradually decrease in initial bacterial species (Jung et al. 2011). The latter were replaced by three genera *Leuconostoc*, *Lactobacillus* and *Weissella*. These results were also confirmed by a metatranscriptomic kimchi study which used unbiased RNA sequencing to analyse complexity of microbial communities (Jiang et al. 2016).

Thus, taking into account the dietary fibre content of kimchi, it can be considered that kimchi represents a main prebiotic food source present in Korean daily diet. At the same time, metagenomic analysis indicated that kimchi contained a large number of different bacterial species predominantly lactic acid bacteria. These when ingested can complement the action and composition of human gut microbiome and therefore constitute the main source of probiotics consumed by Koreans (Park et al. 2015).

As mentioned above, lactic acid bacteria can take over some of the vital biochemical functions in our bodies. They have an ability to digest plant-derived fibre and bake it down to SCFA. The latter constitute precursors for gluconeogenesis and lipid synthesis. Moreover, SCFA are involved in lowering of the intestinal pH and mucin production, enhancing the absorption of various nutrients and improving gut integrity as such having an impact on bacterial cell adhesion, possibly preventing colonization of the gut by pathogenic bacteria. It was also reported that SCFA have the ability to protect against the development of colorectal cancer, with most studies pointing out the versatile role of butyrate. This SCFA was shown to promote colon motility, inhibit tumour cell progression and induce apoptosis (Ríos-Covián et al. 2016). Thus, kimchi-derived SCFA could be considered as major nutrition-derived molecules that impact on gut physiology and pathology. Kimchi-derived bacteria also may contribute to de novo biosynthesis of vitamins.

Finally, kimchi effect on the development of various diseases deserved additional attention. It was reported that ingredients in kimchi and lactic acid bacteria can cause inhibition of carcinogenesis. These bacteria were shown to suppress the activity of carcinogen-activating enzymes such as azoreductase, nitroreductase, 7-α-dehydrogenase, β-glucosidase and β-glucuronidase (Kwak et al. 2014). Secreted by *Leuconostoc mesenteroides*, *Weissella cibaria* and *Weissella confusa* exopolysaccharides have anti-inflammatory properties as well as blood cholesterol-decreasing functions (Park et al. 2013; Badel et al. 2011). In particular anticancer activity was observed in case of *Weissella cibaria.* It was demonstrated using colorectal cancer cells that this kimchi-derived species is able to inhibit cell growth in vitro and induce high levels of nitric oxide, TNF-a, interleukine-1b and NF-κB (Ahn et al. 2013; Kwak et al. 2014). Moreover, *Lactobacillus plantarum* was shown to have an antimutagenic effect against aflatoxin B1, N-methyl-N′-nitro-N-nitrosoguanidine and 4-nitroquinoline-1-oxide (Son et al. 1998; Rhee and Park 2000).

Studies in mice with the azoxymethane and dextran sulphate sodium-induced colon cancer indicated that mice treatment with kimchi extracts had the lowest number of tumours in the large intestine. This treatment was also able to suppress colonic mucosal damage and neoplasia accompanied by the decrease in mRNA levels of TNF-α, IL-6, IFN-γ, p53 and p21. The mRNA expression of the inducible nitric

oxide synthase and cyclooxygenase-2 (COX-2) was also downregulated as compared to control mice (Kim et al. 2014a).

Various ingredients present in kimchi such as capsaicin in red pepper or garlic-derived organosulfur compounds were shown to exert anticancer effect. Capsaicin was able to induce apoptosis in breast cancer cells by having an impact on the cell cycle arrest and apoptosis by modulating EGFR/HER-2 pathway (Thoennissen et al. 2010; Jeong et al. 2013). Organosulfur compounds from garlic were shown to have anti-proliferative activity via induction of caspase-3 and cell cycle arrest (Pinto and Rivlin 2001; Xiao et al. 2003).

In vitro studies with various tumour cell lines and mouse models were successful in pointing out some of the beneficial properties of kimchi. However, the conducted randomized clinical trials can prove to be more challenging in order to demonstrate significance of potential beneficial immunomodulatory properties of kimchi. Healthy students received daily portions of kimchi for four weeks followed by the analysis of various cell populations, cytokine secretion and induction of immunoglobulin secretion. There were no significant differences in levels of immunoglobulins, IL-6, TNF-α, IL-4 and IL10 as well as numbers of T-cell, B-cell and NK cells between the groups that received kimchi and non-kimchi controls (Lee et al. 2014). As such daily ingestion of kimchi had no short-term immunomodulatory effects in healthy volunteers.

The consumption of kimchi also raises some safety concerns as various preparations may contain pathogenic bacterial strains. In 2012, the outbreak of the food-borne enterotoxigenic *E. coli 0169* was reported in South Korea (Cho et al. 2014). This incident was followed by the occurrence of kimchi-derived norovirus GI4 in 2013 (Park et al. 2015). Various studies confirmed that pathogenic bacteria such as *E.coli 0157*, *Salmonella enteritidis*, *Staphylococcus aureus* and *Listeria monocytogenes* can survive and multiply in various kimchi preparations (Inatsu et al. 2004; Cho et al. 2011). As kimchi-making process is often done at home by the family members, monitoring of the appearance of different bacterial species might be advisable in order to avoid spreading of food-borne diseases. The recent availability of metagenomic data pointed out the high abundance of unidentified sequences of bacterial origin as well as high representation of sequences belonging to DNA phages in commercially available kimchi (Jung et al. 2011). It means that the lactic acid bacteria present in kimchi might harbour bacteriophages, and the impact of those on human health remains to be further elucidated and considered.

15.8 The Role of Lactic Acid Bacteria in Fermented Vegetables

Lactic acid fermentation plays an important role in preserving fresh vegetables, fruits and other food items for feeding humanity in developing countries. However, several fermented fruits and vegetables (sauerkraut, kimchi, gundruk, khalpi, sinki, etc.) have a long history in human nutrition from ancient times and are also

well associated with social aspects of different communities. Among the food items, fruits and vegetables are easily perishable commodities due to their high water activity and nutritive values. These conditions are more critical in tropical and subtropical countries which favour the growth of spoilage microorganisms. Lactic acid fermentation increases the shelf life of fruits and vegetables and also enhances several beneficial properties, including nutritive value and flavours, reducing toxicity at the same time. Fermented fruits and vegetables can be used as a potential source of probiotics as they harbour several lactic acid bacteria such as *Lactobacillus plantarum*, *L. pentosus*, *L. brevis*, *L. acidophilus*, *L. fermentum*, *Leuconostoc fallax* and *L. mesenteroides*. As a whole, traditionally fermented fruits and vegetables not only serve as food supplements but also attribute towards health benefits. This review by Swain et al. (2014) aims to describe some important Asian fermented fruits and vegetables and their significance as a potential source of probiotics.

Asian traditional fermented foods are generally fermented by LAB such as *Lactobacillus plantarum*, *L. pentosus*, *L. brevis*, *L. fermentum*, *L. casei*, *Leuconostoc mesenteroides*, *L. kimchi*, *L. fallax*, *Weissella confusa*, *W. koreenis*, *W. cibaria* and *Pediococcus pentosaceus*, which are considered as the probiotic source of the food practice. Availability of certain specific nutrients such as vitamins, minerals and acidic nature of fruits and vegetables provides conducible medium for fermentation by LAB.

With the popularity and success of sauerkraut, fermentation of many other vegetables has emerged, such as cucumbers, beets, turnips, cauliflower, celery, radishes and carrots (Roberts and Kidd 2005).

Depending on the type of raw materials in final fermented products, vegetable fermentation is characterized accordingly. Sauerkraut, fermented cucumbers and kimchi are the most studied lactic acid fermented vegetables mainly due to their commercial importance. Canning or freezing is often a too expensive method in food preservation which cannot be afforded by millions of world's economically deprived people and lactic acid fermentation (Paramithiotis et al. 2010).

LA fermentation of vegetable products applied as a preservation method for the production of finished and half-finished products is considered as an important technology and is further investigated because of the growing amount of raw materials processed in the food industry (Montet et al. 2006), and these foods are well suited to promoting the positive health image of probiotics (Heller 2001).

Some of the fermented fruits and vegetables contain coloured pigments such as flavonoids, lycopene, anthocyanin, β-carotene and glucosinolates, which act as antioxidants in the body by scavenging harmful free radicals implicated in degenerative diseases like cancer, arthritis and ageing (Kaur and Kapoor 2001). Lactic acid fermentation of vegetables has an industrial significance only for cucumbers, cabbages and olives (Montet et al. 2006). In Italy, the industrial production of fermented vegetables is limited to sauerkrauts and table olives (Di Cagno et al. 2008).

According to Kim et al., the Chinese cabbage, cabbage, tomato, carrot and spinach provide relatively higher fermentability than other vegetables (okra and gourds) because they have more fermentable saccharides (Kim et al. 2000a).

15.9 Conclusions

Different fermented vegetables have been described in this chapter. For example, soybean tempe and other soybean paste products, sauerkraut, fermented olives, fermented cucumber and kimchi. Moreover, salting procedures are well explained here along with the role of lactic acid bacteria in fermented vegetables.

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Part III
New Technologies

Chapter 16
New Technologies and Edible Coatings for Minimally Processed and Refrigerated (MPR) Fruits and Vegetables (Fresh Cuts and Freshly Squeezed Juices)

Gürbüz Güneş and Deniz Turan

16.1 Introduction

Fresh fruits and vegetables have a lot of well-known health benefits to consumers through their rich nutritional constituents. This has led to increased consumption of fresh produce products. It is known that the conventional processing technologies cause destruction of nutritional constituents as well as loss of organoleptic quality of fresh produce products. Consumers' lifestyle has been changed significantly during the last couple of decades in that they are more aware of the consequences of diet on their health and they look for more convenient foods, i.e., ready-to-cook or ready-to-eat foods. They also demand for products with fresh or fresh-like quality and reduced synthetic additives in food. Therefore, fresh produce product in convenient forms with retained fresh or fresh-like quality attributes has been highly demanded. Such products are also known as minimally processed refrigerated fruits and vegetables (MPRFV) which include various fresh-cut fruits and vegetables and freshly squeezed juices. Production of MPRFV has been a challenge for both food manufacturers and researchers, because conventional processing technologies are insufficient to produce such products with reasonable shelf life and acceptable quality. Fresh-cut processing involves unit operations such as peeling, slicing, dicing, and shredding, all of which cause excessive tissue damage and make the product more favorable to microbial contamination and growth.

Thus, they are more perishable compared to their whole uncut counterparts. Moreover, fresh-cut products can be contaminated by foodborne pathogens and carry a safety risk as they are often served raw. Several foodborne disease outbreaks associated with consumption of fresh-cut leafy greens, cantaloupes, cucumbers, and fenugreek seeds have been reported in the US and Europe

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Table 16.1 Typical new technologies applicable at different production stages of minimally processed refrigerated fruits and vegetable products

Production stage or objective	Applicable new technologies
Washing and disinfection of raw material prior to processing	Ozone Electrolyzed water Ultrasound
Treatment of fresh-cut products before packaging	Ultraviolet light Pulsed light Ultrasound
Treatment of fresh-cut product after packaging	High-pressure processing Irradiation
Reducing cutting force	Pulsed electric field
Increasing juice yield	Pulsed electric field
Treatment of freshly squeezed juice before packaging	Pulsed electric field High-pressure processing Dense CO_2 processing Ultraviolet light Pulsed light Ultrasound
Treatment of freshly squeezed juice after packaging	High-pressure processing Irradiation

(Muranyi 2012). MPRFV products have to be fresh; thus, conventional preservation technologies such as thermal processing, freezing, or drying are not appropriate for them. Thus, their production requires novel technologies to make the product stable and safe, but at the same time to retain fresh-like quality attributes. Fruit juices are being manufactured and thermally processed for their storage stability and safety. However, the applied thermal process generally results in undesirable effects on flavor, and consumers demand for juice product with freshly squeezed flavor and quality. Minimally processed fruits and vegetable juices also require novel nonthermal technologies to achieve this demand. Typical new technologies applicable at different production stages of minimally processed refrigerated fruits and vegetable products are given in Table 16.1. The following sections briefly describe these novel technologies as applied in production of the minimally processed refrigerated fresh produce products.

16.2 Novel Technologies for Minimally Processed Refrigerated Fruits and Vegetables

16.2.1 Ozone

Ozone is a strong antimicrobial agent with high reactivity and penetrability. It gives nontoxic decomposition products (Gras et al. 2003). It can be used in decontamination of whole or fresh-cut commodities as part of washing treatment prior to

processing and packaging as an alternative to traditional disinfectants like chlorine. Ozone can be used in gaseous form (fumigation) or in aqueous form (ozonated water). It is rapidly oxidized to oxygen leaving no residues in foods. Ozone is more soluble and thus more effective at low temperatures. The efficacy of ozone is also dependent on its concentration, pH, and the amount of organic constituents in the medium (Priyanka et al. 2014). Lower pH, higher concentration, and less organic solids in the medium would increase the microbial inactivation by ozone treatment. On the other hand, higher ozone concentration may have adverse effects on organoleptic and nutritional quality of the product.

Treatment with ozone extended the storage life of fresh whole commodities such as broccoli, cucumber, apples, grapes, oranges, pears, raspberries, and strawberries by reducing microbial populations and by oxidation of ethylene (Skog and Chu 2001). Ozonated water has been applied also to fresh-cut products, and significant reduction in microbial populations and extension of shelf life of the products have been reported in various studies (Beltrán et al. 2005). Ozone treatments on fresh produce reduced various types of decays caused by *B. cinerea*, *Penicillium digitatum*, *A. alternata*, *C. coccodes*, and *Sclerotinia sclerotiorum* on various fresh produce such as strawberries, tangerine fruit, tomatoes, kiwifruit, and carrots (Hildebrand et al. 2008; Minas et al. 2010; Nadas et al. 2003; Tzortzakis et al. 2008; Whangchai et al. 2010). Prevention of fungal decay and maintenance of color were observed in blackberries treated with 0.3 ppm gaseous ozone (Barth et al. 1995). Ozone in gaseous form at 5 ppm resulted in significant inactivation of total microbial count, coliform, *S. aureus*, and yeast/molds (Najafi and Khodaparast 2009). Ozone treatment has also been suggested for treating grapes to inhibit growth of mold (Gabler et al. 2010). Ozone treatment also provided odor control and extended storage life of fresh produce by destroying the ripening-/senescence-promoting hormone ethylene (Palou et al. 2001; Skog and Chu 2001). Combination of ozone and chlorine had beneficial effects on quality and shelf life of fresh-cut lettuce (Garcia et al. 2003). Ozone in aqueous form resulted in up to 2.9 log reduction in inoculated *E. coli* and *Listeria* on fresh-cut lettuce, spinach, and parsley, which was comparable to chlorine treatment (Karaca and Velioglu 2014).

Ozone in gaseous form can also be bubbled into fruit juice to inactivate microorganisms. Ozone applied to apple juice resulted in 5 log inactivation of inoculated *E. coli* and extended shelf life (Patil et al. 2010). Torres et al. (2011) reported that ozone treatment affected color, rheological properties, and phenolic content of treated apple juice, and thus, the impact of the treatment on the quality aspects must also be considered in ozone treatment (Torres et al. 2011).

Based on the available information in literature, ozone seems to be an effective disinfectant and can be used in both fresh-cut product and juices to inactivate spoilage and pathogenic microorganisms as an alternative to chlorine. The effects of ozone on the desired objective depend on several factors including temperature, pH of the product, pulp content, and the target microorganisms. It should be noted that the ozone treatment can have an adverse effect on product quality, and thus its application must be optimized considering this aspect as well.

16.2.2 Electrolyzed Water

Electrolyzed water (EW) is conventionally generated by electrolysis of aqueous sodium chloride to produce an electrolyzed basic aqueous solution at the cathode and an electrolyzed acidic solution at the anode (Kim et al. 2000). Negatively charged ions, such as hydroxide ions and chloride ions in the salt solution, move to the anode to give up electrons and form oxygen gas, chlorine gas, hypochlorite ion, hypochlorous acid, and hydrochloric acid, while positively charged ions such as hydrogen ions and sodium ions move to the cathode to take up electrons and form hydrogen gas and sodium hydroxide (Hsu 2003).

Fresh-cut vegetables including carrot slices, trimmed spinach leaves, shredded Japanese radish, chopped bell pepper, and diced potatoes have been treated with electrolyzed water and found that it was highly effective in surface decontamination without any adverse effect on color and taste of the products (Izumi 1999). Mildly heated (45 °C) and slightly acidic (pH 5.5) electrolyzed water was highly effective on decontamination of carrot slices (Koide et al. 2011).

Acidic EW (pH 2.1–4.5) has a stronger bactericidal effect against pathogens and spoilage microorganisms than chlorine due to its high oxidation-reduction potential (Bari et al. 2003). Acidic EW has higher effectiveness in reducing viable aerobes on whole produce (Koseki et al. 2001), but its use on fresh-cut product was limited due to adverse effects on quality (Wang et al. 2004). The use of neutral EW offers the advantage over acidic EW in that the first does not affect the pH, surface color, or general appearance of the treated fresh-cut products (Izumi 1999). EW treatment was shown to preserve quality and extend shelf life of mushrooms, minimally processed cabbage, lettuce and chicory, and apples (Aday 2016; Graca et al. 2011; Pinto et al. 2015).

All of these research showed that EW seem to be an effective decontamination process with reasonable cost and can replace chlorine in washing treatments. However, more studies focusing on effects of EW on organoleptic quality of fresh-cut products are needed.

16.2.3 Gamma Irradiation

Food irradiation involves exposure of food products to ionizing radiation from either gamma rays or electron beams to extend their shelf life and improve safety. The process involves use of either gamma rays, generally from Co-60 radioisotopes, or electron beams generated by electron accelerator machines. The irradiation process is a nonthermal treatment that is highly effective in inactivation of microorganisms and pests. It also affects physiological processes in fresh produce, contributing to their control for shelf life extension. Because the ionizing radiation has high penetration power, it is applicable to food products in their final packaging. Minimally processed fruits and vegetables are prone to microbial contamination and growth as well as increased metabolic activities such as respiration and ethylene

production due to mechanical damage caused by applied processes like peeling, cutting, slicing, etc. Appropriate preservation methods are required to inactivate pathogenic and spoilage microorganisms as well as biochemical degradations while keeping their fresh-like quality attributes. Being a nonthermal treatment, ionizing radiation can be an effective process for MPRFV products. There have been several studies reporting the use of ionizing radiation in MPRFV products. FDA approved irradiation of potatoes at maximum of 0.15 kGy for sprout inhibition in 1963, fresh produce at maximum of 1 kGy for insect control and delay of physiological growth in 1986, seeds for sprouting to inactivate pathogens at maximum of 8 kGy in 2000, and fresh iceberg lettuce and fresh spinach at maximum of 4 kGy for inactivating pathogens and shelf life extension in 2008 (Stefanova et al. 2010). The applied dose is usually limited by softening in fresh produce. Irradiation caused breakdown of pectic substances in the middle lamella of tissues causing softening depending on the type of produce and the applied dose (Gunes et al. 2001). Thus, the applicable dose has to be determined by considering both the desired and undesired effects of irradiation on the product. Irradiation can be used for fresh-cut product for controlling physiological changes, inactivating pathogenic and spoilage microorganisms at appropriate doses in their final packaging under refrigerated conditions.

Significant amount of information on the use of irradiation on fresh produce products exist. The irradiation of minimally processed carrots improved their color and flavor, although it impaired their texture (Chervin and Boisseau 1994). In minimally processed lettuce, doses of up to 0.5 kGy did not adversely affect quality, but some adverse effects have been reported upon irradiation at 0.81 or 1.1 kGy (Foley et al. 2004; Goularte et al. 2004; Niemira et al. 2002). Microbiological studies carried out in cantaloupes showed that samples irradiated had a lower and more stable rate of respiration and lower microbial counts than non-irradiated control samples through storage (Boynton et al. 2006).

Irradiation of fresh-cut apples stimulated respiration rate, and this was affected by applied dose, cultivar, and maturity level (Gunes et al. 2000). The authors reported that irradiation doses less than 1.2 kGy had no effect, and doses between 1.2 and 2.4 kGy had minimal effect on respiration rates of apple slices from four different cultivars. They also observed that stimulatory effects of irradiation on respiration rate were not significant in postclimacteric apple slices, but significant in preclimacteric slices in their study. Irradiation had an inhibitory effect on ethylene production rate of apple slices. On the other hand, the same researchers reported that irradiation beyond 0.34 kGy resulted in softening in apple slices, which was positively correlated with water-soluble pectin content in the tissues (Gunes et al. 2001). They found that 2% calcium treatment prior to irradiation at 1 kGy prevented irradiation-induced softening in the tissues.

Irradiation was shown to be effective in inactivation of various potential pathogens such as *E. coli* O157:H7, *Salmonella*, *Shigella*, and *Listeria* in various fresh-cut product such as lettuce, spinach, parsley, chives, and cabbage at doses up to 1.5 kGy without having any adverse effects on their organoleptic quality (Banerjee et al. 2016; Junqueira-Gonçalves et al. 2012; Mahmoud 2012; Mintier and Foley 2006; Zhang et al. 2006). The combined use of MAP and irradiation reduced adverse

effects of irradiation on fresh produce product (Fan et al. 2012; Smith et al. 2016). It has been also shown that irradiation can increase phenolic content and total antioxidant activity of some fresh produce products (Fan 2005; Patil et al. 1999; Oufedjikh et al. 2000). Irradiation has also been tested on fruit juices such as mango and sour cherry juice recently. Irradiation up to 3 kGy resulted in significant microbial inactivation, increase in phenolic content and total antioxidant activity, decrease in ascorbic acid content, and no change in sensory quality in mango juice (Naresh et al. 2015). Sour cherry juice has been irradiated at various doses, and a maximum of 3 kGy was suggested to effectively inactivate microorganism without causing significant adverse effects on quality (Arjeh et al. 2015).

Overall, irradiation can be an effective tool to assure safety of fresh-cut product and extend their shelf life, but the applicable dose must be carefully determined considering its potential adverse effects on quality especially the texture. Irradiation treatment requires special radiation source (radioisotopes or electron accelerator) and facility (irradiation plant), which requires high capital investment as well as compliance with strict regulations. In practice the packaged products are shipped to centralized food irradiation plants to be irradiated. This can be inconvenient for the producers of MPRFV products as these products are highly perishable. Thus, proximity of the manufacturing facility to irradiation plant becomes critical. On the other hand, on-site installation of electron accelerators can be feasible for manufacturers with large capacities.

16.2.4 Ultraviolet Light

Ultraviolet light (UVC) at 254 nm has germicidal effect and thus can be used to inactivate microorganisms in foods. However, UVC has no penetration power on opaque foods, and thus it is effective only at the surfaces where the light can reach. Special mercury lambs are used to generate UVC. UVC inactivates microorganisms mainly due to the formation of dimer which inhibits the formation of new DNA chains in the cell (Gomez-Lopez et al. 2007). It can be applied to whole or fresh-cut product for disinfection of microorganisms, but the system must be designed properly so that all the surfaces are exposed to UVC effectively. Special UVC treatment chambers can be designed to process whole or fresh-cut products in a continuous system. Although, UVC would cause a considerable microbial inactivation and thus extend shelf life of fresh-cut product, it is not sufficient to ensure microbial safety of these products due to improper exposure of the product to UVC, which may allow survival of potential foodborne pathogens. Thus, it should be used in combination with other treatments for the safety assurance of the product.

UVC treatment of melon cubes at 1200 J/m^2 resulted in 2 log inactivation of microbial load with no adverse effects on organoleptic quality, thus contributing to shelf life extension (Manzocco et al. 2016). Allende et al. (2006) reported a significant microbial decontamination of fresh-cut lettuce by two-sided UVC treatment at 2.7 kJ/m^2, but it caused softening and browning in the tissues at higher doses

(Allende et al. 2006). Conflicting and unclear effects of UVC treatments on microbial growth on pomegranate arils have been reported (López-Rubira et al. 2005). UVC treatment at 8 kJ/m^2 delayed yellowing and chlorophyll degradation, retained better tissue integrity, increased phenolic content, and decreased microbial population in broccoli during storage (Lemoine et al. 2007). UVC treatment also resulted in shelf life extension through microbial inactivation in fresh-cut melon and pineapple (Manzocco et al. 2011, 2016). Exposure to UVC also induces the synthesis of health-promoting compounds such as anthocyanins and stilbenoids (Lu et al. 2016). The UVC treatment systems are relatively inexpensive and easy to use, and thus even small manufacturers can have them installed in their plants (Bintsis et al. 2000). However, high UV doses can cause damage to the treated tissue (Ben-Yehoshua et al. 1992; Nigro et al. 1998). UVC treatment of minimally processed lettuce reduced microbial deterioration, but increased respiration rate and lignification-like process depending on the applied doses (Allende and Artés 2003).

UVC can also be used for freshly squeezed juices for pasteurization purpose. There is special flow through UVC reactor designed for liquid products. The design of these systems is critical to ensure total exposure of the juice (must be in thin layer) to UVC at target levels to achieve the desired microbial inactivation. Several types of continuous UVC treatment reactor design with laminar or turbulent flow have been described (Koutchma 2009). Shelf life extension of freshly squeezed orange juice and apple juice by UVC treatment has been reported with no significant quality loss compared to the fresh samples (Donahue et al. 2004, Torkamani and Niakousari 2011). UVC treatment of fresh apple cider resulted in 5 log inactivation of pathogenic microorganisms in a specially designed system (Duffy et al. 2000). Further studies on quality changes and enzyme inactivation by UVC are needed.

Overall, UVC treatment can be highly effective for surface decontamination of whole or fresh-cut product as well as treatment of fresh juices. Being simple, easy to use, flexible, and relatively inexpensive, UVC systems can be installed in fresh produce processing plants even at small capacities. More research and development work is required to design UVC systems applicable to various types of fresh-cut product with different surface qualities as well as for different fresh juices to have effective microbial inactivation. Moreover, more studies on organoleptic quality aspects of the UVC treatment are required.

16.2.5 Pulsed Light

Pulsed light treatment involves the use of inert gas flash lamps emitting a broad spectrum including UV, visible, and infrared. A moderate amount of energy is stored in a capacitor and delivered within extremely short time (fractions of a second) with high intensity in pulsed light applications. This treatment is rapid and effective on microbial inactivation in both solid and liquid foods. Pulsed light has both photochemical and photothermal effects depending on the spectrum involved (Gomez-Lopez et al. 2007). UVC-rich pulsed light has photochemical effects which are the

intensified effects mentioned for the continuous UVC treatment described in the previous section. Inclusion of high levels of visible and infrared wavelength would have photothermal effects which include significant temperature increase on the treated surfaces causing thermal sterilization. However, nonthermal treatments are required for production of MPRFV products to maintain fresh-like quality. Thus, UVC-rich pulsed light would be applicable for them. As similar to UVC treatment, pulsed light has no penetration power, and thus, it is also a surface treatment. The total exposure of the product surface to pulse is critical for an effective decontamination. Significant inactivation (3–4 log reduction) of *E.coli* O157:H7 and salmonella on strawberries and raspberries by pulsed light treatments of 72 J/cm^2 without any noticeable quality degradation has been reported (Bialka and Demirci 2007). Fungal inactivation on fruit surfaces required much less treatment doses (Lagunas-Solar et al. 2006). Treatment up to 47.8 kJ/m^2 maintained texture and inhibited fungal growth on strawberries during storage (Duarte-Molina et al. 2016). A temporary off-odor has been reported in minimally processed cabbage upon pulsed light treatment (Gomez-Lopez et al. 2005). Effective microbial inactivation and inhibition of tissue browning were observed on apple slices treated with pulsed light at 221 J/cm^2 (Gómez et al. 2012). Similarly, pulsed light treatment at 12 J/cm^2 inactivated *E.coli* and *Listeria* by 2–3 logs but caused substantial adverse effects on quality (Ramos-Villarroel et al. 2012). Treatment at 7.8 J/cm^2 significantly inactivated microorganisms and increased the shelf life of fresh-cut cantaloupe with retention of nutritional quality (Koh et al. 2016). Pulsed light treatment has also been shown to be effective in inactivation of various microorganisms in juices like apple and orange (Pataro et al. 2011; Sauer and Moraru 2009).

Like continuous UVC treatments, PL treatment also has high potential to be used in production of various MPRFV products. Pulsed light treatment must be optimized for each product to have maximum microbial inactivation and simultaneously have minimum adverse effects on quality. The overall system is also simple and relatively inexpensive and, thus, can be afforded by the industry. The design of treatment systems for both fresh cuts and juices plays a key role in this application and is subject of research.

16.2.6 Ultrasound

Ultrasound is a form of energy generated by sound waves of frequencies higher than 20 kHz. It causes cavitation which includes formation, growth, and collapse of bubbles that generate a localized and instantaneous shear, high pressure, high temperature, and chemical effects. Ultrasound through cavitation can decontaminate food surfaces, aid in removing dirt and breaking biofilms, and also inactivate microorganisms. Ultrasound can be used in minimally processed fresh fruits, vegetables, and juices. It can be used to improve washing process prior to minimal processing operations. Moreover, it can be combined with antimicrobial treatments (dipping, spraying systems) to improve the disinfection process. Minimally processed juices

can also benefit from ultrasound treatment in these applications as well as in pasteurization process. Manothermosonication which combines thermal and pressure treatment with ultrasound is known to have significant microbial inactivation in various foods including juices.

Reduction in *S. typhimurium*, *E. coli*, *S. aureus*, *S. enteritidis*, and *E. coli O157:H7* up to 6 logs was reported after 32–40 kHz for 10–45 min of ultrasound treatment on several fresh-cut products (Birmpa et al. 2013; Forghani et al. 2013; Seymour et al. 2002). Ultrasound alone or in combination with peracetic acid was effective in removing *Salmonella* from cherry tomatoes (de São José et al. 2014; São José and Vanetti 2012). Ultrasound at 40 kHz for 10 min at 20 °C inhibited decay incidence and number of microorganisms on fresh strawberries as well as better overall quality maintenance (Cao et al. 2010). For fresh-cut products, the pH, vitamin C, acidity, and total soluble solids were retained better between 30 and 40 min, while firmness and color of the fruit were found to be optimum between 20 and 30 min after ultrasound treatment at 33 kHz, 60 W (Amaral et al. 2015).

Treatment with a high-power sonicator working at 20 kHz under various conditions (treatment time, 3, 6, and 9 min; sample temperature, 20, 40, and 60 °C; amplitude, 60, 90, and 120 mm) resulted in reduced yeast and mold counts in apple juice, papaya juice, blackberry juice, orange juice, and kiwifruit juice (Athmaselvi 2008; Bevilacqua et al. 2013; Simunek et al. 2013; Tomadoni et al. 2015; Yuan et al. 2009). Carrot juice sonicated at 58 °C (24 kHz, 120 μm amplitude) maintained good quality and low microbial growth: 3 logs for mesophiles, 4.5 logs for yeasts and molds, and 2 logs for enterobacteria after 20 days of refrigerated storage (Martínez-Flores et al. 2015). A multifrequency power ultrasound was applied to cloudy apple juice and found that treatment was effective against pathogens such as *E. coli* O157:H7, *Salmonella* spp., *L. monocytogenes*, and yeast including *Debaryomyces hansenii*, *Torulaspora delbrueckii*, *Clavispora lusitaniae*, *Pichia fermentans*, and *Saccharomyces cerevisiae* (Gabriel 2012). Increased level of pulp in the juice reduced the benefits of sonication. Ultrasonic treatment at a frequency of 135 kHz, temperature of 31 °C, and duration of 40 min on red grape juice resulted in maximum inactivation of *Saccharomyces cerevisiae* and resulted in juice with high organoleptic and nutritional qualities (Nafar et al. 2013).

Ultrasound also has inhibitory effects on enzymes. Costa et al. (2013) treated pineapple juice with ultrasound (376 W/cm^2 and 10 min) and found a 20% reduction in polyphenol oxidase (PPO) activity and 75% reduction in juice viscosity, but enhanced stability for 42 days of storage compared to the non-sonicated juice (Costa et al. 2013). A study on inactivation kinetics of PPO in mushroom (*Agaricus bisporus*) by ultrasound at 55–75 °C showed that the thermosonication decreased the D values of PPO by 1.3–3 times compared to thermal treatment alone (Cheng et al. 2013). Fresh-cut potatoes treated by ultrasound at 40 kHz, 200 W, for 5 min had more uniform firmness and inhibited polyphenol oxidase activity (50%) for 4 days, but this treatment did not affect enzymatic browning on the product (Amaral et al. 2015). Ultrasound treatment at 20 kHz, 1000 W, for 15 min improved the physical properties of peach juice: increased stability in cloudiness, maintained or increased consistency, and insignificant color changes during the storage (Rojas et al. 2016). Nutritional

quality (total phenol content, antioxidant activity, vitamin C, carbohydrates, etc.) of ultrasound-treated carrot juices was as good as fresh juices and had >98% of carotenoids and 100% of ascorbic acid retained (Khandpur and Gogate 2016; Martínez-Flores et al. 2015).

Based on the available information, ultrasound has significant potential in washing of raw material, disinfection of fresh-cut product, and direct treatment of fresh juices. Ultrasound treatment systems with flexible designs applicable for various processing systems at reasonable cost are available, which further increase its potential use in fresh produce industry.

16.2.7 Cold Plasma

An emerging antimicrobial technology for decontaminating infected surfaces is the use of nonthermal ionized gases known as cold plasmas. The plasma is composed of gas molecules, which have been dissociated by an energy input. Nonthermal plasma can be generated in different systems including dielectric barrier discharge (DBD), atmospheric pressure plasma jet (APPJ), and corona discharges (CD), in which various gas mixtures such as air, oxygen, nitrogen, helium, argon, etc. are utilized (Ehlbeck et al. 2010). It is constituted by photons, electrons, positive and negative ions, free radicals, and excited or non-excited molecules that, in combination, have the ability to inactivate microorganisms (Fernández et al. 2012). The primary modes of action are due to UV light and the reactive chemical products of the cold plasma ionization process. Niemira (2012) described three types of plasma systems for treatment of foods. *Remote treatment* involves generation of plasma in a distant location and transfer to the food product to be treated. *Direct treatment* involves application of the active plasma directly to the food. *The electrode contact system* involves placement of food between the plasma generating electrodes. Each of these systems provides different degrees of flexibility in operation and the intensity of the energy transferred to the food (Niemira 2012). Cold plasma treatment is a nonthermal process effective only on surfaces.

Studies on strawberry, lettuce, and potato had shown that cold plasma (60–80 kV dielectric barrier discharge (DBD) pulsed at 50 Hz, across a 40 mm electrode gap) reduced the background microflora (aerobic mesophilic bacteria, yeast, and mold) and human pathogens, such as *E. coli O157:H7* and *Salmonella* spp. by 2 logs within 24 h (Fernandez et al. 2013; Misra et al. 2015, 2014). In fresh lettuce, cold plasma treatment (400 and 900 W for 10 min) showed inactivation of *Escherichia coli O157:H7* and *Salmonella typhimurium* pathogens by up to 2.8 log CFU/g, while sensory properties of the lettuce were not affected (Song et al. 2015). The degree of inactivation can be affected by the type of microorganisms, inactivation medium, number of cells, operating gas mixture, gas flow, and physiological state of cells, among others (Bermúdez-Aguirre et al. 2013).

Besides microbial inactivation, cold plasma treatment inhibited PPO enzyme activities by 42% and reduced browning and metabolic activities in fresh-cut apple during

storage (Tappi et al. 2014). Plasma treatment resulted in better maintenance of color, texture, and antioxidants in fresh-cut kiwifruit and strawberries (Misra et al. 2015; Ramazzina et al. 2015). Study on fresh-cut melon showed inhibition of peroxidase and pectin methyl esterase activities up to 17 and 7%, respectively, by cold plasma treatment (Tappi et al. 2016). Metabolic activity and respiration rate of cherry tomatoes, strawberry, fresh-cut apples, and melon decreased without any textural changes upon treatment by cold plasma (Misra et al. 2015, 2014; Tappi et al. 2014).

Cold plasma treatment, as discussed above, has significant microbial inactivation effects and thus can be applied to surface decontamination of MPRFV products without having negative effects on quality. However, commercial size systems must be designed and produced for various processing systems.

16.2.8 Dense-Phase CO_2 Processing

Dense CO_2 at especially supercritical state has microbial inactivation properties and can be used in minimally processed juice production. It involves mixing CO_2 with a product and adjusting temperature and pressure of the mixture to certain levels to have CO_2 in dense phase in the mixture for a certain time and then depressurize the mixture to separate CO_2 from the product. CO_2 exist in supercritical state above 31 °C and 7.34 MPa, which include relatively low temperature and pressure making the process nonthermal and less energy intensive keeping the operating cost low. Significant amount of information on effects of dense CO_2 on juices exist in literature, which shows its great potential in juice manufacture through its very high microbial inactivation power. Spore and enzyme inactivation requires higher temperature and pressure (Damar and Balaban 2006). The inactivation mechanism of pressurized CO_2 mainly include intercellular pH reduction, extraction of cellular constituents, and mechanical damage induced by volume fluctuation of CO_2 induced during pressurization and depressurization (Damar and Balaban 2006; Zhang et al. 2006). The reported results in literature have been conducted in various types of batch and/or continuous systems in lab or pilot scale equipment.

Dense CO_2 is also effective in inactivation of enzymes. Treatment of orange juice at 26.9 MPa and 56 °C for 145 min resulted in 100% inactivation of pectinesterases (Balaban et al. 1991). Dense CO_2 treatments at 2.94 and 4.9 MPa at 5 °C for 10 min resulted in more than 70% inactivation in lipoxygenase and 61% inactivation in polyphenol oxidase in carrot juice, respectively (Park et al. 2002).

Critical process factors affecting the result of the process include temperature, pressure, the amount of CO_2, and the process time. It has been shown in several studies that CO_2 in its supercritical state had highest microbial inactivation effects. Research done on apple cider and grape juice showed more than 6 log inactivation on *E. coli* and *S. cerevisiae* and *C. albicans* (Gunes et al. 2005, 2006). These works proved that the inactivation was mainly due to CO_2 (not the pressure), and both the amount of CO_2 and being in supercritical state were highly effective on microbial inactivation. More than 6 log reduction in various microorganisms in various juices

by dense-phase CO_2 has been reported (Arreola et al. 1991; Bermúdez-Aguirre et al. 2013; Kincal et al. 2005; Lecky and Balaban 2005; Park et al. 2002; Sims and Estigarribia 2002; Yagiz et al. 2005).

Substantial amount of information on dense CO_2 processing is accumulated in literature, which shows that it has significant potential as a nonthermal treatment for fresh juices to inactivate pathogens and spoilage microorganisms. Currently, due to a lack of commercial scale process equipment for large capacities, dense CO_2 is not used as a nonthermal pasteurization process in juice industry. The design of dense CO_2 processing systems and their integration with the commercial scale juice processing line are required for its full commercialization.

16.2.9 High-Pressure Processing

High-pressure processing (HPP) involves exposure of food products to elevated hydrostatic pressure ranging from 100 to 900 MPa. It has been scientifically and commercially proven that HPP as a nonthermal treatment can produce microbiologically safe and stable products with improved quality characteristics such as enhanced flavor, color, and fresh-like sensory quality (Barba et al. 2015; Huang et al. 2015; Hurtado et al. 2015; Picouet et al. 2016; Tribst et al. 2016). It is highly effective on vegetative cells of pathogenic and spoilage microorganism as well as spores when combined with mild thermal treatment. Both solid and liquid foods can be treated with HPP. Fresh-cut product and juices can be packaged and treated with HPP. The juices can also be treated first by HPP then aseptically packaged to have stable products.

HPP treatment of fresh-cut product can cause cell disruption, increase cell membrane permeability, and thus may cause quality degradations such as browning and textural changes. Thus, the HPP treatments must carefully be optimized for each type of product. It has been shown that HPP treatment at 300 MPa resulted in up to 50% loss in firmness mainly due to turgidity loss (Araya et al. 2007). Fresh-cut peaches in vacuum packaging have been treated with HPP at various pressures and exposure time, and 584 MPa for 1 min treatment was reported to be the best condition to retain product quality (Denoya et al. 2016). HPP together with vacuum packaging (VP) has been suggested for fresh-cut peaches, in which anaerobic metabolism induced by VP was prevented by HPP (Denoya et al. 2015). Although HPP under certain conditions shows promise for preserving some fresh-cut products, more research is needed to determine optimum treatment conditions and interactions with various quality aspects of each product.

HPP is more applicable to freshly squeezed juices and purees with significant microbial inactivation and fresh quality maintenance. These products can be treated by HPP either in package or prior to packaging. HPP treatment of 600 MPa significantly reduced aerobic bacteria, coliform, and yeast counts in sugarcane juice, mulberry juice, multifruit smoothies, and pumpkin puree (García-Parra et al. 2016; Huang et al. 2015; Hurtado et al. 2015; Picouet et al. 2016; Zou et al. 2016). No significant changes due to HPP were observed related to color degradation, pH,

and brix for several fruit juices and smoothies (Andrés et al. 2016; Barba et al. 2013; Perera et al. 2010; Rodrigo et al. 2007; Samaranayake and Sastry 2010).

Besides microbial spoilage, quality degradation due to enzyme activity (PPO, POD, LOX, PG, PME, etc.) in MPRFV products can also be controlled to some extent by HPP (Chakraborty et al. 2014). HPP inactivated polyphenol oxidase activity up to 100% percent for carrot and peroxidase activity up to 81% percent for cocoyam (Tribst et al. 2016) as well as spinach sauce, pumpkin puree, coriander paste, and beetroot slices (García-Parra et al. 2016; Medina-Meza et al. 2015; Nath et al. 2016; Paciulli et al. 2016).

High-pressure treatment is among the greatest potential process for nonthermal pasteurization of fresh juices. Industrial scale equipments are available for manufacturers to produce premium quality fresh juices. This process has also some potential for fresh-cut products, but further research is needed to develop effective treatment to retain quality of the products.

16.2.10 Pulsed Electric Field

Pulsed electric field (PEF) processing involves application of high-voltage energy in the form of very short pulses to food products. The product is placed between two electrodes through which the high voltage is applied, which generates electrical field in the food. Because the treatment time is very short (less than a second), it is a nonthermal process. PEF primarily affects cell membranes where it causes electrical breakdown or electroporation. These effects are used for various purposes such as microbial inactivation, extraction of cellular constituents (juice, bioactive compounds, etc.), as well as tissue softening to aid in cutting operation in fresh produce processing.

PEF is highly effective on inactivation of microorganisms and thus can be used as a nonthermal pasteurization treatment in freshly squeezed juices. The information in literature includes various types of PEF systems and treatment conditions, and thus it is generally hard to make a comparison among them. PEF treatment (34 kV cm^{-1}, 150 μs) resulted in 5.4 log and 6.3 log reduction in *E. coli* in fruit smoothies and orange juice, respectively (McNamee et al. 2010; Walkling-Ribeiro et al. 2008). PEF treatment (22 kV/cm, 59 μs) at 45 °C showed 1.59 log reduction in *Escherichia coli O157:H7*, 2.05 log reduction in *Salmonella typhimurium*, and 4.5 log reduction in *Lactobacillus lactis* in orange juice (Gurtler et al. 2010). Grape and tomato juice treated at 35 kV/cm for 1000 μs resulted in up to 4 log inactivation of bacteria, yeasts, and *S. enteritidis* (Marsellés-Fontanet et al. 2009; Mosqueda-Melgar et al. 2008).

Enzymes such as polyphenol oxidase, lipoxygenase, peroxidase, polygalacturonase, and pectin methyl esterase can affect color, flavor, and texture of MPRFV products, and they are effectively inactivated in thermal processed products. PEF can result in substantial inactivation of enzymes depending on the origin and types of enzyme and process condition, but some of these inactivation may not be sufficient or no effects at all for some enzymes (Terefe et al. 2015). The required energy input is usually higher for enzyme inactivation than microbial inactivation.

These can become a limitation for replacing thermal process by PEF in some product. PEF treatment of 24 kV/cm, 8000 μs at 15 °C resulted in 94% inactivation in tomato pectin methyl esterase (Giner et al. 2000). About 52% inactivation in watermelon lipoxygenase was possible with 35 kV/cm, 1000 μs PEF treatment at 35 °C (Aguiló-Aguayo et al. 2010).

The efficiency of PEF treatment in juice expression, extraction, and recovery of valuable compound enhancement by cellular permeabilization from fruits and vegetables has been studied by many authors. On lab scale, the combination of ohmic heating (60 kV/cm, 50 Hz) and PEF treatment (0.6 kV/cm, 0.04 s) leads to a synergetic effect, with an 85% enhancement of juice extraction, due to the combination of the electro-permeabilization of cell membranes and the thermal softening of tissues (Praporscic et al. 2007). Increase in apple juice yield from 2% to 8% was reported as a result of 1–5 kV/cm/ 30 pulses PEF treatment (Schilling et al. 2007). Treatments of 1.25 and 2.5 kV/cm on alfalfa, followed by pressing at 40 bar for 2 min, resulted in an increase in the extracted protein (57%) and minerals (73%) (Gachovska et al. 2009). Juice yield increased from 51 to 67% after PEF treatment of carrot (0.25–1 kV/cm/100 pulses/5 bar) with higher content of β-carotene (Grimi et al. 2007). Enhanced carotenoids extractability for carrot puree (up to 66%) with PEF treatment ((0.1–1.1 kV/cm) 20 μs, 150 pulses) was also reported (Leong et al. 2015).

PEF can be used to replace conventional thermal process for fruit juices as it effectively inactivates microorganisms and retains fresh-like quality attributes such as flavor, color, and bioactive compounds to a large extent (Odriozola-Serrano et al. 2013). PEF has also potential to increase juice yield in the extraction process. It has also significant potential in fresh-cut products as a processing aid such as reducing cutting force and thus providing energy saving. However, insufficient inactivation of enzyme by PEF can be a limitation for some of these applications, and thus, further research on combination of PEF with other treatments using hurdle approach is needed.

16.2.11 Edible Coatings

Edible coating involves covering the surface of a product with a thin layer of material to preserve quality of the product. Edible coatings can be applied to products with different techniques including dipping, spraying, brushing, etc. These materials are edible and provide some barriers to the transfer of gas and moisture and to microbial contamination. Whole fresh fruits and vegetables are coated with edible films to extend their storage life by controlling respiration and transpiration rate as well as fungal decay. Fresh-cut product has already lost their natural protective skins and their tissues are damaged, and cellular constituents are exposed to ambient environment. This facilitates biochemical degradative reactions such as enzymatic browning, softening, and microbial spoilage. Edible coating provides a protective layer to fresh-cut products against gases, moisture loss, and microbial contamination and growth and thus provides extension of shelf life.

Table 16.2 Different types and characteristics of edible coatings for fresh produce products

Types of edible coatings	Typical materials	General properties
Polysaccharide based	Chitosan Starch Alginate Cellulose Pectin Pullulan Carrageenan Gellan gum Gum arabic Aloe gel	Excellent gas barrier properties Not a good barrier for moisture
Protein based	Casein Whey protein Zein Gluten Egg albumen Collagen Gelatin	Excellent barrier for aroma, oil, and gas (O2) Good mechanical properties Not a good barrier for moisture
Lipid based	Carnauba wax Beeswax Paraffin wax Mineral oil Vegetable oil	Excellent moisture barrier
Composites	Protein/protein Polysaccharide/protein Lipid/lipid Lipid/polysaccharide	Combination of two or more materials Enhanced mechanical strength Enhanced gas barrier properties

There are three main classes of edible coatings: hydrocolloids, lipids, and composites (Table 16.2). Various types of polysaccharides and proteins have been proposed as hydrocolloid materials for edible coating. They have excellent gas barrier properties but they have a poor moisture barrier property. A number of lipids have been used as edible coating material as well. Lipid-based edible coatings (especially waxes) have excellent moisture barrier property. Composites involve various combinations of hydrocolloids and lipids as edible coating. The composite coatings are designed and used to enhance and improve gas barrier and mechanical properties.

Polysaccharide-based edible coatings such as cellulose, hydroxypropyl methylcellulose, chitosan, alginate, carrageenan, gum arabic, and starch have been applied on several fresh and minimally processed fruits and vegetables, in which respiration rate and weight loss were decreased significantly through creation of good barriers to moisture, O_2, CO_2, and ethylene (Duran et al. 2016; Fai et al. 2016; Fakhouri et al. 2015; Gol et al. 2013; Maftoonazad and Ramaswamy 2005; Mali and Grossmann 2003; Oz and Ulukanli 2012; Shiri et al. 2013). Edible coating based on gellan gum, pectin, and alginate incorporated with active components such as ascorbic acid, calcium chloride, and dietary fiber maintained color, firmness, sensory, and microbial quality and contribute to nutritional quality in coated fresh-cut apples (Moreira et al. 2015). Chitosan-based edible coating combined with sodium chlorite and

MAP decreased microbial growth and maintained quality of minimally processed pomelo fruit (Ban et al. 2015). A chitosan derivative commercial coating, Nutrisave (Nova Chem, Halifax, NS, Canada), was shown to reduce the respiration rate and weight loss and microbial decay in fresh-cut pears and apples (Elson et al. 1985). Silver-montmorillonite nanoparticle was added to alginate coating and applied to fresh-cut melon, in which increased antimicrobial effect against bacteria, yeast and molds, and, thus, shelf life of coated product was observed (Danza et al. 2015).

Protein-based edible coatings from milk, soybeans, corn, wheat, peanut, cottonseed, etc. can have excellent barrier properties for aroma, oil, and oxygen. However, their moisture barrier property is generally low except insoluble ones such as zein and gluten. The characteristics (barrier, mechanical, thermal) of the protein-based edible coatings are affected by the molecular characteristics and the origins of the specific proteins (Vargas et al. 2008). Fresh-cut apples and cantaloupe coated with zein or soy protein incorporated with malic and lactic acid maintained color and quality effectively (Bai et al. 2003; Eswaranandam et al. 2006).

The lipid-based edible coatings such as carnauba wax, beeswax, or vegetable oil have good water barrier capacity and provide shiny and glossy appearance to fruit and vegetable products. Recently, nano- and micro-emulsions were studied for fresh-cut apples, and coatings exhibited a faster and greater microbial inactivation and reduced respiration rates and ethylene production (Salvia-Trujillo et al. 2015).

Combination of lipid, polysaccharides, and protein are used in coating material to improve their mechanical and barrier properties (Abugoch et al. 2016; Ayranci and Tunc 2004; Lee et al. 2003; McHugh and Senesi 2000; Moldao-Martins et al. 2003; Toğrul and Arslan 2004; Zapata et al. 2008). Edible coatings with multicomponent from extracts of fruit and vegetable residues were applied to fresh-cut carrot and showed potential in quality maintenance (Fai et al. 2016). Composite coating with starch/gelatin significantly improved appearance and overall quality and decreased weight loss in grapes (Fakhouri et al. 2015).

There are several commercial edible coating products such as Semperfresh (AgriCoat Industries Ltd., Berkshire, UK), Pro-long (Courtaulds Group, London), Nature-Seal (Ecoscience Product System Division, Orlando, FL), Nutrisave (Nova Chem, Halifax, NS, Canada), Natural Shine 9000 (Pace International, Seattle, USA), FreshSeal, Christp-Coat 868, Food Coat, etc. Most of these commercial coatings have cellulose derivatives. These products have been used in various fresh produce successfully (Olivas et al. 2008).

Edible coating formulations can have plasticizers such as glycerol, fatty acids, sorbitol, propylene glycol, etc. to increase their mechanical properties. Moreover, specific compounds having antimicrobial or antioxidant activities can also be included in edible coating formulations to control microbial growth and oxidation and thus to extend the shelf life of the coated products. Among the antimicrobials, potassium sorbate, citric acid, lemongrass, oregano, cinnamon, clove, eugenol, and chitosan have been used in various coating materials and applied to fresh-cut products, and significant antimicrobial effects have been reported (Rojas-Graü et al. 2009). Similarly, antioxidants including ascorbic acid, citric acid, oxalic acid, 4-hexylresorcinol, cysteine, and glutathione have been used in edible coating formulations and inhibited enzymatic browning on fresh-cut products (Rojas-Graü et al. 2009). Calcium chloride in edible coating had significant contribution to maintenance of

texture in coated products (Olivas et al. 2008; Rojas-Graü et al. 2009). Natural compounds such as essential oils, herbal or spice extracts, and dietary fibers can also be used in edible coating to have nutritional contribution in addition to preserve the coated products. For instance, calcium and vitamin E have been incorporated into chitosan coating and applied to strawberries and raspberries successfully (Han et al. 2004). The active compounds with specific functions should be selected based on their compatibility with the fresh-cut product as they may affect flavor and aroma. There is a substantial amount of information on application of edible coating to fresh fruits and vegetable products; selected ones are given in Table 16.3.

Table 16.3 Selected studies showing the applications of edible coatings on fresh produce products

Coating type	Composition	Application	Effects	References
1. *Hydrocolloids*				
	Chitosan and Tween 80	Strawberry, grape, cherry, litchi, peach, Japanese pear, kiwi		Vargas et al. (2006), Park et al. (2005), Han et al. (2004)
		Sliced mango fruit		Chien et al. (2007)
	Chitosan, nisin, natamycin, pomegranate extract, grape seed extract	Strawberry	O_2 consumption was reduced and respiration rate was decreased. Antimicrobial effect against bacteria, yeast, and molds was increased. Shelf life of fruit was increased	Duran et al. (2016)
	Chitosan, pectin, sodium caseinate, glycerol	Fresh-cut nectarine	Ripening of nectarines was delayed	Ramirez et al. (2015)
		Strawberry	Weight loss was decreased	Gol et al. (2013)
		Raspberry, table grape, sweet cherry	Respiration rate and weight loss were decreased	Han et al. (2004), Shiri et al. (2013)
Alginate	Silver-montmorillonite nanoparticles (Ag-MMT), sodium alginate	Fresh-cut melon	The active coating was effective from the microbiological and the sensory point of view. Shelf life prolongation	Danza et al. (2015)
	Alginate, gellan gum, pectin, calcium chloride, ascorbic acid, and dietary fiber (apple fiber, inulin)	Fresh-cut apple	Improved firmness and color Positive effect on the sensory properties Increased nutritional value Gellan gum reduced mesophilic and psychrophilic counts on fresh-cut apples	Moreira et al. (2015)

(continued)

Table 16.3 (continued)

Coating type	Composition	Application	Effects	References
Carrageenan	Carrageenan glycerol, PEG 200	Fresh-cut apples		Lee et al. (2003)
	Carrageenan, glycerol, and Tween 80	Strawberry		Ribeiro et al. (2007)
Gum arabic		Tomato	Ethylene production, respiration rate, and weight loss were decreased	Ali et al. (2013)
Aloe vera, Shellac		Tomato	Ethylene production, respiration rate, and weight loss were decreased	Chauhan et al. (2015)
Cellulose	MC and glycerol	Strawberry, avocado		Maftoonazad and Ramaswamy (2005)
Starch		Strawberry	Weight loss was decreased	Mali and Grossmann (2003)
	Corn starch, gelatin, glycerol/ sorbitol	Red crimson grapes	Water vapor permeability was decreased so weight loss was reduced. Appearance was improved during 21-day storage	Fakhouri et al. (2015)
		Pomegranate	Respiration rate and weight loss were reduced	Oz and Ulukanli (2012)
Soy protein	Soy protein, glycerol, malic acid, lactic acid	Apple, cantaloupe, melon cubes		Eswaranandam et al. (2006)
Zein	Zein and propylene glycol	Fresh-cut apple		Bai et al. (2003)
2. *Composites*				
Polysaccharide lipid	MC, PEG, stearic acid, citric acid, ascorbic acid	Apricot		Ayranci and Tunc (2004)
	Paraffin wax, beeswax, soybean oil; CMC from sugar beet pulp; emulsion PE, oleic acid, and sodium oleate	Peach, pear mandarin		Togrul and Arslan (2004)
	HPMC, beeswax, shellac, stearic acid, and glycerol	Plum		

(continued)

Table 16.3 (continued)

Coating type	Composition	Application	Effects	References
	Carrageenan, pectin, cellulose, alginate, monoglycerides	Fresh-cut apple cylinders	O_2/CO_2 barrier, gloss	Wong et al. (1994)
	HPMC-lipid	Plum	Weight loss was unaffected	Perez-Gago et al. (2002)
		Mandarin	Respiration rate and weight loss were decreased	Pérez-Gago et al. (2002)
Polysaccharide Protein	CMC, WPI, caseinates, and glycerol	Strawberry		Vachon et al. (2003)
	WPC, glycerol, CMC, CaCl2, apple puree, ascorbic acid, citric acid, soy oil	Fresh-cut apple Pieces		McHugh and Senesi (2000)
	Quinoa protein, chitosan, sunflower oil	Fresh blueberries	Fruit ripening was delayed and growth of molds and yeast controlled during 32-day storage	Abugoch et al. (2016)
	Zein, alginate	Tomato	Ethylene production, respiration rate, and weight loss were decreased	Zapata et al. (2008)
	Gelatin, alginate, CMC	Fresh-cut apple	$O_2/CO_2/H_2O$ barrier	Moldao-Martins et al. (2003)
	Carrageenan, WPC	Fresh-cut apple	O_2/H_2O barrier	Lee et al. (2003)
	Calcium caseinate, WPI, pectin, CMC	Peeled carrot	H_2O barrier	Lafortune et al. (2005)
3. *Lipids*				
Nanoemulsion	Sodium alginate, lemongrass essential oil (LEO), Tween 80	Fresh-cut *Fuji* apples	LEO nanodroplets exhibited a faster and greater inactivation of *Escherichia coli* during storage time Reduced respiration rates and ethylene production At higher LEO concentrations, browning was observed	Salvia-Trujillo et al. (2015)
Emulsion	WPC, WPI or HPMC, stearic acid, beeswax, or carnauba wax	Fresh-cut apple pieces		Perez-Gago et al. (2005)

16.3 Combined Technologies

Combination of new and conventional technologies using a hurdle approach can have synergistic effects on preservation of MPRFV products. As described above, each of new technologies can have limitations in achieving the desired results which can be succeeded by combined treatments. Combination of treatments can be used to decrease undesirable effects of one treatment on product quality or to increase the level of microbial or enzyme inactivation. When selecting the treatments to be combined, the main objective, the mechanistic effects of the treatment, and the practical applicability in terms of the system requirements and cost must be considered. For example, treatments with different microbial inactivation mechanisms may have synergistic effects on overall microbial inactivation. For instance, bacterial spores and some enzymes can be inactivated by combining HPP or PEF with mild thermal treatment in fruit juices. Natural antimicrobials can be used in combination with the nonthermal treatments including HPP, PEF, ultrasound, UVC, or PL to achieve better microbial inactivation. Edible coating can be used together with the nonthermal treatments such as PL, PEF, and irradiation to have improved quality in the treated product. Some of these technologies can be combined with modified atmosphere packaging in all fresh-cut products. More research is needed to develop effective processes for fresh-cut product and fresh juices. Selected studies from literature on the use of combined technologies in fresh produce products are summarized in Table 16.4.

16.4 Concluding Remarks

Minimally processed fresh fruits and vegetable products have been highly demanded by consumers due to their fresh quality, nutritional value, and health benefits. Preservation technologies applicable to these products must maintain these characteristics. The emerging nonthermal technologies discussed in the chapter have great potential for preservation of these products. Thus, the developments in these technologies in terms of system and equipment design and commercial availabilities at reasonable cost would certainly contribute to their use in production of minimally processed refrigerated fruits and vegetables. Research on the use of these new technologies alone or in combination with others on minimally processed products focusing on both quality and safety are still needed to determine optimum process conditions for various types of products.

Table 16.4 Selected studies showing the effects of combined technologies on quality of fresh produce products

Product	Treatment conditions	Main effect	References
Fresh-cut apple	Edible coating + pulsed light: Gellan gum, glycerol, apple fiber, calcium chloride, ascorbic acid 2.5 kV/30 pulses of duration of 0.3 ms with an emitted fluence of 0.4 J/cm^2 The emitted spectrum from 180 to 1100 nm	Reduced softening and browning of apple pieces through storage. Decontamination and shelf-life extension	Moreira et al. (2015)
Ready-to-eat cauliflower florets	Antimicrobial edible coating + gamma irradiation + negative air ionization + ozone: Methylcellulose, maltodextrin, and antimicrobial compound Irradiation doses from 0 to 2.4 kJ/kg The ionizer/ozonator was set to produce minimal amount of ozone (volume of 428 mg/m^3) and negative ions (ranging from −0.2 to −0.4 mV)	Each treatment alone was effective on *Listeria innocua*, *Escherichia coli*, and mesophilic bacteria After 7 days, treatment with γ-radiation reduced *L. innocua* and *E. coli* of 1.8 and 3.6 log CFU/g, respectively. NAI + ozone reduced *L. innocua* and *E. coli* of 2.0 and 2.8 log CFU/g, respectively. Mesophilic bacteria were reduced of 1.8 log CFU/g after γ -radiation and 1.4 log CFU/g after NAI + ozone. The combination of bioactive coating and NAI + ozone induced an additive effect on *L. innocua*, *E. coli*, and mesophilic bacteria	Boumail et al. (2016)
Fresh-cut mango	Edible coating + pulsed light: Alginate, malic acid PL 20 pulses at fluence of 0.4 J.cm^{-2}/pulse	Combined treatment reduced *L. innocua* counts by 4.5 logs. Firmness of alginate-coated slices sharply increased. Color parameters and total soluble solids content decreased in all treated mango slices throughout 14 days	Salinas-Roca et al. (2016)

(continued)

Table 16.4 (continued)

Product	Treatment conditions	Main effect	References
Fresh-cut avocado, watermelon and mushrooms, and apple	Edible coating + pulsed light/UV light: PL (180–1100 nm, 12 J/cm^2) or UVC (11.2 kJ/m^2) with antibrowning dips (malic acid, citric acid, ascorbic acid/calcium chloride solution) through refrigerated storage	More than 5 log reductions of *L. innocua* and *E. coli* populations No significant effect on the firmness and total phenolic content but increased the polyphenol oxidase activity of fresh-cut apples The content of chlorogenic acid, epicatechin (+), catechin, and caffeic acid in CA + UV-treated sample was higher than the control after 15 days storage	Ramos-Villarroel et al. (2015)
Fresh-cut "Royal Gala" apple, "Rocha" pear, and "piel de sapo" melon	UV light + electrolyzed water: UVC (0–10 kJ.m^2), acidic electrolyzed water (EW), and sodium hypochlorite (SH) (100 ppm chlorine)	*C. sakazakii* did not grow at 4 °C. The UVC 7.5 and 10 kJ/m^2 produced greater *C. sakazakii* population decreases (2–2.4 log CFU/g) than AEW (1.3–1.8 log CFU/g)	Santo et al. (2016)
Peach	Edible coating + gamma irradiation: Carboxymethyl cellulose (CMC) coatings and 1.2 kGy gamma irradiation followed by refrigerated storage	Prevented disease incidence of peach up to 7 days during post-refrigerated storage at 25 ± 2 °C, RH 70% following 30 days of refrigeration	Hussain et al. (2016)
Apple juice	Ultrasound (US) + pulsed light (PL) (600 W, 20 kHz, and 95.2 mm wave amplitude; 10 or 30 min at 20, 30, or 44 ± 1 °C) and (xenon lamp; 3 pulses/s; 0.1 m distance; 2.4 J/cm^2–71.6 J/cm^2; initial temperature 2, 30, 44 ± 1 °C)	3.0 log cycles of spore reduction in commercial apple juice and 2.0 log cycles in natural juice, while for *S. cerevisiae*, 6.4 and 5.8 log cycles of reduction were achieved in commercial and natural apple juices, respectively Sensory shelf life was determined by 6 days (25% rejection) with fresh natural apple taste	Ferrario et al. (2015), Ferrario and Guerrero (2016)

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Chapter 17
Use of Geographical Information Technologies in a Precision Agriculture Management System for Food Traceability

Muzaffer Kahveci

17.1 Introduction

Agriculture and food sector is one of the most important sectors which brings service from the field to the table. In many countries, agricultural areas have been divided into thousands of different shapes and sizes. And maybe thousands of tractors and other similar machineries are being used in those fields. On the other hand, the use of modern technologies such as GIS, GNSS and remote sensing in agriculture increases in many countries. Thus, the use of such modern technologies in numerous and scattered agricultural areas is inevitable due to the fact that they are both cost-effective and time-saving. Consequently, to implement an effective and dynamic PAMS, it is only possible with the integration of GITs (photogrammetry, remote sensing, GIS and GNSS). This integration requires a professional approach and state policy (Kahveci 2012).

Precision agriculture (PA) may be defined as observation, impact assessment and timely strategic response to fine-scale variation in causative components of an agricultural production process. Therefore, PA may cover a range of agricultural enterprises, from dairy-herd management through horticulture to field crop production (McBratney and Whelan 2001). In addition to the above definition, it can also be said that PA is the scientific approach for the correct and reliable decision-making in PAMS by means of GITs. Actually, the starting point of PA is the requirement of accurate position information in the farming areas. This position information is used in almost all agricultural applications such as conventional cadastral surveys, improvement of the field use policies, state support purchases, effective harvest analysis, use of AG machinery equipped with technology, seeding, spraying and real-time monitoring of livestock, and food traceability (Yildiz 2008). So, a PAMS established for the above-mentioned goals can be called as *precision agriculture/*

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farming or *site-specific farming* or *site-specific crop management* (*SSCM*) or *variable rate agriculture* (*VRA*). As it can be seen from above, there are different definitions for PA applications due to the fact that definitions and their contents may show some changes depending on the region/country. But here, for providing a unique and comprehensive definition which is supposed to cover all of the descriptions above, the name "precision agriculture management system (PAMS)" has been preferred to use. So, PAMS may be explained as the integration of resource and agronomic practices with environment, soil attributes and crop requirements as they vary across a field.

Georeferenced mapping and surveying works for agriculture purpose are applied in many developed/developing countries. The data collected by means of mapping and surveying are used for making effective decisions in PAMS in order to collect accurate and reliable information on crops, livestock and other relevant sources. To accomplish this work, geographical information technologies (GITs) are used for collecting the necessary data. GITs involve photogrammetry, remote sensing, GIS and GNSS technologies. Thus, using GIT in PAMS means collection, analysis and interpretation of the georeferenced data obtained via the above technologies. Necessary data are collected via remote sensing (i.e. satellite images), photogrammetric (aerial photos and UAV data) and GNSS (positioning) methods and technologies. Some basic info about GIT components can be given as follows:

Satellite Images: In precision agriculture (PA) applications, high-resolution satellite images (e.g. WorldView-2, WorldView-3, QuickBird, etc.) are valuable tools (Fig. 17.1). It is possible to obtain satellite image data at different spatial, temporal and spectral resolutions for agriculture implementations. Satellite images can be utilized in PA applications for the determination of crop health, crop assessment, yield monitoring, variations in organic matters and drainage patterns, environmental and soil analyses and orchard identification. As a result, with the help of capabilities provided by remote sensing, farmers can use aerial and satellite imagery to help them monitor their fields more efficiently and dynamically by determining precisely the way their fields reflect and emit energy in the visible and infrared wavelengths (Wu et al. 2010).

Fig. 17.1 Satellite image (WorldView-2, 2015)

Unmanned Aerial Vehicles (*UAVs*): Several classifications for micro-UAVs (also called microdrones) can be made. One of the main classifications is according to their wing types, namely, rotary wing and fixed wing (Figs. 17.2 and 17.3). The use of UAVs in agriculture is an emerging and promising technology. And in the near future, they will be the main actors in a successful PAMS application. UAVs can be used for capturing, displaying and managing georeferenced imagery and video data. UAVs play an important role and make an important contribution to the precision agriculture applications such as precise spraying of fields, determining the areas accurately where pesticides may be used or whether there is a lack of water, reducing the massive use of fertilizers, etc. Being equipped with several sensors, cameras, GNSS receiver and microcontrollers, they support farmers for dynamic management and effective use of their fields by providing important data on the type of soil and environmental conditions and thus help the farmers to work targeted, ecological and profitable. The use of UAVs in agriculture has huge potential and numerous fields of application. Data collected with UAVs using NIR (near-infrared), RGB (red, green and blue), multispectral and thermal sensors/cameras provide detailed info about the crop. So, it is always possible to see the impact of variations on crop

Fig. 17.2 Rotary wing UAV (Source: Google Earth)

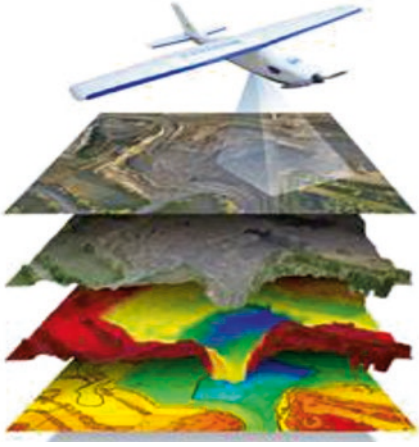


Fig. 17.3 Fixed wing UAV (http://www.mavinci.de)

productivity with UAV data. Professional UAVs provide very efficient way to assess crop health. One just flies over the field of interest, and once the necessary data and images have been collected, then they can be downloaded into a GIS database for management and control of AG resources. Then it becomes possible to generate NDVI (normalized difference vegetation index) maps of the field for the purpose of vegetation classification, soil analysis and crop management. Due to the technological advancements in camera systems, today, it is possible for users to collect imagery data on different sections of the electromagnetic spectrum, from the RGB (red, green and blue) to the near-infrared imagery and multispectral imagery. It is also possible to collect thermal data using thermal cameras via UAVs. RGB images are used for GIS and mapping purposes which provide high-resolution orthophotos, digital elevation/surface models (DEM/DSM) and 3D point cloud data. NIR (near-infrared) images can be used for NDVI maps in PA applications. Besides, UAV equipped with a NIR camera supplies information about the leaf structure or the water content of the plants. Examining the reflected wavelengths of the plants, it becomes possible to determine the need for fertilizer, the nutritional status and the population density of the plants. Multispectral images can also be used for NDVI map productions and provide important data related to the state of the soil (Fig. 17.4). Additionally, thermal imaging sensors are devices that translate thermal energy (heat) into visible light in order to analyse surface temperature of a particular object or scene (Grainger 2016) (www.grainger.com.) In thermal imagery, a temperature value is assigned to each individual pixel in an image. Thermal imaging technique has been used in medicine, electrical, mechanical and civil engineering for a long time (Agerskans 1975). Reasonable cost of the equipment and simple operational procedure have created opportunities for the application in several fields of the agricultural and food industries. This technology can be used in all agricultural materials and processes, where heat is generated or lost in space and time (Hellebrand et al. 2002). Potential use of thermal imaging in agriculture and food industry includes predicting water stress in crops, planning irrigation scheduling, disease and pathogen detection in plants, predicting fruit yield, evaluating the maturing of fruits,

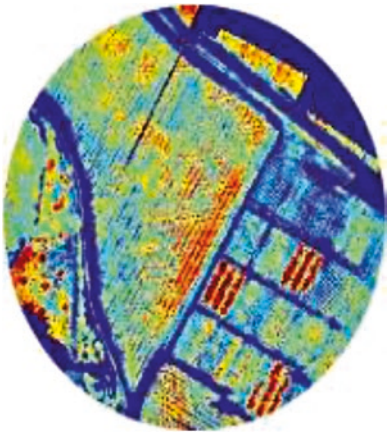


Fig. 17.4 Multispectral image (Source: Google Earth)

bruise detection in fruits and vegetables, detection of foreign bodies in food material and temperature distribution during cooking (Vadivambal and Jayas 2011).

Global Navigation Satellite System (*GNSS*): Essential developments on computer systems and space technologies have given rise to GPS (Global Positioning System), which is one of the most important technological products of the last three or four decades, to enter in our daily life. Positioning and navigational advantages provided by GPS have caused it to be perceived as a basic requirement from the application areas' point of view particularly in developed countries. GPS provides us 3D (three-dimensional) position, velocity and time in any weather condition, anywhere in the world, anytime (24/7), in real time and in a unique coordinate system (i.e. WGS84). On the other hand, nations' dependency on the GPS system has been a vital factor particularly in transportation and in most of the military and civil infrastructure investments. Hence, in order to prevent dependency on the US GPS only, Russia (GLONASS), EU (European Union) (Galileo) and other states (China, BeiDou; India, GAGAN; Japan, QZSS) which have necessary technology and resources have established their own GNSS programmes. With the advent of these new satellite systems, the term GPS, which denotes a US trademark, has been replaced by the more general term of GNSS (Global Navigation Satellite Systems) (Kahveci et al. 2011). GNSS stands for Global Navigation Satellite System and is the standard generic term for satellite navigation systems that provide autonomous geospatial positioning (position, velocity and time) with global coverage. In addition to its widespread military uses, GNSS has been also widely used by civilians for scientific (e.g. earthquake prediction studies, GNSS meteorology, surveying and geodesy, precision agriculture, GIS data collection, search and rescue operations, etc.), navigational (air, land, sea and rail transportation, fleet management, LBS, etc.), infrastructure (datum definitions, IGS studies, RTK CORS networks, etc.) and recreational (hunting, skiing, hiking, climbing, fishing, etc.) purposes until the present. Precise positioning with GNSS CORS networks (or commercial satellite-based augmentation services) is the most important part of a successful PAMS due to the fact that it provides accurate, precise and reliable coordinate information of the data collected in the field (georeferenced data) which are the basis of a site-specific crop management (SSCM) system. On the other hand, GNSS CORS networks and/or single-base RTK applications support the accurate navigation and positioning of agricultural vehicles such as tractors, combines, sprayers, etc. (Fig. 17.5). So, GNSS equipment (GNSS antenna mounted on vehicle, in-cabin autosteering console, etc.) used for vehicle guidance or steering is called as "vehicle navigation aids". Vehicle navigation aids provide real-time benefits to the farmers by eliminating the unnecessary overlaps during fertilizing, spraying and sowing.

Geographical Information System (*GIS*): The art, science, engineering and technology associated with answering geographical questions is called GIS. GIS is a generic term denoting the use of computers to create and depict digital repre-

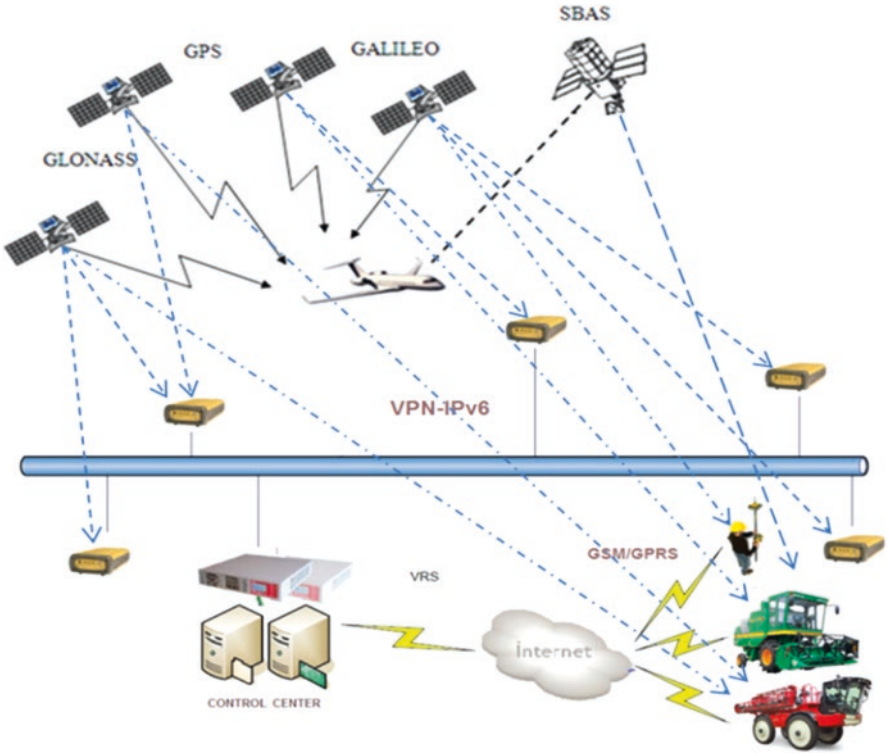


Fig. 17.5 CORS network for real-time positioning (SBAS, satellite-based augmentation system)

sentations of the Earth's surface (Berry 1998). Many other detailed GIS definitions can be made. But a comprehensive definition can be as follows: GIS is a computer system used for assembling, storing, checking and displaying georeferenced data stored in a database. Here, stored data are displayed via maps. By examining these maps, one can easily see, analyse, compare and understand the relations of the objects. GIS includes data about people, lands, agriculture, buildings, roads, dams, weather, rivers, forests, energy, etc. which, in turn, means GIS has many applications in agriculture, transportation, forestry, municipality, economic planning, water, energy, land use, cadastre, public health and safety, governmental issues and many other fields. In agriculture, the main use of GIS is for surface modelling, spatial analysis and statistics. A GIS database established for PAMS should store data related to soil and vegetation characteristics (such as yield, moisture, texture, structure, nutrient status, landscape position data, etc.) in layers and assign that information to the particular field location. A professional GIS software should be used to analyse above given characteristics between layers to develop efficient, accurate and dynamic prescription/application maps (e.g. Figs. 17.6 and 17.7). These digital maps specify where and how much input to apply before the application. After creating these digital maps, they are loaded into a computerized variable rate controlling system of the agricultural machinery. During the applications using navigation aids, variable rate check map loca-

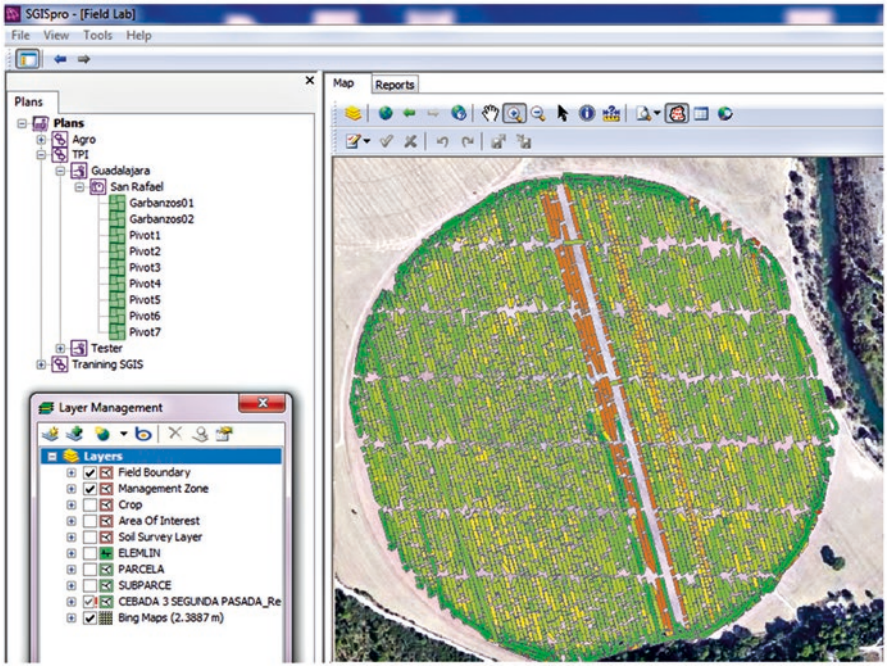


Fig. 17.6 GIS software screenshot (Source: SGISpro Screenshot)

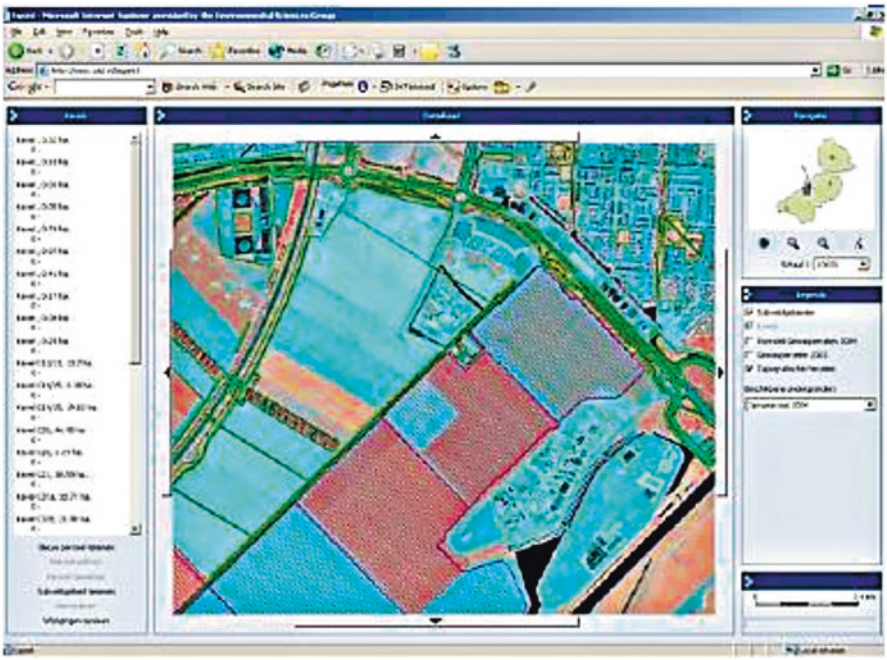


Fig. 17.7 GIS software screenshot (Source: Van der Mal 2010)

tions and the required rates at those locations and match them with the changing position of the vehicle during applications.

On the other hand, because the food safety is directly related to people's health, it is vital to build a food traceability system. For food traceability Radio Frequency Identification (RFID) technology can be used effectively. RFID transmits product information using radio waves. This technology is used in agri-food industry to enhance food quality, safety and traceability. It provides information on food materials (e.g. product identity, supplier, serial number, etc.) as they enter and move through the farm-to-consumer distribution chain. In order to be able to build an efficient and effective food traceability system, geographical information and RFID technologies should be used in combination, because efficient or complete traceability of food means having accurate and updated information about any food (including fishery and hunting) through all steps of production, processing and distribution which means the "history information of food".

17.2 GIT Applications in a Precision Agriculture Management System

It is a known fact that utilizing maps for agricultural purposes even in developed countries was not so common until the 1990s. One of the reasons of this reality is that most soil and topographical maps were containing so generalized info that it was impossible to use them effectively at the field/farm level. As a result of this, traditional agriculture management system has been applied based on the average values of field data (Tekin and Sındır 2006). In another saying, in those days, "uniform" treatment of the fields with the assumption of uniform crop and soil management was in use instead of "differential treatment" of field variation. In this method, nutrient status of the field is determined by performing some measurements related to the yield performance and soil samples of the field. Farmers use this info in order to determine the seed type and fertilizing rate and to make decision by accepting the whole field as homogeneous (i.e. having the same characteristics). Consequently, even if farmers know that they get different yield rates from different parts of their fields, they cannot use this info appropriately due to traditional agriculture principles (Güçdemir et al. 2004), because the field is treated as a whole (one unique parcel) regardless of its size and thus almost the same fertilizing and spraying inputs are applied to the vegetables everywhere in the field. Then this approach causes some areas to have more fertilizing and spraying than necessary, whereas some other areas have less than necessary.

Goals of a project to establish an effective and dynamic PAMS in a country may be as follows:

a. To develop simple and user-friendly GNSS applications in small-scale fields and farms

b. To establish a management system for large-scale agricultural fields, setting up an integrated positioning (e.g. RTK CORS) network for the field and the collected crop/harvest
c. To integrate GITs, namely, GNSS, GIS, UAV, satellite imagery, etc.
d. To create the national agricultural infrastructure and database of the country by using the info and data obtained from these technologies

The starting point for such a project is the requirement for accurate and reliable positioning info for agricultural works. Areas of usage of these needed data and info in agriculture can be as follows: conventional cadastral works, developing land-use policies for agricultural areas, state support purchases, agricultural support, effective crop/harvest analysis, technological use of machinery, seeding, spraying, real-time monitoring of livestock and herds, etc. Management system to be established for the above-mentioned purposes is called PAMS. Benefits of a PAMS integrated with GITs can be as follows (Güçdemir et al. 2004; Davis et al. 2004):

a. Reducing cost of fertilizing and spraying
b. Reducing environmental pollution (by preventing random and unnecessary spraying)
c. Increase in crop quality and yield
d. Making right and appropriate decisions by providing accurate and reliable info and data
e. Creating reliable and correct agricultural records in an archive to be needed for agricultural production process

Consequently, PAMS is a system based on GITs to improve crop production efficiency. Here, a field is divided into subareas (management zones), and measured/collected farming inputs obtained from the GIS database are adjusted (variable rate) to specific conditions within each management zone. Variable rate technology in PAMS can also be used to adjust seeding rate. Seeding rate may need to be higher or lower depending on the soil's ability to support the target plant population, potentially increasing ground cover and erosion protection. By applying PAMS, it has been stated in many papers that it ensures economy, increases yield, saves environment and provides efficient management (Kahveci 2012; Güçdemir et al. 2004; Davis et al. 2004; USDA: Precision Agriculture 2007; EC Regulation 2005).

There are many different ways to show PAMS management cycle in figures or charts. As an example, an effective PAMS can be expressed in six main steps, namely, georeferenced data collection, point sampling, data analysis and spatial modelling, preparation of prescription maps, GNSS-guided implementation and planning and documentation steps which are illustrated in Fig. 17.8. In the first (data collection/logging) step, georeferenced data collection (e.g. crop yield) is a continuous activity, and this is performed as the tractor and/or other machinery moves through a field. Because the PAMS is a continuous process, the site specificity (georeference) is provided by the use of GNSS. GNSS provides precise and accurate coordinates which enable the farmer to know where he is during the farming applications. If an RTK CORS network is established which covers the field (only

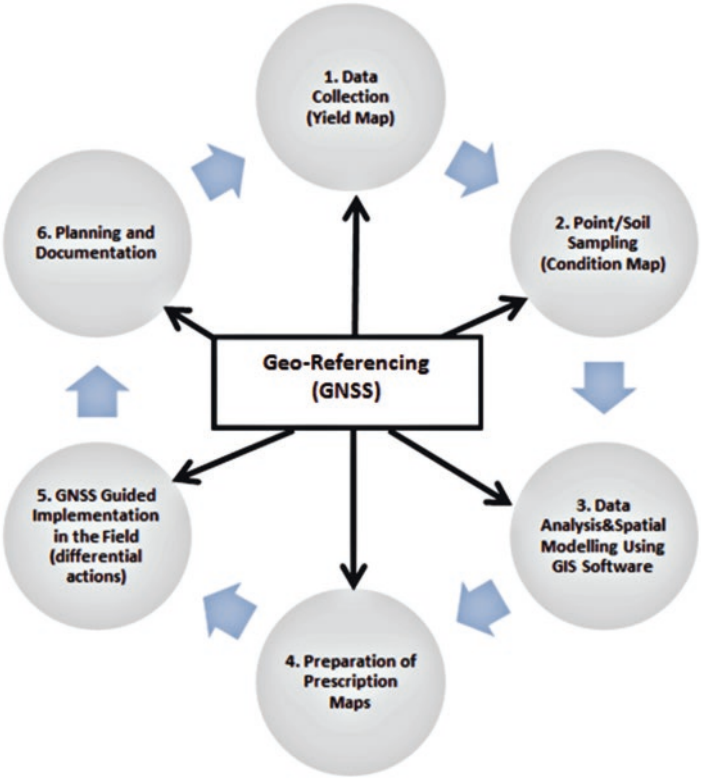


Fig. 17.8 The PAMS process

reasonable for very big farming areas) or single RTK GNSS is used or directly corrections from a national CORS network are used in this step, then it becomes possible to obtain real-time positions accurate to 2–4 cm on every part of the field in question. In the point sampling step, dispersed sample data (e.g. phosphorus, potassium and nitrogen levels) are used in order to model field conditions and thus to prepare condition maps. Here, users are concerned about estimation techniques such as frequency and interpolation method between sample points. Here, analysis grid must be determined suitably. Because if the grid is too big, then some information may be lost. Combination of data collection and the point/soil sampling steps can also be called as "crop, soil and climate monitoring" step.

In data analysis step, the collected data are reviewed, and the validity of statistical techniques applied to mapped data is tested. Thus, crop production functions can be obtained via the regression analysis of field data. This step is performed using a GIS software. In the spatial modelling step, the relationships established during the data analysis step are evaluated, and thus the optimal solutions are determined. The blend of phosphorous, potassium and nitrogen to be applied at each location in the field can be given as an example. As can be concluded from Fig. 17.5, the whole

process is completely based on georeferenced data logging which means GNSS is in the heart of a PAMS. Finally, there is an implementation step based on prescription maps prepared. Here, a GNSS guidance system is needed for the pesticide application equipment. For example, variable rate spray equipment controlled by GNSS is needed to control pesticide application. Yield monitoring equipment is needed to develop background/historical data and evaluate the precision agriculture plan.

Almost all issues mentioned above are also valid for livestock and grazing industry. Similarly, regardless of the differences such as cattle, sheep/goat or winged, it is assumed that all animals provide equal yield in average and need the same amount of water and feed. Besides, it is also assumed that there is no difference in their sheltering requirement and condition. Hence, based on these assumptions, all decisions related to feeding, watering, aeration, etc. are taken accordingly. But, in reality, the situation is quite complex. That is the reason that PAMS is not only implemented for agriculture but also for irrigation and livestock. For tracking applications, GNSS has been used in agri-logistics (e.g. harvest collection) and in control (e.g. fisheries vessel tracking, manure transport tracking). Since 2005 long-distance livestock transporters are obliged to have a GNSS-based onboard trip recorder for control on animal welfare (Van der Mal 2010, 2013; Trotter et al. 2010). GNSS tracking devices can be successfully deployed to identify and quantify the spatial grazing patterns of livestock, and there is potential for the development of site-specific management strategies using this information. Preliminary results of a trial performed in Australia are given in Berry (2013). Here, it has been concluded and suggested that spatial monitoring of livestock behaviour can provide graziers with the tools to similarly manage their grazing land culminating in reliable measures of true pasture utilization and facilitate site-specific management to account for nutrient removal and redistribution.

17.3 Examples of Data Collection Using GNSS

GNSS is often used in precision agriculture and provides extremely accurate positioning information. In another saying, GNSS allows the farmer to know accurately where they are in their field in real time. As a result of this, GNSS systems allow the farmer to create a yield/prescription maps after completing the process given in Fig. 17.1. An agriculture machinery equipped with necessary GIS software and GNSS sensor may be used automatically as seen in Fig. 17.9.

If one uses a hand-held GNSS receiver for data collection, then the real-time accuracy one will get will vary between 1 and 10 m depending on the observation day. If one uses a conventional differential GNSS (DGNSS), then the accuracy one will get will be around 50 cm. For single-base RTK applications, one will get 1–3 cm accuracy depending on the distance from the base station to the machinery (rover) which should not exceed 10–15 km. But if a project requires more accuracy and precision, then the establishment of a small CORS network is inevitable (or a

Fig. 17.9 The use of GNSS on a tractor and tractor cabin (Source: Van der Mal 2010)

national CORS network can also be used for this purpose if it has an adequate distance separation which is about 50 km). So, if there is a CORS network covering agriculture area, then the real-time accuracy may be obtained between 2 and 4 cm (if 1 cm accuracy is requested for an automatic steering application, then a dense network having less than 20 km spacing must be established depending on the field characteristic). If the GNSS receiver mounted on a tractor or combine harvest is connected to any crop sensor, all the crops collected in the field will be georeferenced. Thus, it will be possible to map the existing conditions using this digital data (e.g. to show the weed and insector disease infestation on an aerial image or digital map and to monitor their spread). There are many different uses of GNSS together with GIS in agriculture such as surveying and geo-fencing of agricultural area, machine guidance, harvest monitoring, soil sampling, tracking of livestock and agricultural machinery, determining and checking parcel areas, etc. Below are some examples of these uses:

17.3.1 Precision Point Sampling (Zonal Soil Sampling)

In point sampling (or zonal soil sampling), small amounts of soil are taken as discrete samples to characterize field conditions such as phosphorous. Meanwhile data logger continuously records measurements (e.g. crop yield) as a machine moves through a field. Then these soil samples are chemically analysed in a laboratory in order to determine crop nutrient needs for each sample. Then the fertilizer application map is plotted on a GIS database using all the soil samples collected in the field. Plotted application map is loaded into a computer on the machine (e.g. spreader) and is implemented in the field with the help of GNSS (i.e. variable rate application). Grid sampling is accomplished using GIS software. In sampling, sample size (i.e. sampling intensity), sampling grid (i.e. grid resolution) and

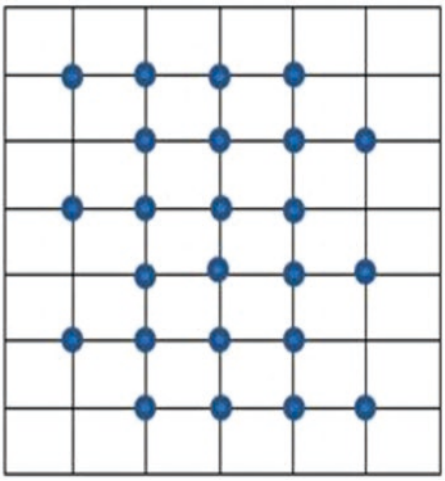


Fig. 17.10 Grid pattern sampling (Source: Van der Mal 2010)

Fig. 17.11 Sampling using GNSS (Source: Van der Mal 2010)

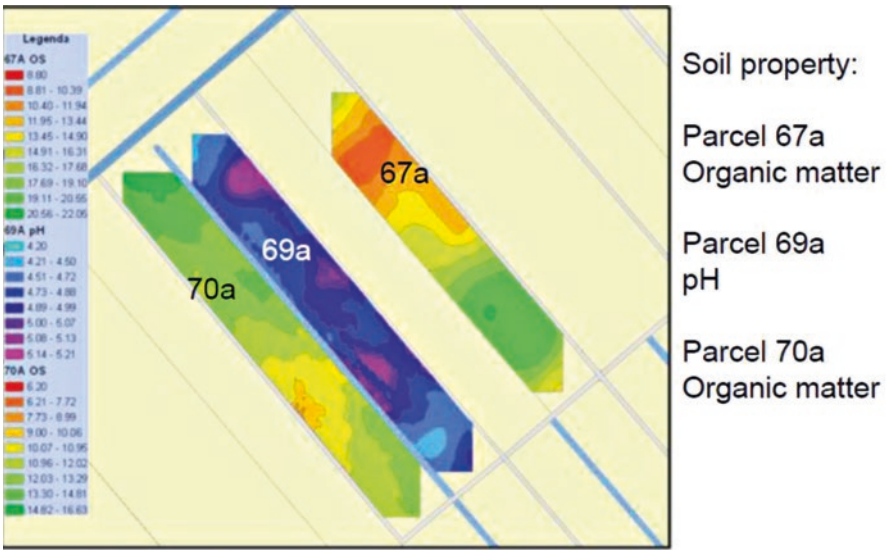


Fig. 17.12 GNSS-based soil fertility map (Source: Van der Mal 2010)

Fig. 17.13 Point guidance in the field (Source: Google Earth)

sampling patterns (i.e. regular, random, etc.) are important issues in a sampling design (Figs. 17.10, 17.11, 17.12).

17.3.2 Finding Sampling Points in the Field

Point to be measured is selected on the digital map on a console mounted in the tractor cabin. Or the coordinates of this point are entered manually. Tractor moves to the target point by showing the direction and the distance on the console (Fig. 17.13).

17.3.3 Topographic Mapping of the Field

Topographic maps show the outlines of selected natural and man-made features of the Earth. Although topography refers to the shape of the surface, represented by contour lines, topographic maps also show the geographic features such as roads, buildings, railways, rivers, forest, etc. Thus, topographic mapping is very useful and necessary in agricultural applications. For example, while the tractor moves in the field, GNSS-determined coordinates are stored either by time or by distance (e.g. every 5 s or every 10 m) into the computer/console mounted in the tractor cabin. If CORS (or commercial satellite-based augmentation services) correction data are used, these coordinates will be on the order of 2–4 cm accuracies depending on the terrain conditions and CORS station spacings. Once the 3D coordinates of an area/field are determined using GNSS, then it is possible to compute all other products necessary for agricultural implementation. These products can be DEM (digital elevation model), elevation map (contour lines), slope map, area and volume computations, cross-section computations and boundary determination. All these are possible using GNSS-derived coordinates and a GIS software in a grid map structure. Then, for example, a DEM can be used for an effective irrigation management

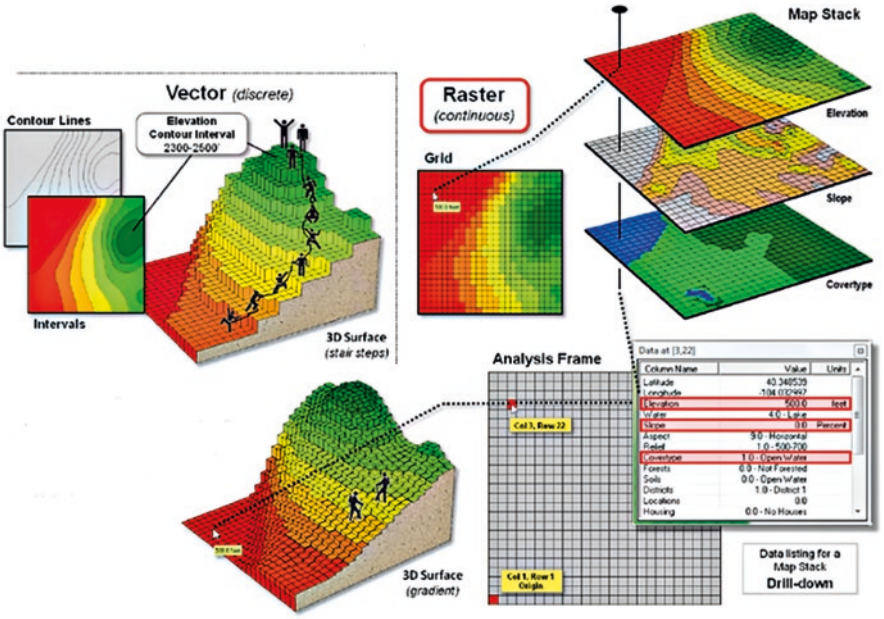


Fig. 17.14 Topographic mapping of the field (Source: Berry 2013, p. 6)

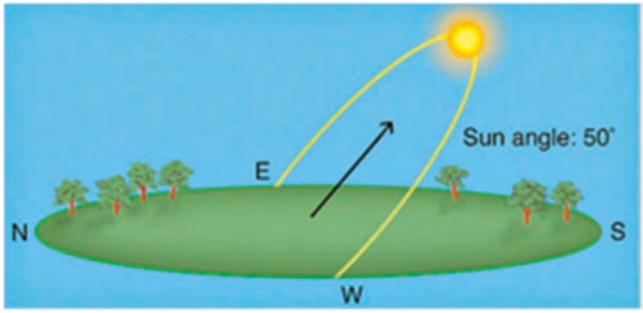


Fig. 17.15 Movement of the sun over a field (source: www.sunearthtools.com)

system, effective crop planning, determination of the erosion areas, slope map of the area, etc. (Fig. 17.14). In the below figure, grid map constitutes a set of georeferenced map layers (stacks) which consist of numbers with a value indicating the condition at each cell (location) (www.sunearthtools.com 2016; www.satconsystem.de Satcon System 2016).

Besides, using GIT it is also possible to compute and prepare maps for the sun's position along the field in question by determining the azimuth and the elevation angles of the sun during its movement (Fig. 17.15). An example of elevation and azimuth values of the sun is given in Table 17.1. If one wants healthy vegetables

Table 17.1 Elevation and azimuth values of the sun

Date	23 February 2016 / GMT + 2	
Coordinates	40° 30.0′, 32° 28.8′	
Location	Ankara, Turkey	
Time (h)	Elevation (°)	Azimuth (°)
06:25	−0.82	101.9
07:00	2.78	104.3
08:00	14.64	117.6
…	…	…
…	…	…
17:00	5.9	250.8
17:34	−0.82	257.2

and/or sweet fruit, one needs to place his/her gardens, greenhouses and orchards where they will get adequate sunshine for the parts of the year that they are growing or fruiting. Thus, mapping where the sun falls on the site at different times of the year is important, because plants need sunshine.

Elevation and azimuth are angular measurements in a spherical coordinate system. The solar elevation angle (or its complementary angle, the solar zenith angle) defines the altitude of the sun (i.e. how high the sun is). It is defined as the angle between the horizon and the centre of the sun's disc. Azimuth is the angle between a projected vector and a reference vector on the reference plane. The solar azimuth angle is the azimuth angle of the sun. It defines in which direction (from the north or south) the sun is. These two angles are shown in Fig. 17.16.

After applying and integrating GITs in agriculture, final products are the maps and analysis results archived in a GIS environment and also printout of a field sheet (field passport) giving detailed info about the related field. Such a field sheet is shown in Table 17.2.

17.4 Conclusion and Suggestions

Currently, most of the researches and applications are being focused on using GITs for PAMS in order to apply a successful SSCM. PAMS applications show a very good example of effective GITs' use in agriculture both in the field and in office which can be named as agricultural revolution. On the other hand, RFID use is also very important for food traceability. Thus, utilizing GITs and RFID together in agriculture for food traceability makes it possible to establish an accurate database which will constitute the grounds of the decisions to be taken by the farmers and/or governments. Thus, well-trained farmers will perform their works more consciously by using technology, in another saying, agricultural works, from the field to the table, including food traceability, which have been equipped with technology. Consequently, the main goal of establishing PAMS is not to burden farmers with

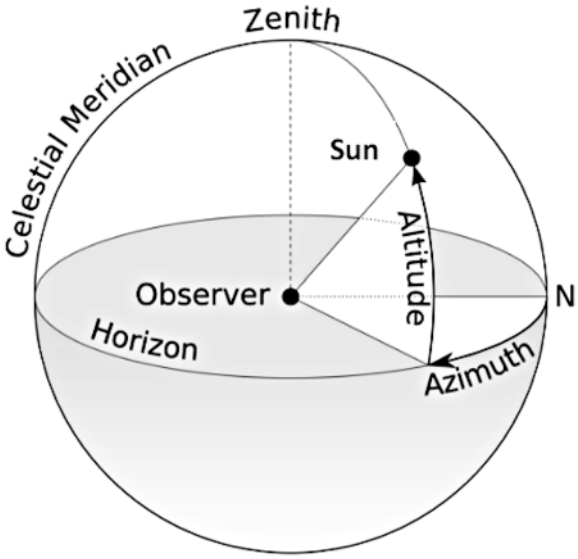


Fig. 17.16 Elevation and azimuth angles of the sun

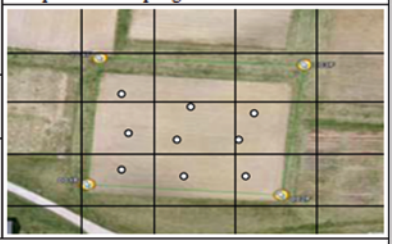
Table 17.2 Example of a field sheet

c.f. Field passport, source: www.satconsystem.de

Farmer Name: Muzaffer Kahveci — Farm ID: 18055416
Address: Safranbolu Cad. Nr:15
City: Ankara
State: Turkey

Field Data
Field Name: Camlidere
Arable Area: 2,970
Land Register Size: 3,052
Land Register No.: 1218

Middle coordinates (GNSS):
Field circumference: 875 m
Soil sampling: 10.02.2015
Deep of soil sampling: 25 cm



Crop: w-wheat — Crop Year 2015:
Soil Type: SL — Soil Classification: 54

Soil sample results from 18.04.2008

pH:	5,9	Ton in %:	8
P_2O_5:	13,8 mg	Humus in %:	1,27
K_2O:	14,3 mg	N_{min}:	48 kg
MgO:	6,1 mg	Micronutrients:	

Seed
Brand: Hybrid — Treated with: FasTac
Date: 30.10.2008 — kg/ha: 176 kg/ha — Technic: Airseeder

Date	Fertilizer	kg/ha m/ha	N kg/ha	P_2O_5 kg/ha	K_2O kg/ha	MgO kg/ha	CaO kg/ha	S kg/ha
02.03.2009	Kmex	370,6	-	-	150	22	-	15
24.03.2009	TSP	151,7	-	70	-	-	26	-
18.04.2009	KAS+Mg	202,8	55	-	-	7	31	-
23.05.2009	ASS	208,8	54	-	-	-	-	29
			109	70	150	29	57	44

Chemicals

Date	Type	Applied (l,kg,g/ha)
10.04.2009	CCC 720+Pronto+Cercobin+N36+OpticaDB	2+0,8+0,67+2+1,5
15.05.2009	CCC 720+Composan+Juwel Forte	1+0,5+1
07.06.2009	JuwelTop	0,5

Harvest
Date: 14.09.2009 — Yield in dt/ha: 69,3
Moisture: 12 — Protein: 15,2 — Sugar:

REMARKS: Heavy rain in April and May, very high temperature in July and August, wett in September

data and processing but to provide accurate and up-to-date information for supporting them to give correct decisions.

Acronyms

CORS	Continuously Operating Reference Stations
DEM	Digital elevation model
DGNSS	Differential GNSS
DSM	Digital surface model
EU	European Union
GIS	Geographical information system
GITs	Geographical information technologies
GNSS	Global Navigation Satellite System
GPS	Global Positioning System
LBS	Location-based system
NDVI	Normalized difference vegetation index
NIR	Near infrared
PA	Precision agriculture
PAMS	Precision agriculture management system
RFID	Radio frequency identification
RGB	Red, green and blue
RTK	Real-Time Kinematic
SBAS	Satellite-based augmentation system
SSCM	Site-specific farming or site-specific crop management
UAV	Unmanned aerial vehicles
VRA	Variable rate agriculture
WGS84	World Geodetic System 1984

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Chapter 18
DNA Barcoding for MPR Fruits and Vegetables

Remziye Yilmaz

18.1 Introduction

MPR fruits and vegetables are essential elements for human diet, that's why their origin and traceability throughout all stages of production, processing, and distribution are key elements in the field of food safety. Most MPR fruits and vegetables producers go through significant effort and expense to provide products that are easily traced back to their source. In the last 20 years, several DNA (deoxyribonucleic acid) based methods have been used for tracing the botanical origin of fruits and vegetables (Pasqualone et al. 2003; Ren et al. 2006; Salem et al. 2007). These methods are useful for both producers, who are interested in protecting and certifying their products, and consumers, who are interested in the quality and origin of their food (Yildiz 2010; Galimberti et al. 2013; Madesis and Ganopoulas 2014; Espineira and Santaclara 2016).

In general, DNA-based methods use specific DNA sequences as markers such as conventional Polymerase Chain Reaction (PCR) and sequencing, quantitative real-time PCR, High Resolution Melting (HRM) analysis, capillary electrophoresis (CE), microarrays, and Next Generation Sequencing (NGS). Some of these methods, species-specific DNA profiles are discovered by hybridizing DNA digested by restriction enzymes and comparing it with labeled probes (DNA fragments of known origin or sequence). The PCR-based methods involve the amplification of target loci by using specific or arbitrary primers and a DNA polymerase enzyme. Fragments are then separated electrophoretically, and banding patterns are detected by different staining methods (Madesis and Ganopoulas 2014). Last decade, a new identification system, DNA barcoding, was developed by researchers at Canada. This approach is based on the analysis of the variability within a standard region of the genome called "DNA barcode" (Hebert et al. 2003). This approach proved useful in solving

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taxonomic problems in several theoretical and practical applications (Valentini et al. 2009; Hollingsworth et al. 2011). Literally, DNA barcoding is not completely innovative, because molecular identification approaches were already in use. However, it has the advantage of combining three important innovations: molecularization of identification processes (i.e., the investigation of DNA variability to discriminate among taxa), standardization of the procedure (from sample collection to the analysis of molecular outputs), and computerization (i.e., the not redundant transposition of the data using informatics) (Casiraghi et al. 2010).

The aim of the present chapter is to summarize the state-of-the-art use of DNA barcoding as a universal tool for food traceability of MPR fruits and vegetables and cultivar identification.

18.2 DNA Barcoding

DNA barcoding is a method that uses a short genetic marker in a plant organism's DNA to identify it as belonging to particular species (Larranaga and Hormaza 2015). Plant DNA barcode cycle includes DNA extraction, PCR amplification, visualization, and sequencing. Prior to species discrimination, checking of the barcode sequences quality is essential step for this procedure (Fig. 18.1). The gene regions that are being used as the standard barcode for almost all plant groups are two gene regions in the chloroplast, matK and rbcL. They have been also approved as the barcode regions for MPR fruits and vegetables. It is explained that alternative barcoding regions as variety of candidate DNA barcode loci (trnH-psbA, rpoB, rpoC1, ndhJ, accD, ITS, ycf5, and TS2) and various two-loci combinations have been tried for plant authentication. The combination of matK and rbcL was shown to discriminate at the species level with only a probability of 72%. (https://www.npainfo.org/

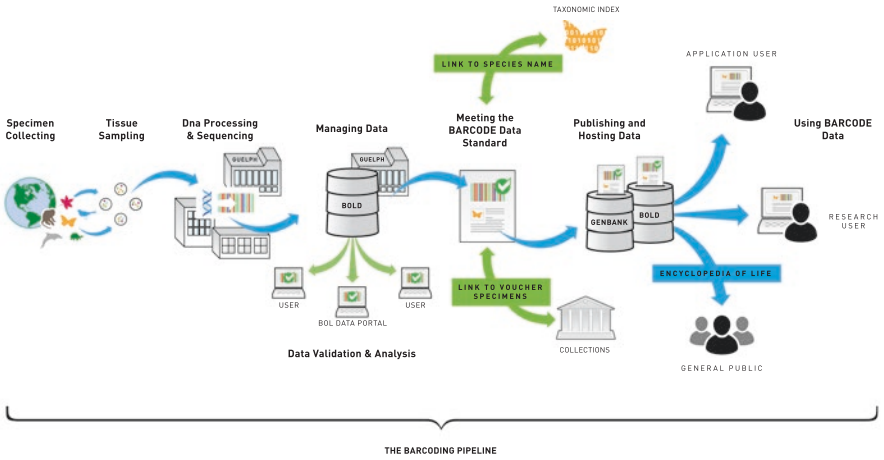


Fig. 18.1 The barcoding pipeline (http://www.barcodeoflife.org/content/about/what-dna-barcoding)

App_Themes/NPA/docs/regulatoryLegislative/White%20Paper/NPAWhitePaper_DNABarcoding.pdf). On the other hand, the two primer sets have employed for lepidopterans recover the barcode region from more than 99% of animal species or two primer sets for fishes have had about 97% success (Collins and Cruickshank 2013; Ivanova et al. 2016).

18.3 DNA Extraction

Detection of traceability of MPR fruits and vegetables requires the isolation of DNA free of inhibitors, and in sufficient quantity and quality. However the DNA extraction from MPR fruits and vegetables possesses several problems and limitations and requires special treatment (Cheng et al. 2003; Martins-Lopes et al. 2013; Madesis and Ganopoulas 2014). MPR fruits and vegetables are going through a number of procedures which result in DNA degradation. Thus it may not result in satisfactory PCR amplification because the method requires intact targets. Moreover, food products contain a large range substances including carbohydrates and chemicals which are often inhibits the PCR reaction leading to false negative or false positive results. Thus optimization procedures should be applied in a case by case manner. Different methods have been proposed for DNA extraction (Bernardo et al. 2007; Pafundo et al. 2011) from plant tissues. Yet, CTAB (cetyl trimethylammonium bromide) and phenol chloroform is the most commonly described (Box 18.1) (Doyle and Doyle 1987; Köchl et al. 2005). In addition most of the biotechnology companies have developed commercial kit for high-throughput DNA extraction, such as foodproof® Sample Preparation Kits (Biotecon Diagnostics, Potsdam, Germany), NucleoSpin® Food Kit and NucleoSpin Plant II (Macherey-Nagel GmbH, Germany), Wizard Magnetic DNA Purification for Food (Promega, WI, USA), DNeasy® Plant Mini Kit, DNeasyTissue Kit and QIAamp DNA Mini Kit (Qiagen GmbH, Germany), and Gene Elute Plant Kit (Sigma-Aldrich, MO, USA) with good results in terms of quality and quantity.

18.4 Polymerase Chain Reaction (PCR) Amplification of Barcode Region

PCR is widely held as one of the most important inventions of the twentieth century in molecular biology. Small amounts of the genetic material can now be amplified to be able to identify, detect genetic variations, including mutations, in any organism genes. PCR involves the following three steps: denaturation, annealing, and extension. First, the genetic material is denatured, converting the double stranded DNA molecules to single strands. The primers are then annealed to the complementary regions of the single stranded molecules. In the third step, they are extended by the action of the DNA polymerase. All these steps are temperature sensitive and the

Box 18.1 Cheng et al. (2003) Described a Simple and Efficient Method for Genomic DNA Extraction from Woody Fruit Crops Containing High Polysaccharide Levels

CTAB method is widely used for purifying DNA from plant tissues. CTAB, a cationic detergent, facilitates the separation of polysaccharides during purification while additives, such as polyvinylpyrrolidone, can aid in removing polyphenols.

Materials

Citrus spp. and its wild relatives were tested, including orange, mandarin, tangerine, grapefruit, pummelo, kumquat, trifoliate orange, Chinese box-orange, and 40 kinds of Citrus somatic hybrids (~300 individuals). Recently, more than 20 tropical and subtropical fruit crops were tested. Leaves were harvested at different developmental stages (young, mature, old, frosted, withered).

Equipment and reagents

- Mortar and pestle
- Waterbath
- Beckman centrifuge (J6-HC)
- UV-1601 spectrophotometer (SHIMADZU)
- Liquid nitrogen
- Extraction buffer: 100 mM Tris-HCl (pH 8), 1.5 mM NaCl, 50 mM EDTA
- (pH 8), 0.5% β-mercaptoethanol, 1.5% (w/v) CTAB (added just before use)
- Chloroform-isoamyl alcohol (24:1)
- Phenol-chloroform-isoamyl alcohol (25:24:1)
- TE buffer (pH 8): 10 mM Tris-HCl, 1 mM EDTA
- 10 mg/mL RNase A (free of DNase)
- Water-saturated ether
- Ethanol
- 5 M NaCl
- 70% ethanol
- Agarose

DNA extraction protocol

- Grind 5–8 g of clean tissue with a mortar and pestle in the presence of liquid nitrogen.
- Transfer the ground powder into a clean autoclaved 50 mL centrifuge tube and add 15 mL of boiling extraction buffer.
- Mix well and incubate at 65 °C for 60 min with occasional inversion.
- Cool the mixture to RT. Add 10 mL of chloroform-isoamyl alcohol (24:1) and invert gently for 10 min.
- Centrifuge in a Beckman centrifuge at 5500 g for 15 min at RT.

(continued)

Box 18.1 (continued)

- Transfer the top aqueous layer to a fresh 50 mL tube. Precipitate the DNA by adding an equal volume (~15 mL) of isopropanol, mix by gentle inversion, and incubate at −20 °C for 30 min.
- Deposit DNA by centrifuging at 5000 g for 10 min at RT. Wash the pellet 2–3 times (2–3 h each time) with 3 mL of 70% ethanol.
- Air-dry the pellet for 20–30 min at RT. Add 3 mL TE buffer and 25 μL of 10 mg/mL DNA-free RNase A. Incubate at 37 °C for 3 h.
- Transfer the DNA solution into a fresh 10 mL tube. Add 3 mL of phenol-chloroform-isoamyl alcohol (25:24:1), mix by using gentle inversion for 5–10 min, and centrifuge for 15 min at 5000 g at RT.
- Transfer the top aqueous layer into a new 10 mL tube. Add 1 mL of 5 M NaCl (final concentration of 1.25–1.3 M) and 4 mL water-saturated ether. Mix well by using gentle inversion and centrifuge for 10 min at 5000 g at RT.
- Discard the top ether layer. Carefully transfer the bottom aqueous layer with a 20 μL sterile pipette tip against inside the rim of the tube. Bend the tip, so as to form a slot, and pour the bottom aqueous layer from the slot into a new 10 mL tube.
- Add 4 mL of chilled isopropanol, mix well by using gentle inversion, and freeze at −20°C for 30 min. Remove the DNA pellet with a hook.
- Wash the pellet three times for 1 h each time with 3 mL of 70% ethanol.
- Air-dry the pellet for 20 min at RT. Add 1 mL TE buffer to dissolve the pellet.
- Dilute 15 μL of DNA solution to qualitative assay with a UV-1601 spectrophotometer (SHIMADZU).
- Adjust the concentration of the samples to 200 ng/μL. Add 5 μL of each sample to test on a 0.8% agarose gel. Store the DNA solution at −20 °C until use.

common choice of temperatures is 94–95 °C, 55–60 °C, and 72 °C respectively (Fig. 18.2). Good primer design is essential for successful reactions and minor adjustments can have large impacts on barcode recovery. The important design considerations described below are a key to specific amplification with high yield in barcode recovery (Ivanova et al. 2016). To maximize the chance of finding primers specific for short and universal DNA regions as barcode gene template, most important step is using refseq accession (raw DNA sequence) as template whenever possible. Some example for refseq accessions of ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) and maturase K (matK) genes are given by Table 18.1. To assure primer specificity, primers can be Basic Local Alignment Search Tool (BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi) searched from any of

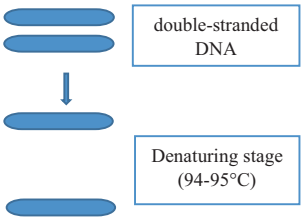
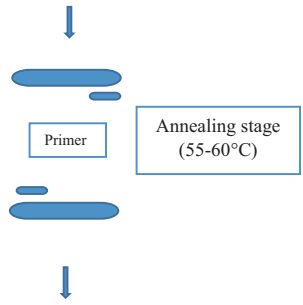
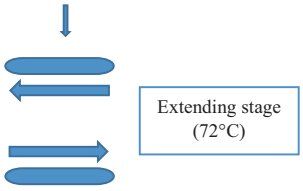
	(I)	Strand separation or denaturation. The two strands of the parent DNA molecule are separated by heating the solution to 94-95°C for 15-20s.
	(II)	Hybridization or annealing of two oligonucleotide primers (one is a reverse primer and the other is a forward primer). The solution is quickly cooled to 55-60°C to let the primers anneal to a DNA strand. One primer anneals to the 3′ end of the target (template strand) while the other primer anneals to the 3′ end of the complementary target strand. Then each copy will be the template in the next cycle. This primer annealing also depends on the melting temp (T_m) of the primer.
	(III)	DNA synthesis or elongation or extension. The solution is then heated to 72°C the optimal temperature for *Taq* DNA polymerase. This is a polymerase from a thermophilic bacterium, *Thermus aquaticus*, which lives in hot springs. This polymerase elongates both primers in the direction of the target sequence because DNA synthesis is in the 5′ to 3′ direction. DNA synthesis continues on both strands and continues beyond the target sequence.

Fig. 18.2 From there the PCR reaction goes through three steps

the genomic databases available at National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/).

18.5 Barcoding Gene Sequencing

The other important step in DNA barcoding of plants is to collect of sequences data as the molecular markers to be used. The classical chain-termination sequencing method requires a single-stranded DNA template, a primer, a DNA polymerase, normal deoxynucleosidetriphosphates (dNTPs), and modified di-deoxynucleoside-triphosphates (ddNTPs), the latter of which terminate DNA strand elongation.

Table 18.1 The rbcL and matK gene regions that are being used as the standard barcode

Barcode gene	Source	Accession
Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL)	*Malus domestica* rbcL gene, partial cds; plastid	GenBank:KM360872.1
	Citrus reticulata voucher *USDA* PI109635 rbcL gene, partial cds; chloroplast	GenBank: EF590512.1
	Citrus chloroplast rbcL gene, partial cds	GenBank: AB505951.1
	Cucurbita maxima voucher *PI* 532715 rbcL gene, partial cds; chloroplast	GenBank: HQ438627.1
Maturase K (matK)	*Vitis vinifera* chloroplast partial matK gene	GenBank: AJ429274.1
	Citrus x paradisi matK gene, partial cds	GenBank: AB071304.1
	Salix pulchra voucher *Bennett*_06-364_CAN matK gene, partial cds; chloroplast.	GenBank: KC475783.1
	Cucurbita maxima voucher *PI* 532715 matK gene, partial cds; chloroplast	GenBank: HQ438602.1

These chain-terminating nucleotides lack a 3′-OH group required for the formation of a phosphodiester bond between two nucleotides, causing DNA polymerase to cease extension of DNA when a modified ddNTP is incorporated. The ddNTPs may be fluorescently labeled for detection in automated sequencing machines (Sanger and Coulson 1975).

The DNA sample is divided into four separate sequencing reactions, containing all four of the standard deoxynucleotides (dATP, dGTP, dCTP, and dTTP) and the DNA polymerase. To each reaction is added only one of the four dideoxynucleotides (ddATP, ddGTP, ddCTP, or ddTTP), while the other added nucleotides are ordinary ones. The dideoxynucleotide is added to be approximately 100-fold lower in concentration than the corresponding dinucleotide (e.g. 0.005 mM ddATP: 0.5 mM dATP) allowing for enough fragments to be produced while still transcribing the complete sequence. Putting it in a more sensible order, four separate reactions are needed in this process to test all four ddNTPs. Following rounds of template DNA extension from the bound primer,

Table 18.2 Different type genetic analyzers available for sequencing

ABI PRISM® 3100 genetic analyzer	The ABI PRISM® 3100 genetic analyzer is an automated capillary electrophoresis system that can separate, detect, and analyze up to 16 capillaries of fluorescently labeled DNA fragments in one run
Applied Biosystems 3730 or 3730XL DNA Analyzer	The Applied Biosystems 3730/3730xl DNA Analyzers are automated, high throughput, capillary electrophoresis systems used for analyzing fluorescently labeled DNA fragments. The 3730 DNA Analyzer is compatible with the 48-capillary array only. The 3730xl DNA Analyzer is compatible with both the 48- and 96-capillary arrays
Amersham Biosciences MegaBACE™ 500 and MegaBACE™ 1000	MegaBACE™ DNA Analysis System is a fluorescence-based system utilizing capillary electrophoresis. The system performs gel matrix replacement, sample injection, DNA separation, detection, and data analysis for DNA sequencing, microsatellite genotyping, and single nucleotide polymorphism (SNP) analysis. For throughput flexibility, MegaBACE 1000 can be configured to use 16, 32, 48, 64, 80, or 96 capillaries, MegaBACE 500 can be configured to use 16, 32, or 48 capillaries
Amersham Biosciences MegaBACE 4000	MegaBACE™ 4000 is a fluorescence-based capillary electrophoresis system that simultaneously analyzes 384 DNA samples. The system replaces the gel matrix, performs simultaneous injection of up to 384 samples, and carries out the electrophoresis, detection, and analysis for DNA sequencing, genotyping, and general fragment analysis
Beckman-Coulter CEQ 8000/8800	The Beckman Coulter GenomeLab Dye Terminator Cycle Sequencing Kits contain dye-labeled terminator ddNTPs which are incorporated at the final nucleotide position of each synthesized DNA strand. Each ddNTP (A, C, G, T) has a different dye assigned to it. The DNA strands containing fluorescent dye terminators are separated, detected, analyzed, and the complete DNA sequence is reported using Beckman Coulter Inc's CEQ and GenomeLab Genetic Analysis Systems

the resulting DNA fragments are heat denatured and separated by size using gel electrophoresis.

There are different type genetic analyzers available for plant DNA sequencing techniques (Table 18.2). Capillary sequencers have now largely displaced slab gel instruments, but ABI PRISM® 377 and 373 sequencers provide a low-cost sequencing solution for DNA barcoding that seek to analyze no more than 50 templates a day. However, for higher production goals and greater automation, a multicapillary instrument is critical. Applied Biosystems has long dominated the sequencer marketplace and they produce several highly reliable instruments with varied production capacities (Ivanova et al. 2016).

Whenever possible, barcode products should be sequenced bidirectionally if they are destined for inclusion in the barcode reference library. Bidirectional sequencing aids the generation of full-length barcode sequences by avoiding problems in signal deterioration that often occur near the end of a read. It has also allowed the creation of specialized software that generates a consensus sequence from the two reads and determines a quality score for each position. However, there

Table 18.3 Some examples for DNA analysis software packages

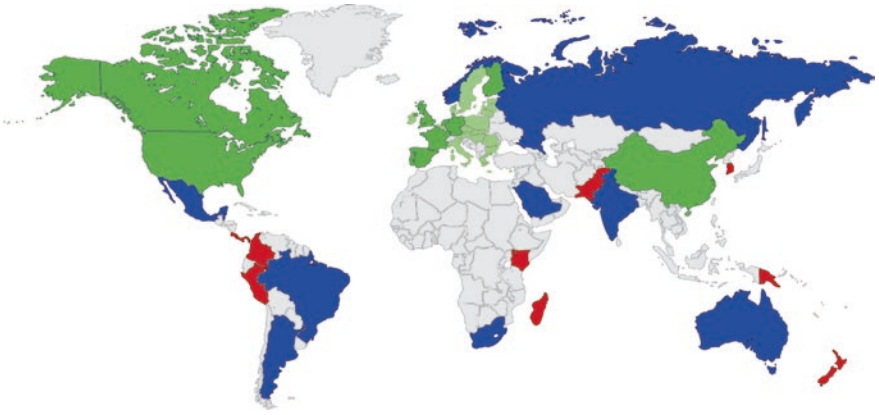
International nucleotide sequence database collaboration (INSDC)	INSDC covers the spectrum of data raw reads, though alignments and assemblies to functional annotation, enriched with contextual information relating to samples and experimental configurations
BioBarcode sequence information	A general DNA barcode data processing system, BioBarcode, with open source software – which is a general purpose database and server. It uses mySQL RDBMS 5.0, BLAST2, and Apache http server. An exemplary database of BioBarcode has around 11,300 specimen entries (including GenBank data) and registers the biological species to map their genetic relationships. The BioBarcode database contains a chromatogram viewer which improves the performance in DNA sequence analyses
BOLD (barcode of life data systems) sequence information	The Barcode of Life Data Systems is designed to support the generation and application of DNA barcode data. The platform consists of four main modules: a data portal, a database of barcode clusters, an educational portal, and a data collection workbench
Sequencher™ (Gene Codes Corporation)	Gene Codes Corporation is an international software firm specializing in bioinformatics software for DNA sequence analysis. Sequencher software for sequence analysis have been served the needs of industrial, academic and government-based biotechnology groups across the globe
SeqScape® (Applied Biosystems)	Applied Biosystems SeqScape Software v2.5 is expressly designed for mutation detection and analysis, SNP discovery and validation, pathogen subtyping, allele identification, and sequence confirmation
DNA Star (Lasergene®)	Lasergene molecular biology suite is essential sequence analysis software for performing alignments, identifying genes, assembling contigs, creating virtual clones, designing primers, etc.

remains a need for manual sequence editing to fully extract information from sequence records.

One of the most important components of the DNA barcoding studies is the construction of a public reference library of species identifiers which could be used to assign unknown specimens to known species. There are widely used DNA analysis software packages are effective for the analysis of barcode sequences in Table 18.3.

18.6 DNA Barcoding Bodies and Resources

Many DNA barcoding bodies and resources have been formed since 2003. Researchers across the globe have joined hands to establish some of the major barcoding initiatives. The primary goal is to develop an efficient DNA barcoding–based species identification system which will be universally applicable. The two major international barcoding initiatives are the International Barcode of Life (iBOL) and Consortium for the Barcode of Life (CBOL) (Ratnasingham and Hebert 2007; Bhargava and Sharma 2013).



Central	Regional	National
Canada	Argentina	Colombia
China	Australia	Costa Rica
European Union (France, Finland, Germany, Netherlands, Portugal, United Kingdom)	Brazil	Kenya
	India	Korea
	Mexico	Madagascar
	New Zealand	Pakistan
United States	Norway	Panama
	Russia	Papua New Guinea
	Saudi Arabia	Peru
	South Africa	

Fig. 18.3 Networks of leading researchers and key organizations affiliated to iBOL and engaged in DNA barcoding and/or in funding and advancing biodiversity science in a country or region

iBOL was organized by the Biodiversity Institute of Ontario at the University of Guelph, Canada, it was formerly activated in 2010. Its mission is the maintenance of the barcode reference library BOLD (Barcode of Life Data systems) and its aim is to establish around five million barcode records by 2015, including animal, plant, and fungal species. The barcode reference library currently contains records for more than 100,000 species derived from well over a million specimens. iBOL invites countries to participate as Nodes of the project. Nodes are networks of leading researchers and key organizations affiliated to iBOL and engaged in DNA barcoding and/or in funding and advancing biodiversity science in a country or region (Fig. 18.3) (http://www.ibol.org/about-us/partner-nations/).

CBOL is an international initiative devoted to the development of DNA barcoding as a global method for the identification of flora and fauna constituting the earth's biodiversity. It was established in May 2004 and is supported by grants from Alfred P. Sloan foundation. At present, over 130 organizations from 43 countries are members of CBOL from various countries promoting DNA barcoding through conferences, workshops, and trainings.

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Part IV
Health and Food Safety

Chapter 19
Microorganisms Found in MPR and Packaged Produce and Their Detection Methods

Sinem Yavaş Acar and Yeşim Soyer

19.1 The Safety of MPR and Packaged Produces

The minimally processed refrigerated (MPDR) and packaged produces are the vegetables and fruits (and their products, i.e., juices, ready-to-eat salads, sauces, fermented and dried products) that became an important meal for consumers nowadays. The consumption of fresh fruits and vegetables is highly related with health and nutritional benefits. There are several campaigns advising to consume at least five daily servings of fruits and vegetables in the USA and EU states. Besides, considerable changes in lifestyles and consumption trends such as "staying healthy," "eating correctly," and "in all seasons" have led to the increase in imports of fresh produce (Warriner et al. 2009). At the same instant, there is an increase in the number of foodborne outbreaks related with uptake of fresh fruits and vegetables. This increase is also associated with extensive produce production worldwide and insufficient sanitary practices.

Biological, chemical, and physical hazards in MPR are at allowable level. Thus, these products cannot be considered as 100% sterile, since a heat treatment to provide 12-D *Clostridium botulinum* killing is not applied.

Therefore, the refrigeration temperature and time controls are specific concerns of these produces to prevent pathogen and spoilage growth and, hence, to provide safe food to the consumers (Snyder 2003).

The production and packaging process of these products change according to product specifications. Mild disinfection is used for some products such as sprouts, berries, and cut leafy and root vegetables. Pasteurization can also be applied for

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some packaged products such as sauces and salad dressings. In mild disinfection, a reduction of 10^{-2} to 10^{-5} of pathogenic bacteria can be done and this can be achieved by washing or blanching. Alternative methods can also be practiced to reduce 10^{-5} to 10^{-7} of pathogenic bacteria such as irradiation, pulsed light, ultraviolet light, or high pressure. On the other hand, fermented produces such as kimchi and sauerkraut are classified as formulated products, and their safety is attained by fermentation, drying, salting, or acidification.

Foodborne disease pathogens are the leading cause of human illness with approximately 230,000 hospitalizations and 2600 deaths in every year in the USA (Scallan et al. 2011). In addition, a total of 5262 foodborne outbreaks were reported in the European Union, causing 43,473 human cases, 4695 hospitalizations, and 25 deaths in 2010 according to the European Union Summary Report (EFSA and ECDC 2012). The annual expenses due to bacterial foodborne infections in the USA have been figured out to be between 2.9 and 6.7 billion dollars, and this number is highly related with loss of wedges, medical care costs, and loss of life (Buzby et al. 1996).

19.2 Pathogens Related with MPR and Packaged Products

A variety of pathogens that can be found on MPR depends on the characteristics of pathogens such as being able to grow/sustain at refrigeration temperatures or able to contaminate during preharvesting (soil, water) and post-harvesting (processing, packaging, storage). The foremost pathogens for fresh produce are *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella* spp., *Clostridium botulinum*, *Aeromonas hydrophila*, viral pathogens (hepatitis A, *Norwalk virus*), and protozoan pathogens (*Giardia lamblia*, *Entamoeba histolytica*) (Bassett and McClure 2008).

Microbial contamination is the main reason of foodborne outbreaks through fresh produce. The contamination can happen through any step of farm-to-consumer process. Because of tendency toward eating raw or minimally processed foods, fresh produce has become one of the main sources of foodborne diseases due to the pathogens present on the raw material. Tissue damage that may happen during peeling, cutting, or slicing causes release of nutrients and supports growth of microorganisms (Harris et al. 2003). The growth of the most commonly observed pathogens on MPR and packaged produce depends on several factors such as characteristics of microorganisms, intrinsic and extrinsic properties of produces (pH, water activity, and growth temperature) (Table 19.1), and also the effect of pre- and post-harvesting processes. For instance, temperature control is an important measure during storage and in the selection of packaging type. Contamination may also happen as a result of unhygienic personnel or through machine-to-product strikes (insufficiently cleaned instrumentation) during processing and packaging of fresh produce.

Table 19.1 The optimum growth conditions of possible pathogens that can be observed on MPR and packaged produce (Snyder 2003)

Microorganism	Growth temperature (°C)	pH	Water activity (a_w)
Listeria monocytogenes	−1.5–44	4.5–9.5	0.92
Clostridium botulinum	3.3–45[a] 10–47.8[b]	5.0–9.0[a] 4.6–9.0[b]	0.97[a] 0.94[b]
Escherichia coli	7.0–49.4	4.0–9.0	0.95
Salmonella spp.	5.5–45.6	4.1–9.0	0.95
Campylobacter jejuni	30–45	4.9–8.0	0.987
Yersinia enterocolitica	−1.5–44	4.6–9.0	0.945
Aeromonas hydrophila	0.0–50	5.0–7.0	0.97

[a]nonproteolytic
[b]proteolytic

Table 19.2 Major listeriosis outbreaks associated with contaminated fresh produce and produce products

Food type	Observations	Location	Year	Reference
Whole cantaloupes	147 infections, 33 deaths, 1 miscarriage	28 states of USA	2011	CDC[a]
Sprouts	20 infections, 16 hospitalizations	7 states of USA	2008	CDC

[a]https://wwwn.cdc.gov/foodborneoutbreaks/

19.2.1 Listeria monocytogenes

Listeria monocytogenes, Gram-positive rod-shaped bacterium, causes the foodborne illnesses including mild flu symptoms that have the possibility to cause meningitis, septicemia, stillbirths, and abortions. The foodborne disease, listeriosis, has a mortality rate of about 24% and is effective mostly among pregnant women, their fetuses, and immunocompromised people (Farber and Peterkin 1991). The nonspore-forming, facultatively anaerobic rod has the ability to grow between −0.4 and 50 °C, and thus *L. monocytogenes* is a possible pathogen that can survive and grow at refrigeration temperatures and under low-oxygen concentrations within modified atmosphere packages of MPR produces (Gandhi and Chikindas 2007) (Table 19.2).

Listeria monocytogenes is abundant in nature but also present in silage, sewage, and milk, and can be carried by various animals such as sheep, goat, poultry, and cattle (Gunesena et al. 1995; Weis and Seeliger 1975; Welshimer and Donker-Voet 1971). Recent studies point out that *L. monocytogenes* can be present in individual salad ingredients such as beansprouts (Arumugaswamy et al. 1994), cabbage, potatoes, radish (Heisick et al. 1989), tomato, cucumber (Vahidy et al. 1992), salad dressings (Velani and Roberts 1991), and also in an even ratio of prepacked mixed salads. The contamination can occur, due to agricultural practices, from soil,

water, animal manure, decaying vegetation, and wastewater from sewage treatment plants. The incubation period of invasive diseases that is observed with meningitis and neonatal sepsis is 2–6 weeks; on the other hand the time period for diarrheal disease that comes up with abdominal cramps and fever is shorter, usually in the range of 18–27 hours (Swaminathan et al. 2001).

Listeria monocytogenes is evaluated to cause almost 1600 illnesses annually in the USA, with more than 1400 hospitalizations and 250 deaths. Surveillance studies about *L. monocytogenes* show that a wide range of intact vegetables (bean sprouts, cabbage, lettuce, tomato, radish, cucumber, etc.) and ready-to-use vegetables (cucumber slices, prepacked salad mixes, fresh cut salad packages, processed vegetables and salads) are under threat. The most significant case was observed in cantaloupes (known as mushmelon, or Persian melon) in 2011 in the USA. One hundred forty-seven people were infected with any of the four outbreak-associated strains of *Listeria monocytogenes* according to the Center for Disease Control and Prevention (CDC) report from 28 states (http://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/120811/index.html). Unfortunately, 33 deaths were reported at the end, and one pregnant woman had a stillbirth at the time of illness. It was observed that the disease mainly had affected older adults, immunocompromised people, and pregnant women and their newborns. According to 2012 recall records of FDA, ready-to-eat salads, specific packages of products containing onion, cantaloupes, prepared salads, dressings, and sandwich rolls are pointed out to be in the list of risk containing foods. Also, the *Listeria* counts were found to be above the legal safety limits for ready-to-eat (RTE) foods at retail, especially RTE salads with 4.2% presence rate in 25 gram of samples, for the European countries (EFSA and ECDC 2012).

19.2.2 Clostridium botulinum

Botulism is caused by a neurotoxin produced by the Gram-positive, rod-shaped, anaerobic, endospore-forming bacterium *Clostridium botulinum*. Botulism was firstly recognized as “sausage poisoning” due to outbreaks during the eighteenth and nineteenth centuries. The disease is described by symmetric, descending, flaccid paralysis of motor and autonomic nerves. Botulism is observed with blurred vision, dysphagia, bulbar weakness, and dysarthria. Neurological symptoms and changes because of toxin-induced barricade of the voluntary motor and automatic cholinergic junctions (arm, leg weakness, diplopia) are the most seen effects of foodborne botulism in humans. Gastrointestinal symptoms are generally as nausea, vomiting, abdominal cramps, and constipation after onset of neurological indications (Shapiro et al. 1998). The incubation period of botulism depends on severity, but generally the effects can be seen between 12 hours and 72 hours of the consumption (Arnon et al. 2001).

The bacilli produce neurotoxins in a similar pharmacological behavior, but the serological properties of the toxins are different (toxin types A, B, C, D, E, F, and G). Human botulism is proven to be related with the toxin types A, B, and E and from time to time with type F. The spores of *C. botulinum*, highly resistant to heat,

cannot be eliminated by many preserving methods. The neurotoxins are produced under anaerobic, low acid (pH < 4.6), and low solute concentrations (Lund 1990).

The endospores of *C. botulinum* can be found in soil, aquatic environments, and digestive track of animals, and thus vegetables and fruits can be contaminated during growth, harvesting, and processing (Rhodehamel 1992).

Although it is very omnipresence, recently 0.36% of precut modified atmosphere-packaged vegetables (Lilly et al. 1996) and less than 0.08–0.16 organisms per 100 g of mushroom (Notermans et al. 1989) are found to be contaminated with the spores of *C. botulinum*. In 1994, the largest outbreak of botulism has been recorded in the USA (El Paso, Texas) because of aluminum foil-wrapped baked potato consumption (Angulo et al. 1998). Thirty people were estimated to be affected and four of them had needed mechanical ventilation. Botulism toxin type A was present in patients and in both dips. Toxin formation was due to maintaining aluminum foil-wrapped baked potatoes at room temperature, evidently for several days, before they were used in the dips. Recent incidences due to *C. botulinum* in the USA are given in Table 19.3.

19.2.3 *Shiga Toxin-Producing* Escherichia coli *(STEC)*

Escherichia coli, a Gram-negative, facultative anaerobic, nonsporulating microorganism, was first discovered in the gut in 1885 and called *Bacterium coli commune*. Although most *Escherichia coli* strains are nonpathogenic members of the intestinal

Table 19.3 Major botulism outbreaks associated with contaminated fresh produce and produce products

Food type	Observations	Location	Year	Reference
Home-canned vegetable	4 infections, 4 hospitalizations	Ohio, USA	2008	CDC[a]
Chili peppers[b]	4 infections, 3 hospitalizations, 1 death	Colorado, USA	2007	CDC
Canned Chili Sauce	8 infections	3 states of USA	2007	CDC
Home-canned carrot	2 infections, 2 hospitalizations	California, USA	2006	CDC
Pasteurized carrot juice	4 infections, 4 hospitalizations, 1 death	Multistate of USA	2006	CDC
Canned mushroom	2 infections, 2 hospitalizations	Oregon, USA	2004	CDC
Home-canned pepper	3 infections, 3 hospitalizations, 1 death	Ohio, USA	2000	CDC
Garlic and oil	3 infections, 3 hospitalizations	Florida, USA	1999	CDC
Fresh raw onions served on a patty melt sandwich	28 infections, 20 hospitalizations and 1 death	Illinois, USA	1983	CDC

[a]https://wwwn.cdc.gov/foodborneoutbreaks/
[b]**Suspected**

microbiota of humans and animals, some strains have gained virulence factors so that they are able to give rise to important gastrointestinal diseases, such as diarrhea, hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), urinary tract infections (UTI), septicemia, and neonatal meningitis. Recently, there are five classes or pathogroups of diarrheagenic *E. coli*: typical enteropathogenic *E. coli* (tEPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), Shiga toxin (verotoxin)-producing *E. coli* (STEC/VTEC), and enteroaggregative *E. coli* (EAEC) (Mora et al. 2011). Among these strain groups, enterohemorrhagic *E. coli* (also known as STEC or VTEC) have emerged as a highly significant foodborne pathogen. STEC/VTEC strains that are *eae*-positive are responsible for several diseases such as gastroenteritis and hemorrhagic colitis, thrombocytopenic purpura, and HUS (Martin et al. 1986). Diarrhea (often bloody) and severe abdominal cramps are inspected after 1–10 days, but the symptoms are commonly observed after 3–4 days. The bovine gastrointestinal tracts of ruminants are the main reservoir of STEC, especially cattle, sheep, goat, and deer. Feces-contaminated produce or water becomes a vehicle of infection in humans (Fairbrother and Nadeau 2006).

STEC has been recorded frequently in CDC outbreak records due to contaminated fresh produce in the USA. Recently, Romaine lettuce outbreak affected people in ten states of the USA (Arizona, Arkansas, Georgia, Illinois, Indiana, Kansas, Kentucky, Minnesota, Missouri, and Nebraska), and raw clover sprout outbreak was observed in 11 states of the USA (Alabama, Arkansas, Iowa, Kansas, Michigan, Missouri, Ohio, Pennsylvania, Washington, Wisconsin, and West Virginia) (Table 19.4). The outbreak records indicate that *Escherichia coli* O104 is becoming very common in produce products, and the occurrence of STEC is usually multistate (Cho et al. 2006).

The agent of the most severe outbreak through fresh produce was STEC O104:H4. This outbreak was observed in Germany in 2011. The source of the outbreak was suspected as contaminated fenugreek sprouts. The World Health Organization (WHO) declared the outbreak as the biggest ever seen in Europe, the second biggest ever reported worldwide, and the most deadly EHEC outbreak ever reported due to its size and virulence. The outbreak had some unusual features such as the following: adults had higher risk and women developed more HUS than men. It was observed that the outbreak strain is highly virulent, and standard methods to test for STEC used in most EU laboratories could not detect this rare serotype (ECDC 2011).

19.2.4 Salmonella

Salmonellosis is a critical medical problem that causes symptoms of gastroenteritis including diarrhea, nausea, abdominal pain, vomiting, mild fever, and chills caused by *Salmonella enterica* subsp. *enterica* nontyphoidal serotypes (Harvey 1937). The number of salmonellosis infections reaches up to approximately 40,000 infections for each year in the USA according to CDC records. *Salmonella* are Enterobacteriaceae, Gram-negative, zero-tolerant, rod-shaped, facultatively

Table 19.4 Major *Escherichia coli* outbreaks associated with contaminated fresh produce and produce products

Food type	Serotype	Observations	Location	Year	Reference
Raw clover sprouts	O26	29 infections, 7 hospitalizations	11 states of the USA	2012	CDC[a]
Romaine lettuce	O157:H7	60 infections, 30 hospitalizations, 2 HUS infections	10 states of the USA	2011	CDC
Sprouts (possibly fenugreek seeds)	O104:H4	852 HUS cases, 3078 non-HUS cases, and 48 deaths	Germany	2011	CDC
Shredded Romaine lettuce	O104	30 infections, 12 hospitalized, and 3 HUS infections	5 states of the USA	2010	CDC
Leeks and potatoes	O157 (Phage type 8/PT8)	250 infections, 1 death	UK	2010	Uyttendaele et al. (2012)
Lettuce-based salad	O157:H7	26 infections, 11 hospitalizations, 1 death	Alabama, USA	2007	CDC
Fresh Spinach	O157:H7	238 infections, 103 hospitalizations, 31 HUS infections and 5 deaths	26 states of the USA	2006	CDC
Shredded lettuce	O157:H7	77 infections, 55 hospitalizations	5 states of the USA	2006	CDC
Fruit salad	O157:H7	18 infections, 6 hospitalizations, 1 death	Ohio	2005	CDC
Apple cider	O111	212 infections, 14 hospitalizations	New York, USA	2004	CDC
Watermelon	O157:H7	736 infections, 23 hospitalizations, 1 death	Wisconsin, USA	2000	CDC
Red grapes	O157:H7	14 infections, 8 hospitalizations	California, USA	2000	CDC

[a]https://wwwn.cdc.gov/foodborneoutbreaks/

anaerobic bacteria that are able to survive in low-oxygen atmospheres and also at low temperature below 15 °C (Garcia et al. 2010).

Symptoms of salmonellosis (i.e., diarrhea, often fever and abdominal cramps) are seen after incubation period of 6 hours to 10 days. Differently, *Salmonella enterica* subsp. *enterica* Typhi causes high fever, anorexia, malaise, headache, and myalgia, sometimes diarrhea or constipation in 3–60 days after consumption of contaminated food or water. *Salmonella* Typhi, a host-restricted serotype, causes infection only in humans; thus its spread is limited compared to host-independent (i.e., *Salmonella enterica* subsp. *enterica* serovar Typhimurium) and host-adapted (i.e., *Salmonella enterica* subsp. *enterica* serovar Dublin) serovars (Grassl and Brett 2008).

Salmonella infections start with the ingestion of organisms that are found in contaminated food or water. Conditions that cause an increase in gastric pH reduce

the *Salmonella* infectious dose; thus the gastric acidity plays a significant initial barrier for infection. In an interesting manner, salmonellae demonstrate an adaptive acid-tolerance response on exposure to low pH, possibly encouraging the organism to be alive in acidic host environments such as the stomach. After entering the small bowel, salmonellae must pass over the intestinal mucus layer before adhering to cells of the intestinal epithelium. Salmonellae have numerous fimbriae that lead to their capability to adhere to intestinal epithelial cells (Ohl and Miller 2001). All types of foods (meat, milk, ice cream, etc.) play a potential system as a host for *Salmonella*.

According to the CDC and EU records, *Salmonella* outbreaks are identified to be mostly multistate depending on the characteristics of its serological behavior (Table 19.5).

19.2.5 Campylobacter jejuni

The most critical significance of *C. jejuni*, Gram-negative, microaerophilic, spiral bacteria, is to overcome severe conditions at refrigeration temperatures for long time within moderate nutrient accessibility. Thus, together with its ability to live at low-oxygen conditions and affectivity on low dosages, this microorganism becomes a threat for the safety MPR and packaged produce and causes gastrointestinal symptoms in patients. Diarrhea (often bloody), abdominal pain, and fever are noticed after 2–10 days of infection (Jay et al. 2005).

Campylobacter jejuni can be found in different food products as a result of cross contamination. Relating to zoonosis, the microorganism is generally found in intestinal track of animals. Thus it can be observed in produce due to improper food preparation and handling (Kumar et al. 2001).

There are few outbreaks relating comprehensive evaluation of produce and produce products with *C. jejuni* in the USA (Table 19.6). A surveillance study has resulted in that the detection rates from 1564 fresh samples of ten vegetable types from two different retail levels were 3.3 for spinach, 3.1 for lettuce, 2.7 for radish, 2.5 for green onions, 2.4 for parsley, and 1.6 for potatoes. *Campylobacter jejuni* is the predominant species (88%), with the rest being *C. lari* (8%) and *C. coli* (4%) (Park and Sanders 1992). And in a similar study, 2 spinach and 1 fenugreek from a total number of 56 different vegetable samples revealed the ubiquity of *Campylobacter jejuni* biotype I (Kumar et al. 2001).

19.2.6 Yersinia enterocolitica

Yersinia enterocolitica, a Gram-negative, facultatively anaerobic psychrotrophic bacterium, has the ability to produce enterotoxins that cause suppurative and autoimmune complications (Robins-Browne 1997). The gastrointestinal diseases that may be observed after *Y. enterocolitica* infection are generally called as yersiniosis,

Table 19.5 Major *Salmonella* outbreaks associated with contaminated fresh produce and produce products

Food type	Serotype	Observations	Location	Year	Reference
Ready-to-eat sliced watermelon	Newport	At least 30 infections	England, Wales, Germany, and Republic of Ireland	2011–2012	Health Protect Agency (HPA)[a]
Whole fresh imported papayas	Agona	106 infections, 10 hospitalizations	26 states of the USA	2011	CDC[b]
Alfalfa sprouts and spicy sprouts	Enteritidis	25 infections, 3 hospitalizations	5 states of the USA	2011	CDC
Cantaloupe	Panama	20 infections, 3 hospitalizations	10 states of the USA	2011	CDC
Imported Italian tomatoes	Strathcona	55 infections (Denmark 40, Germany 14, Australia 1)	Denmark, Germany, Australia	2011	SSI[c]
Alfalfa sprouts	4,[5],12:i:-	140 infections, 34 hospitalizations	26 states of the USA	2010–2011	CDC
Frozen mamey fruit pulp	Typhi	9 infections, 7 hospitalizations	2 states of the USA	2010	CDC
Alfalfa sprouts	Newport	44 infections, 7 hospitalizations	11 states of the USA	2010	CDC
Alfalfa Sprouts	Saintpaul	235 infections, 7 hospitalizations	14 states of the USA	2009	CDC
Raw Produce (mainly jalapeño peppers and serrano peppers)	Saintpaul	1442 infections, 286 hospitalizations	43 states of the USA	2008	CDC
Cantaloupe	Litchfield	51 (USA) + 9 (Canada) infections, 16 hospitalizations	16 states of the USA	2008	CDC
Peanut butter	Tennessee	425 infections, 71 hospitalizations	44 states of the USA	2007	CDC
Tomatoes	Typhimurium	183 (USA) + 2 (Canada) infections, 22 hospitalizations	21 states of the USA	2006	CDC
Alfalfa sprouts	Chester	26 infections, 3 hospitalizations, 1 death	Multistate	2003	CDC
Cantaloupe	Poona	50 infections, 9 hospitalizations, 2 deaths	Multistate	2001	CDC
Tomatoes	Baildon	86 infections, 16 hospitalizations, 3 deaths	Multistate	1998	CDC

[a] http://www.hpa.org.uk/NewsCentre/NationalPressReleases/2012PressReleases/120202SalmonellaNewportoutbreak/
[b] https://wwwn.cdc.gov/foodborneoutbreaks/
[c] Statens Serum Institut (SSI) http://www.ssi.dk/English/News/News/2011/Salm%20imported%20tomatoes.aspx

Table 19.6 Some major cases due to *Campylobacter jejuni* associated with contaminated fresh produce and produce products

Food type	Observations	Location	Year	Reference
Lettuce	11 infections, 2 hospitalizations	Minnesota, USA	2009	CDC[a]
Peas	104 infections, 5 hospitalizations	Alaska, USA	2008	CDC
Watermelon	15 infections, 1 hospitalization	Virginia, USA	2006	CDC
Lettuce-based salad	13 infections, 1 hospitalization	Connecticut, USA	2000	CDC

[a]http://wwwn.cdc.gov/foodborneoutbreaks/Default.aspx

Table 19.7 Major yersiniosis cases associated with contaminated fresh produce and produce products

Food type	Observations	Location	Year	Reference
Tofu, an oriental soybean curd, packaged in untreated spring water	87 infections, 17 hospitalizations	Washington, USA	1981–1982	CDC[a]

[a]https://wwwn.cdc.gov/foodborneoutbreaks/

and common symptoms are fever, abdominal pain, and diarrhea (often bloody diarrhea) with 1–10 days of incubation. In severe cases, the virulent strains of the bacteria cause lymphadenitis and septicemia.

The microorganism populates at wide spectrum of environments such as intestinal track of animals and terrestrial and aquatic systems. Common serotypes isolated from human cases are O:3, O:9, O:8, O:5, and O:27 (Bialas et al. 2012, Toma et al. 1984. The distribution of the serogroups among people changes geographically (Kapperud et al. 1990). However *Y. enterocolitica* is mostly associated with animal products such as pork and produce (i.e., grated carrots) (Beuchat 1996). Since *Y. enterocolitica* can grow at refrigeration temperatures, especially throughout transport and storage of fresh produce, the control of this pathogen is a fundamental step in terms of safety of MPR and packaged produce products.

Outbreaks due to *Y. enterocolitica*, up to now, that are recorded, are mostly due to pork (i.e., Norway in 2007) and pasteurized milk (Vermont and New Hampshire, USA; 1995) (Grahek-Ogden et al. 2007; Ackers et al. 2000). However in 2006, a *Y. pseudotuberculosis* case was observed arising from carrots in Norway. A prevalence study of *Yersinia* spp. on 673 ready-to-eat vegetables collected from Korea from 2001 to 2002 has demonstrated that the isolation rate of *Yersinia* spp. is 4.0%, while the rate of *Y. enterocolitica* is 2.6%. Corresponding to the serotypes of *Y. enterocolitica* isolates, O:3 (11.1%) and O:5 (11.1%) are the dominant, followed by O:8 (5.6%) and others (72.2%). The biotype analysis reports that the isolates fell into four types (i.e., 1A, 3B, 3, and 5A with 77.8%, 11.1%, 5.6%, and 5.6% of isolates, respectively) (Lee et al. 2004). Also, in the 1980s an outbreak has been recorded due to tofu with the symptoms of gastroenteritis (Table 19.7). *Y. enterocolitica* was found in tofu products, and also from the processing plant's water supply, several sites within the plant (Tacket et al. 1985).

19.2.7 Aeromonas hydrophila

A. hydrophila, being from the family Vibrionaceae, is a Gram-negative, motile, psychrotrophic, rod-shaped bacterium. Septicemia, meningitis, and endocarditis are the main infections caused by this microorganism in human. Traveler's diarrhea is one of the characteristic symptoms of *Aeromonas* (Vila et al. 2003).

The habitat of *Aeromonas hydrophila* is very diverse and thus it can be isolated from various sources: aquatic and soil environments and also animal feces. Hence, fish and shellfish products (ICMSF 1996), grocery store produce (Callister and Agger 1987), and various salads such as ready-to-use vegetable salads (Marchetti et al. 1992), mayonnaise salad samples (Knøchel and Jeppesen 1990), mixed salads (Garcia-Gimeno et al. 1996), and minimally processed fresh vegetable salads (Xanthopoulos et al. 2010) provide good media for the growth of *A. hydrophila.* But, there is no record of *Aeromonas hydrophila* associating with produce-related outbreaks.

19.2.8 Viral Pathogens

Food-related viral infections usually start with direct contact or by contamination of food or water. *Norwalk virus* and hepatitis A virus are the most significant ones according to the outbreak records in the USA (Cliver 1997) (Table 19.8). The incubation times and the symptoms of the two prominent viruses, on the other hand, can be used as a selective factor among them. Hepatitis A, with 15–50 days of incubation period, causes jaundice, dark urine, fatigue, anorexia, and nausea, whereas *Norwalk virus* (*Norovirus*) is responsible for vomiting, cramps, diarrhea, and headache after 15–77 hours of incubation.

19.2.9 Parasites

Parasites, also known as macroparasites, are categorized as protozoa and helminthes. Protozoa are unicellular eukaryotes, whereas helminths are multicellular worms and classified as cestodes (tapeworms), trematodes (flukes), and nematodes (roundworms) (Table 19.9). Up to now, different species of parasites are found to have a role in fruit-/vegetable-related foodborne infections and outbreaks (Newell et al. 2010).

Foodborne pathogens and parasites differ from each other in some ways such as:

(i). Parasites cannot survive and replicate outside the host, whereas bacterial pathogens do not necessitate a host to live.
(ii). Antimicrobial susceptibility rate is too low for parasites compared to pathogens.

Table 19.8 Some major virus-associated outbreaks associated with fresh produce and produce products

Food type	Source of organism	Observations	Location	Year	Reference
Fruit salad	*Norovirus*	139 infections, 1 hospitalization	Missouri, USA	2010	CDC[a]
Green salad	*Norovirus*	45 infections, 2 hospitalizations	New York, USA	2010	CDC
Caesar salad	*Norovirus*	53 infections, 1 hospitalization	Illinois, USA	2010	CDC
Greek salad	*Norovirus*	15 infections	Florida, USA	2010	CDC
Grapes	Hepatitis A	5 infections, 5 hospitalizations	Ohio, USA	2010	CDC
Specialty salads	*Norovirus*	10 infections	Tennessee, USA	2010	CDC
Lettuce wraps	*Norovirus*	151 infections, 1 hospitalization	Oregon, USA	2008	CDC
Lettuce-based salad	*Norovirus*	12 infections, 3 hospitalizations, 1 death	Illinois, USA	2008	CDC
Romaine lettuce	Hepatitis A	22 infections, 4 hospitalizations	California, USA	2008	CDC
Acai, bananas, strawberries, sugarcane juice	Hepatitis A	3 infections, 2 hospitalizations	Florida, USA	2007	CDC
Salad	*Norovirus*	37 infections, 8 hospitalizations	Pennsylvania, USA	2006	CDC
Tomato	Hepatitis A	23 infections, 9 hospitalizations	Tennessee, USA	2005	CDC
Salad bar	*Norovirus*	425 infections	North Carolina, USA	2004	CDC
Green onion/scallion	Hepatitis A	57 infections	Tennessee, USA	2003	CDC
Green onion/scallion	Hepatitis A	297 infections	Georgia, USA	2003	CDC
Cabbage, green onion/scallion	Hepatitis A	16 infections, 3 hospitalizations	North Carolina, USA	2003	CDC
Green onion/scallion	Hepatitis A	565 infections, 128 hospitalizations, 3 deaths	Pennsylvania, USA	2003	CDC
Coleslaw	Hepatitis A	16 infections, 3 hospitalizations, 1 death	Florida, USA	2002	CDC
Guacamole, unspecified; salsa, unspecified	Hepatitis A	4 infections	Washington, USA	2000	CDC
Green onion/scallion	Hepatitis A	32 infections, 15 hospitalizations	Multistate outbreak, USA	2000	CDC
Strawberries	Hepatitis A	8 infections	Massachusetts, USA	2000	CDC
Green salad	*Norovirus*	300 infections	Pennsylvania, USA	2000	CDC

Salads and sandwich	Hepatitis A	40 infections	Michigan, USA	1999	CDC
Salad	Hepatitis A	2 infections, 1 hospitalization	Colorado, USA	1999	CDC
Strawberries	Hepatitis A	29 infections	Texas, USA	1998	CDC
Lettuce-based salads, potato, baked	*Norovirus*	25 infections	Illinois, USA	1998	CDC
Green onion/scallion	Hepatitis A	42 infections, 13 hospitalizations	Ohio, USA	1998	CDC
Salad	Hepatitis A	4 infections	Florida, USA	1998	CDC
Salad	*Norovirus*	67 infections	Virginia, USA	1998	CDC
Salad	*Norovirus*	51 infections	Georgia, USA	1998	CDC
Mixed fruits	*Norovirus*	103 infections, 1 hospitalization	Illinois, USA	1998	CDC

[a]https://wwwn.cdc.gov/foodborneoutbreaks/

Table 19.9 Common parasites that have been studied on raw vegetables and fruits

Phylum	Organism	Vegetable/fruit	Reference
Protozoa	*Cyclospora*	Herb	Tram et al. (2008)
	Cryptosporidium spp.	Raw salad	Amoros et al. (2010)
	Giardia lamblia	Raw vegetables	Anuar et al. (2012)
	Toxoplasma gondii	Green vegetables	Ekman et al. (2012)
	Entamoeba histolytica	Herbs, lettuce, radish samples	Mongre and Arias (1996)
	Balantidium coli	Fresh vegetables	Ogbolu et al. (2009)
	Trypanosoma cruzi	Açai palm	Aglaêr et al. (2009)
Nematodes (roundworms)	*Angiostrongylus*	Raw vegetables	Zanini and Graeff-Teixeira (2001)
Cestodes (tapeworms)	*Taenia solium*	Raw vegetables	Rivero and Navarro (1986)
	Echinococcus	Raw vegetables	Daryani et al. (2008)
Trematodes (flukes)	*Fasciolopsis*	Uncooked vegetables	Lee et al. (2011)
	Fasciola hepatica	Raw vegetable salad, alfalfa juice	Marcos et al. (2005)

(iii). Some pathogens can sporulate, but nearly all parasites have a resting stage such as egg, cyst, or oocyst which gives a resistance for desiccation, disinfections, antimicrobial agents, and other difficulties.
(iv). Very low dosages of parasites have the possibility to cause severe infections, for instance, *Cryptosporidium* spp. (Teunis et al. 2002).
(v). The infection period and effects are different for parasites and pathogens; most of the parasites cause asymptomatic infections, some are responsible for short-lived effects, and the others may live in the host body for a long time showing a chronic disease.
(vi). The detection methods and surveillance studies for parasites are not well developed compared to bacterial pathogens.

As a result of all of these differences, a study related with molecular detection of parasites gains a crucial interest (Newell et al. 2010).

Raw vegetables and fruits are under concern in case of parasite contamination because of possible risk factors such as feces, soil, irrigation water, sewage, human handling, and improper processing of them. For instance, if sewage or slurry is used for fruits and vegetables during irrigation before harvesting, there would be a possible risk of parasite contamination. Kern et al. (2004) have illustrated that eating unwashed strawberries and chewing grass are possible routes of transmission of fox tapeworm *Echinococcus multilocularis*.

Giardia lamblia is the prominent flagellated protozoan parasite among other protozoans, especially important for vegetable contamination (Adam 2001). It colonizes and reproduces in the intestines and it is responsible for the giardiasis (beaver fever) in which loss of appetite, fever, explosive diarrhea, hematuria, watery stool, and stomach cramps are seen. This microorganism is the usual cause of gastroenteritis

Table 19.10 Some parasite outbreaks associated with contaminated fresh produce and produce products

Food type	Organism	Observations	Location	Year	Reference
Lettuce-based salads with parmesan and chicken	*Giardia lamblia*	15 infections	Missouri, USA	2007	CDC[a]
Unspecified vegetables	*Giardia lamblia*	50 infections	New York, USA	2005	CDC

[a]https://wwwn.cdc.gov/foodborneoutbreaks/

in humans within the waterborne outbreaks. In addition, foodborne outbreaks of giardiasis linked to person-to-person transmission have been reported. Outbreaks might be waterborne at the beginning but spread afterward through people by person-to-person transmission (Katz et al. 2006). Thus, it makes this microorganism an important threat for plants due to possibility of unhygienic washing techniques and unsanitary handling procedures. For example, in 2005, a giardiasis outbreak is detected in New York (Table 19.10) during camping because of unhygienic vegetables and it has resulted in 50 infections.

19.3 The Traditional (Analytical) Methods to Detect Pathogens in MPR and Packaged Produce

The analytical methods are still the most known, highly applied, traditional ways to control the food safety. Although the methods have some drawbacks such as being time-consuming and labor-intensive, they have been well standardized and accurate.

The standard methods, which are used in food control and reference laboratories both in EU, the USA, and other countries to detect the pathogens in fresh produce, are summarized in this chapter. The standard analytical methods can be reached from the *Bacteriological Analytical Manual* of US Food and Drug Administration (FDA's BAM), European Committee for Standardization (CEN), International Organization for Standardization (ISO), and Association of Analytical Communities (AOAC International). As it can be seen in Table 19.11, protocols of CEN are basically adaptations of ISO methods. Detection protocols of both ISO and FDA/BAM are mainly based on cultural methods.

The analytical methods consist of several basic steps: sample collection, sample storage, sample preparation, detection and analysis, and finally result interpretation. Before sampling, it is crucial to consider the statistical considerations; for instance, sample size, frequency, and volume should be determined. Then, the sample is gathered by swabbing or by directly grabbing and stored at specific temperatures and for settled time intervals depending on the method and the microorganism. The samples should be processed to be homogenized by centrifugation or filtration. For some infections, purification and decontamination (i.e., from chemicals) may

Table 19.11 The analytical methods applicable to MPR and packaged produce

Microorganisms	FDA[a]	EN[b]	ISO[c]
Salmonella	FDA's BAM Chap. 5, 2011 (a culture-based method for detection and enumeration of *Salmonella* spp. in foods)	EN ISO 6579:2002 EN ISO 6579:2002/A1:2007 EN ISO 6579:2002/AC:2006 (a horizontal method for the detection of *Salmonella* spp. including *S. typhi* and *S. paratyphi* in food and animal feed)	ISO 6579:2002 (a horizontal method for the detection of *Salmonella* spp. including *S. typhi* and *S. paratyphi* in food and animal feed) ISO/NP 6579–1 (a horizontal method for the detection, enumeration, and serotyping of *Salmonella* in food chain) ISO/PRF TS 6579–2 (a horizontal method for the detection, enumeration, and serotyping of *Salmonella* in food and animal feed)
E. coli	FDA's BAM Chapt. 4, 2002 (LST-MUG Method: an assay based on enzymatic activity for detection of GUD positive *E. coli* in chilled or frozen foods via their fluorescence ability under long-wave UV light)		ISO/PRF TS 13136 (a PCR-based horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC) belonging to O157, O111, O26, O103, and O145 serogroups in food and animal feed) ISO 7251:2005 (a horizontal method for the detection and enumeration of presumptive *E. coli* – most probable number technique – in food and animal feed)
E. coli O157		EN ISO 16654:2001 (a horizontal method for the detection of *E. coli* O157 in food and animal feeding stuff)	ISO 16654:2001 (a horizontal method for the detection of *E. coli* O157 in food and animal feeding stuff)
Shigella	FDA's BAM Chap. 6, 2001 (a method based on DNA hybridization and a culture-based method for detection and enumeration of *Shigella* spp. in foods)	EN ISO 21567:2004 (a horizontal method for the detection of *Shigella* species in food and animal feeding stuff)	ISO 21567:2004 (a horizontal method for the detection of *Shigella* species in food and animal feeding stuff)
Yersinia enterocolitica	FDA's BAM Chap. 8, 2007 (a culture-based method for detection and enumeration of Y. *enterocolitica* in foods)	EN ISO 10273:2003 (a horizontal method for the detection of presumptive pathogenic *Y. enterocolitica* in food and animal feed)	ISO 10273:2003 (a horizontal method for the detection of presumptive pathogenic *Y. enterocolitica* in food and animal feed)

L. monocytogenes	FDA's BAM Chapter 10, 2011 (a culture-based method using selective media for detection and enumeration of *L. monocytogenes* in foods)	EN ISO 11290–1:1996 EN ISO 11290–2:1996 EN ISO 11290–1:1996/A1:2004 EN ISO 11290–2:1998/A1:2004 (a horizontal method for the detection and enumeration of *L. monocytogenes* in food and animal feed)	ISO 11290–1:1996 EN ISO 11290–2 (a horizontal method for the detection and enumeration of L. monocytogenes in food and animal feed)
Clostridium perfringens	FDA's BAM Chap. 16, 2001 (a culture-based method for detection and enumeration of *C. perfringens* and/or its enterotoxins in foods)		
Clostridium botulinum	FDA's BAM Chap. 17, 2001 (a PCR-based method for detection of *C. botulinum* and the mouse bioassay or ELISA-based method for detection of botulinum toxins in foods)		
Viral pathogens	FDA's BAM Chap. 26, 2001 (a PCR-based method for detection and quantitation of hepatitis A virus)	CEN TC275/WG6/TAG4 (a horizontal method for detection of hepatitis A virus and *Norovirus* in food using real-time RT-PCR)	
Parasites	FDA's BAM Chap. 19, 2001 (a fluorescent-antibody staining method for detection of parasite and enumeration of a number of cysts in a vegetable sample)	ISO TC34/SC9/WG6 CEN TC275/WG6/TAG7	

[a] US Food and Drug Administration
[b] European Standard
[c] International Organization for Standardization

be required. Generally, the steps of nonselective enrichment, selective enrichment, and selective agar plating are performed. The equipment, agars, and broths are particularly chosen depending on the characteristics of the specific microorganism (growth conditions, able to use a sugar type, etc.) and also the sample form (liquid/solid).

19.4 The Molecular Methods to Detect and Subtype Pathogens in MPR and Packaged Produce

Rapid and accurate identification of pathogens is very crucial for investigating foodborne outbreaks, combined with epidemiological investigation. Recent multistate outbreaks such as *E. coli* 0104:H4 in raw sprouts in 2011 in Europe and *Salmonella* in cantaloupe in 2011 in the USA show the sustained risk of pathogens and also the dispute of discovering the reason of widely spread infections.

19.4.1 Detection Methods

Recent advances in molecular techniques have inspired the detection of pathogens in foods. For instance, polymerase chain reactions (PCR), synthesizing multiple copies of (amplifying) a specific piece of DNA, are the leading and mostly used technology (Naravaneni and Jamil 2005). These PCR-based methods consist of three parts: DNA extraction, DNA amplification, and detection. Sample enrichment, the start point of these assays, is the process where samples are incubated at enrichment broths to make all organisms to grow rapidly. After sample preparation, the cells are lysed to extract DNA and the last process, PCR begins. In thermocycler, the DNA is amplified to produce sufficient copies of target sequence. While some detection methods rely on end-point detection (i.e., 5′ nuclease TaqMan PCR assay and microarrays), many commercial test protocols utilize real-time detection. In end-point detection, the amplified target sequences are separated by electrophoresis and visualized by a staining technique (Call et al. 2003; Hoorfar et al. 2000). However, during real-time PCR, the amplification and detection steps are merged, by the addition of a fluorescent reporter probe to monitor the amplification process (Bustin et al. 2009). Thus it becomes possible to indicate detection when the number of copies produced reaches a threshold level. Real-time detection allows testing for more than one pathogen in the same assay by using probes labeled with different colored dyes. Detailed information about present molecular detection techniques are given in Table 19.12.

Table 19.12 Summary of some prominent molecular detection methods (McLoughlin 2011; Espy et al. 2006; Lazcka et al. 2007)

PCR-based methods	Conventional PCR	Less time-consuming compared to culturing and plating Discrimination between viable and nonviable cells is impossible
	Real-time PCR	Mechanism is based on conventional PCR and Southern blot analysis Contamination risk is low compared to conventional PCR Faster than conventional PCR Low cost compared to new technologies (arrays, WGS etc.) Novel organism detection is inadequate
	Multiplex PCR	Mechanism is based on PCR with multiple primers within in a single PCR mixture Limited capacity of multiplexing Sensitivity is low High degree of sensitivity Specificity is high, but sequence of the organism should be known
Oligonucleotide microarrays	ViroChip	Probe design is required Mechanism is based on spotting synthesized oligonucleotides on a glass slide Low cost of spotted oligo arrays Ability to detect novel viruses within a known family Low density of probes on a slide Inherent noisiness of the data High cost for each array design
	Resequencing pathogen microarrays (RPM)	Probe design is required Mechanism is based on resequencing High specificity for a strain-level identification Short probes at high number Low sensitivity Limited range of microorganism detection on one array Detection of novel organisms is not possible
	Universal detection arrays	Probe design is required Sequence-independent mechanism is based on photocatalytic process Probes are not specific to genomes, theoretically able to detect every organism Predicting a signature (reproducible pattern of probe intensity) for a known sequence organism is not possible Experiment number is high
	GreeneChip	Probe design is required Mechanism is based on Agilent inkjet system Species-level identification for viruses is fine but for bacteria it is poor Sensibility is comparable with ViroChip

(continued)

Table 19.12 (continued)

	Lawrence Livermore microbial detection array (LLMDA)	Probe design is required Mechanism is based on NimbleGen platform (photocatalytic synthesis) and prototyped with Agilent inkjet technology Probe density is high Probes are selected from whole genome sequences; thus strain-level identification is possible High sensitivity and specificity Wide usage for bacteria, viruses, and eukaryotes High cost
Whole genome sequencing	454 (Roche) Solexa (Illumina) and SOLiD (ABI)	Mechanism is based on sequencing all of an organism chromosomal DNA High cost Unbiased information Detection of novel organisms is possible High throughput thus requires large amount of computing power and storage capacity

19.4.2 Subtyping Methods

There are numerous methods to tract the bacterial source and determine the distribution of pathogens isolated from infected people. However, there are some difficulties to design a feasible subtyping method; for example, markers must be stable and reproducible and work for all outbreak isolates (van Belkum et al. 2007). Besides the availability, the technique should have a high discriminatory power and also illustrate similar outcomes with epidemiological results of an outbreak. On account of being operable to perform in different laboratories, the method should be rapid and adaptable to different conditions and pathogens. Likewise, the cost of the equipment, reagents, and consumables should be affordable (van Belkum et al. 2007).

Before the molecular subtyping methods, subspecies characterizations of pathogens have been done by phenotypic methods. The phenotyping methods such as serotyping and phage typing have the ability to characterize bacteria, but they have low discriminatory power compared to subtyping methods. In addition to all, high amount of specialization is needed and their reagents may not be accessible for some laboratories. By the development of molecular techniques, it is now available to detect differences in the nucleic acid sequence of pathogens. Some of these subtyping methods (Table 19.13) are based on restriction analysis of bacterial DNA (i.e., ribotyping, PFGE), and some use polymerase chain reaction (PCR) amplification (i.e., AFLP, MLVA) and the others identify DNA sequence polymorphism at specific loci in the genome (i.e., MLST, SNP analysis).

DNA sequencing has altered analyses of pathogen genomes and improved the understanding of the foodborne pathogens' biology and phylogeny. Genetic differences between serotypes and the acquisition and evolution of virulence and

Table 19.13 Summary of some prominent molecular subtyping methods (Modified from: van Belkum et al. 2007)

Fragment-based genotyping methods	Pulsed-field gel electrophoresis (PFGE)	A gold standard method for bacterial subtyping High discriminatory power Slow, labor-intensive Have some restrictions in usage of whole pathogens
	Multiple-locus variable number tandem repeat analysis (MLVA)	Faster than PFGE Requirement of automated sequencer
	Detection of variable absent or present (VAP) loci	Using the presence or absence of a set of genes or virulence factors A supportive method to other typing techniques
	Ribotyping	Utilizing the differences in the location and number of ribosomal RNA gene sequences Highly reproducible and easy analysis Limited number of rRNA genes in some pathogens
	Amplified fragment length polymorphism (AFLP)	Amplification based Requirement of automated sequencer Discriminatory power is similar with PFGE
Nucleic acid sequencing-based genotyping methods	Multiple-locus sequence typing (MLST)	Analyzing the conserved sequences of multiple housekeeping genes Low discriminatory power Labor-intensive, slow technique
	Multiple-virulence-locus sequence typing (MVLST)	Focusing on virulence loci differently than MLST
	Single nucleotide polymorphisms (SNPs)	Useful for qualifying evolutionary relationships Expensive equipment, labor-intensive Portable output, easily managed interlaboratory comparison, and high throughput
	Whole genome sequencing technology	Practicable for designation of additional polymorphic regions Not feasible for outbreak identification due to time and cost considerations Need of software usage
Microbial characterization techniques	Microarrays	Rapid and accurate analysis of large number of DNA fragments at once Restrictions due to development cost and interpretation
	Mass spectrometry	Measurement of the ratio of mass to charge of ions in a gaseous phase Fast and simple method to detect PCR-amplified regions Large and expensive instrumentation

pathogenic traits among species can be defined by the assistance of whole genome sequencing techniques (Gilmour et al. 2010). Firstly, the bacterial genome sequences have been produced using the Sanger chain termination sequencing technology. But recently, post-Sanger sequencing methods (also known as next-generation sequencing) are widely used due to their ease and time (Medini et al. 2008; Shendure and Ji 2008). Commercialized ones such as 454 or GS FLX™ platforms are generally utilized for rapid bacterial genome sequencing for subtyping purposes (i.e., pyrosequencing *Listeria monocytogenes* isolates as a result of a listeriosis outbreak in Canada during the summer of 2008).

19.4.2.1 Pulsed-Field Gel Electrophoresis (PFGE)

PFGE is known as "gold standard" of molecular typing methods for various bacterial foodborne pathogens such as *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia pestis*, *Vibrio cholera*, and *Shigella*. PulseNet network uses PFGE to characterize widespread outbreaks of bacterial foodborne illness (Swaminathan et al. 2001).

In PFGE, bacterial genome is digested with rare cutting enzyme (for example; Table 19.14), and smaller numbers of DNA fragments are formed and then they are separated with electrophoresis techniques (Fig. 19.1). Genetic comparisons among isolates are performed by comparing the differences in restricted DNA profiles. PFGE power supply, gel boxes, proteases, restriction enzyme(s), and the photography system are the main equipment and reagents of the system. Commercial software packages (i.e., BioNumerics from Applied Maths and Bio-Rad) are used to analyze the digitally captured gel images.

Table 19.14 General information of pathogenic bacteria analyzed by PFGE

Microorganism	Restriction enzyme	Approximate number of restriction fragments	Fragment size (kb)	References
Escherichia coli	*Xba*I	ca. 20	10–500	Arbeit (1995), Barrett et al. (1994), Maslow et al. (1993), Haley et al. (1995)
	*Not*I	12–15	10–1000	
	*Sfi*I	15–20	10–700	
Salmonella spp.	*Not*I	40–50	5–400	Olsen et al. (1994), Olsen et al. (1997)
	*Xba*I	20–30	100–1000	
Shigella	*Xba*I	15–23	10–700	Maslow et al. (1993)
	*Sfi*I	15–20	10–700	
Yersinia pestis	*Xba*I	ca. 20	10–700	Maslow et al. (1993)
Listeria monocytogenes	*Asc*I	10–20	10–500	Miettinen et al. (1999)
	*Sma*I	15–20	10–300	
Campylobacter jejuni	*Sma*I	8–10	40–400	Suzuki et al. (1993), Yan et al. (1991)
Vibrio cholerae	*Not*I	20–30	10–400	Chowdhury et al. (1994)

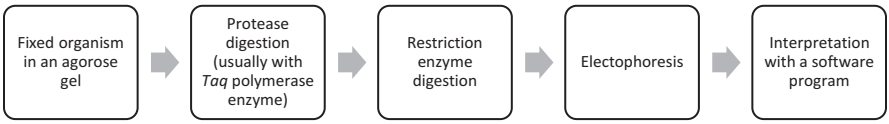

Fig. 19.1 The schematic progress of pulse-field gel electrophoresis

The genetic relatedness of bacterial isolates is determined by the number of band differences. 2–3 band differences are named as closely related and 4–6 band differences are defined as possibly related. On the other hand, more than seven band differences, which means ≥3 genetic differences, is referred as the two isolates are different or unrelated (Tenover et al. 1995). But, the recent studies (Barett et al. 2006) illustrate that this criterion is not appropriate for all foodborne pathogens because of the possibility of horizontal gene transfer. The location of source and the background history of isolates should be considered for determination of discrimination among them.

PFGE is named as "gold standard" based on different criteria: noteworthy discriminatory power, interlaboratory reproducibility, ability to reach standardized protocols, and being based on whole genome. On the other hand, the method is known as labor-intensive and time-consuming. Also, PFGE cannot provide phylogenetic relationship of the organisms. PFGE is used to differentiate genotypes of organisms depending on the band patterns (Foley et al. 2006). In addition, epidemiological evidence is also required to track the source of outbreaks. Methods with more discriminatory power might be needed in outbreaks due to clonal strains.

19.4.3 Multiple-Locus Variable Number Tandem Repeat Analysis (MLVA)

The major advances in the sequencing of bacterial genomes have led the usage of differences in repeated DNA regions in characterization of bacterial species. In epidemiological studies, the variable number of repeated DNA regions can be used for detection, because the size of the repeated sequences can range from few bases to more than 100 base pairs and their number is extremely variable among strains and also among the same species (van Belkum et al. 2007; Lindstedt 2005). For high discrimination, the use of multiple regions of repetitive units is classified as multiple-locus variable number of tandem repeats (VNTR) analysis. PulseNet network uses MLVA as a second major genotyping method and produced standardized protocols for STEC and *Salmonella* serotype Typhimurium for the two platforms: Applied Biosystems Genetic Analyzer 3130xl, 3730xl (3500, 3500xl), and Beckman Coulter CEQ™8000/8800 (GeXP). Protocols for *Listeria monocytogenes* and *Salmonella* serotype Enteritidis are under validation, and according to their program, protocols for non-O157 STEC, *Shigella sonnei*, and *Salmonella* serotype Newport will be done soon after (http://www.pulsenetinternational.org/SiteCollectionDocuments/mlva/MLVA_overview.pdf).

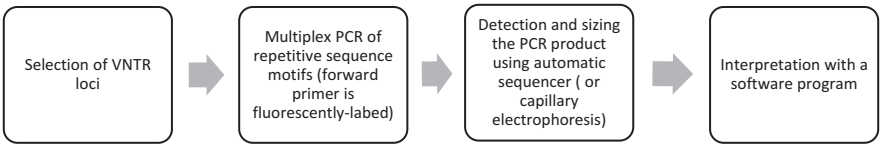

Fig. 19.2 The schematic progress of multiple-locus VNTR analysis

In MLVA, firstly the repeat arrays are screened for the bacterial strain in the whole genome sequence (Fig. 19.2). For screening, software programs are generally used to scan the genome for tandem repeats (Ramisse et al. 2004). After genome identification, PCR primers are designed for an easy amplification of multiple repeated arrays. Then PCR products are analyzed to distinguish the size and the number of repeats and this information is used to differentiate the strains. The amplification is often carried out with fluorescently labeled primers (for example; Table 19.15) which allow product separation and sizing with an automatic sequencer and thus increases the throughput of MLVA. Usage of different fluorescent dyes with primers makes the simultaneous detection possible for multiple loci in one sequencer run (Keim et al. 2000).

The three extensive advantages of using MLVA over PFGE are lack of subjectivity, data portability, and interlaboratory data exchange (Sperry et al. 2008). In addition, protocols are specific to the certain serotypes, not genuses. And, the use of fluorescently labeled primers and automated sequencer increases the accuracy of the result since internal size standards can be included with each set of amplicons which are separated on the sequencer (Lindstedt 2005).

19.5 Legislations on Microbial Loads of MPR and Packaged Produce

As discussed before, two outbreaks (1990 and 1993), involving at least 300 infections in four states attributed to *Salmonella* species, were related with consumption of fresh tomatoes in the USA. When tomatoes from those outbreaks were traced back to a single packing facility, it was outlined that water bath was the likely source of contamination. Since water can be a carrier of many microorganisms including pathogenic strains of *Escherichia coli*, *Salmonella* spp., *Vibrio cholerae*, *Shigella* spp., *Cryptosporidium parvum*, *Giardia lamblia*, *Cyclospora cayetanensis*, *Toxoplasma gondii*, and the *Norwalk* and hepatitis A viruses, the guidelines for fresh fruits are determined mainly according to these hazards. Microbiological quality of minimally processed and refrigerated produces is set by the results obtained from these analytical methods. While *Salmonella* spp. is not allowed, *E. coli* count up to 100 cfu/gram is the limit of safety for 25 g sample of food (Table 19.16).

Table 19.15 MLVA PCR primers

Microorganism	Locus	Forward primer (5′-3′)	Reverse primer (3′-5′)	Fragment size (bp) in LT2/ K1891/EDL933	Repeat length (bp)	Reference
Salmonella serotype Typhimurium	ST2	CAACGCCTGTTCAGCAAC	ATCAACAGCGGG TGGAT	359.9–361.1	39	PulseNet
	ST3	GTTCTTCTGCAACGCAGGCA	GATGGCATGACGCTGCAACG	177.8–179.0	12	PulseNet
	ST5	TTTTCGCTCAACAAACTT	ACAGCACCAGAAGCAAT	221.1–221.7	6	PulseNet
	ST6	AGCAGTGGCTGGCGGGAAACC	GCAGCCGGACAGGGGATAAGCC	267.3–267.7	6	PulseNet
	ST7	CGATTGACGATATCTATGACTT	GTTTTTCACGTTTGCCTTTC	153.6–153.7	9	PulseNet
	ST8	GCAGGTGTGGCTATTGGCGTTGAAA	GATGGTGACGCCGTTGCTGAAGG	554.9–555.7	33	PulseNet
	STTR3	CCCCCTAAGCCCGATAATGG	TGACGCCGTTGCTGAAGGTAATAA	490	27/33	Lindstedt (2005)
	STTR5	ATGGCGAGGCGAGCAGCAGT	GGTCAGGCCGAATAGCAGGAT	259	6	Lindstedt (2005)
	STTR6	TCGGGCATGCGTTGAAA	CTGGTGGGGAGAATGACTGG	342	6	Lindstedt (2005)
	STTR9	AGAGGCGCTGCGATTGACGATA	CATTTTCCACAGCGGCAGTTTTTC	180	9	Lindstedt (2005)
	STTR10	CGGGCGCGGCTGGAGTATTTG	GAAGGGGCCGGGCAGAGACAGC	371	6	PulseNet, Lindstedt (2005)

(continued)

Table 19.15 (continued)

Microorganism	Locus	Forward primer (5′-3′)	Reverse primer (3′-5′)	Fragment size (bp) in LT2/ K1891/EDL933	Repeat length (bp)	Reference
Salmonella serotype Enteritidis	SE1	TGTGGGACTGCTTCAACCTTTGGGC	CCAGCCATCCATACCAAGACCAACAC TCTATGA	194.6–195.4	7	PulseNet
	SE2	GTGCTTCCTCAGGTTGCTTTTAGCCTTGTTCG	GGGGAATGGACGGAGGCGATAGACG	327.4–327.7	7	PulseNet
	SE3	CGGGATAAGTGCCACATAACACAGTC GCTAAGC	CGCCAGTGTTAAAGGAATGAATGAAC CTGCTGATG	211.8–212.4	12	PulseNet
	SE5	GGCTGGCGGGAAACCACCATC	GCCGAACAGCAGGATCTGTCCATTAG TCACTG	202.1–202.5	6	PulseNet
	SE6	CTGGTCGCAGGTGTGGC	GGTGACGCCGTTGCTGAAGGTAATAA CAGAGTC	478.5–479.1	33	PulseNet
	SE8	GGTAGCTTGCCGCATAGCAGCAGAAGT	GGCGGCAAGCGAGCGAATCC	434.0–434.8	86	PulseNet
	SE9	CCACCTCTTTACGGATACTGTCCACCAGC	GGCGTTACTGGCGGCGTTCG	184.3–184.8	9	PulseNet
E. coli O:157 (STEC)	VNTR 3	GGCGGTAAGGACAACGGGGTGTTTGAATTG	GAACAACCTAAAACCCGCCTCGCCA TCG	374.9–375.2	6	PulseNet
	VNTR 9	GCGCTGGTTTAGCCATCGCCTTCTTCC	GTGTCAGGTGAGCTACAGCCCGCTTA CGCTC	531.1–531.4	6	PulseNet
	VNTR 17	GCAGTTGCTCGGTTTTAACATTGCAGTGATGA	GGAAATGGTTTACATGAGTTTGACGA TGGCGATC	157.7–158.3	6	PulseNet
	VNTR 19	GCAGTGATCATTATTAGCACCGCTTTCTGGA TGTTC	GGGGCAGGGAATAAGGCCACCTGTT AAGC	309	6	PulseNet
	VNTR 25	GCCGGAGGAGGGTGATGAGCGGTTATATTTA GTG	GCGCTGAAAAGACATTCTCTGTTTGG TTTACACGAC	135.5	6	PulseNet
	VNTR 34	GACAAGGTTCTGGCGTGTTACCAACGG	GTTACAACTCACCTGCGAATTTTTTAA GTCCC	278.8–279.0	18	PulseNet
	VNTR 36	GGCGTCCTTCATCGGCCTGTCCGTTAAAC	GCCGCTGAAAGCCCACACCATGC	159.2–159.5	7	PulseNet
	VNTR 37	GCCGCCCCTTACATTACGCGGACATTC	GCAGGAGAACAACAAAACAGACAGTAA TCAGAGCAGC	189.4–189.9	6	PulseNet

Table 19.16 Guideline levels for determining the microbiological quality (CFU/g) of ready-to-eat foods[a]

Test	Satisfactory	Marginal	Unsatisfactory	Potentially hazardous
Standard plate count				
Level 1	$<10^4$	$<10^5$	Greater than or equal to 10^5	
Level 2	$<10^6$	$<10^7$	Greater than or equal to 10^7	
Level 3	N/A	N/A	N/A	
Indicators	.	.	.	
Enterobacteriaceae[b]	$<10^2$	10^2–10^4	Greater than or equal to 10^4	
Escherichia coli	<3	3–100	Greater than or equal to 100	[c]
Pathogens				
Clostridium perfringens	$<10^2$	10^2–10^3	10^3–10^4	Greater than or equal to 10^4
Campylobacter spp.	Not detected in 25 g	–	–	Detected
Salmonella spp.	Not detected in 25 g	–	–	Detected
Listeria monocytogenes	Not detected in 25 g	Detected but $<10^2$ [d]	–	Greater than or equal to 10^2 [e]

[a]http://www.foodstandards.gov.au/scienceandeducation/publications/guidelinesformicrobi1306.cfm

[b]*Enterobacteriaceae* testing is not applicable to fresh fruits and vegetables or foods containing these

[c]Pathogenic strains of *E. coli* should be absent

[d]Foods with a long shelf life stored under refrigeration should have no *L. monocytogenes* detected in 25 g

[e]The detection of *L. monocytogenes* in ready-to-eat foods prepared specifically for "at-risk" population groups (the elderly, immunocompromised, and infants) should also be considered as potentially hazardous

N/A – SPC testing not applicable. This applies to foods such as fresh fruits and vegetables (including salad vegetables), fermented foods, and foods incorporating these (such as sandwiches and filled rolls)

19.6 Future Trends

Recent outbreaks have showed that there should be more surveillance studies that are conducted on MPR and packaged produce.

Public health surveillance is defined as continuous, systematic collection, analysis, and interpretation of health-related data needed for the planning, implementation, and evaluation of public health practice according to the World Health Organization (WHO). Thus, correct and continuous data is crucial for understanding the cause of diseases, in correct time and place. At that point, surveillance systems gain importance to collect useful and rapid data. On the other hand, the surveillance systems have not been well-developed in worldwide, and this

leads to misunderstandings in the detection of the agent of a certain foodborne disease. Available resources, knowledgeable staff, organization, and infrastructure for finding and reporting cases do not maintain the requirements of that system. And, it is evident that there is a need of development of rapid identification (detection) methods, and also the usage of standardized, rapid, and comprehensive methods should be broadened to detect the microorganisms related to MPR and packaged produce-associated foodborne diseases in a quick and accurate manner.

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Chapter 20
Comparison of HACCP and ISO 22000 in the Ready-to-Eat Fruit and Vegetable Industry in Conjunction with Application of Failure Mode and Effect Analysis (FMEA) and Ishikawa Diagrams

T. Varzakas and E. Manolopoulou

20.1 Introduction

Fresh-cut produce is defined as any fresh fruit or vegetable or any combination thereof that has been physically altered from its original form, but remains in a fresh state. Regardless of commodity, it has been trimmed, peeled, washed and cut into 100% usable product that is subsequently bagged or packaged to offer consumers high nutrition, convenience and value while still maintaining freshness (IFPA, http://www.fresh-cuts.org).

Other terms to refer to fresh-cut products are minimally processed, lightly processed, partially processed, fresh processed, pre-prepared, ready to use, ready to eat, prêt à consommer (France), panklare groente (Netherlands), frisk *snittet* grønt (Denmark) and färdigskuren sallad (Sweden).

Fresh-cut vegetable salads were developed in the USA about 50 years ago (Treadway and Olson 1953). In Europe, fresh-cut vegetables appeared in Germany in 1970 and in France in 1980 (Mazolier and Scandella 1999).

According to the International Fresh-Cut Produce Association (IFPA), fresh-cut products have been available to consumers since the 1930s in retail supermarkets. The fresh-cut fruit and vegetable market is the fastest growing in the European Union over the past 20 years (Garrett 2002). This segment has weathered the global

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economic downturn rather well since the introduction in the 1980s, and the market for it has been growing by double digits in the European Union (Rabobank 2010).

The United Kingdom (UK) is the largest fresh-cut market with roughly one third of total European consumption. Its fresh-cut retail market recorded 5% growth in 2008 and 4% in 2009 but showed signs of recovery in the first months of 2010 (Rabobank 2010). In UK this category of products is widely acceptable, and the market of fresh-cut fruits and vegetables has a penetration of 85% to the population (Miege and Obertiκ 2007). In fresh-cut fruits and vegetables in the UK, fresh fruit salads hold the highest market share (35%), followed by mix salads (18%), exotic fruit salads (11%), melon and water melon (10%), pineapple (7%) and mango and apple (Miege et Obertiκ 2007).

The Netherlands, Switzerland, Italy and Spain have already established a market and show strong growth in the fresh-cut produce sector; Germany has yet to embrace the sector. Italy is currently emerging as one of Europe's leading fresh-cut markets, where sales of fresh-cut fruits and vegetables have been booming in recent years and are now second only to the UK in terms of value (Jennylynd et al. 2010). In France according to SFPAE (Syndicat des fabricants de fruits et légumes prêts à l'emploi), from 1998 to 2002, the volume of the products increased by 76% or presented an annual increase of 11% (Moras and Merendet 2007).

In France salads comprise of 90% of fresh-cut vegetables (Moras and Merendet 2007). In many cases salad mixes contain pieces of cheese or spices or even pasta.

Successful marketing of lightly processed fresh produce is linked to several factors such as the maintenance of high level of sensory quality for an adequate duration subsequent to harvest (Gertmenian 1992), harvest time to achieve peak quality, quality control for postharvest treatments and effective packaging.

Minimal processing of raw fruits and vegetables has two purposes: First, it is important to keep the produce fresh but convenient without losing its nutritional quality, and, second, the product should have a shelf life sufficient to make distribution feasible within a region of consumption (Huxsoll and Bolin 1989).

Fresh-cut products are an important and ever expanding food category for the produce industry, food processors and retailers and food operators, and the commercial success depends on the maintenance of its fresh state, slowing the loss of nutritional quality, ensuring the microbiological safety and shelf-life enough to make its consumption feasible for consumers.

This class of foods was developed at the beginning to meet the needs of hotels, restaurants and catering centres.

For the food service industry and restaurants, fresh-cut produce presents a series of advantages, including a reduction in the need of manpower for food preparation, the reduced need of special systems to handle waste, and the possibility to deliver in a short time, specific forms of fresh-cut products (Watada et al. 1990).

Organisations such as the World Health Organization (WHO), Food and Agriculture Organization (FAO), US Department of Agriculture (USDA) and European Food Safety Authority (EFSA) recommended an increase of fruit and vegetable consumption to decrease the risk of cardiovascular diseases and cancer (Allende et al. 2006).

Most fruit and vegetables are low-cost food that contain low levels of fat and high levels of a number of nutritionally important compounds, such as vitamins, minerals, fibre, bioactive compounds, etc., many of which cannot be synthesised by the human body.

In recent years, consumers have become more health conscious in their food choices and want to replace unhealthy snack foods with healthier products, but have had less time to prepare healthful meals. As a result the market demand for fresh-cut fruits and legumes has rapidly increased.

Minimally processed vegetables and fruits are purchased by a wide range of consumers in terms of socio-demographic characteristics, though families with young children and higher education take the lead (Ragaerta et al. 2004).

The ready-to-use products include sliced onion, celery strip, cut carrots, sliced cucumbers as snack vegetables, shredded carrots, shredded cabbage for coleslaw, halved and cored pepper, diced onions, sliced red cabbage, shredded lettuce, sliced tomatoes, endive, chicory as salad vegetables, sliced tomatoes, shredded lettuce as sandwich vegetables, strip-cut peppers, sliced mushrooms, sliced tomatoes as pizza vegetables, diced peaches, diced pears, seedless grapes, diced pineapple, pitted cherry halves, sliced bananas, segmented oranges, segmented mandarins as fruit salad, sliced melons, pitted plums and peeled oranges as snack fruits (Yildiz 1994).

Many forms of fresh-cut produce (Table 20.1) and many food groups for ready-to-use products (Table 20.2) are prepared, packaged and marketed.

20.2 The HACCP and ISO22000 Approach

Hazard Analysis Critical Point Control (HACCP) is a structured approach to the identification, assessment of risk (likelihood of occurrence and severity) and control of hazards associated with a food production process or practice.

Design and implementation of a HACCP system involve the well-known seven basic principles or steps including hazard analysis, identification of the critical control points (CCPs) in food preparation, establishment of critical limits for preventive measures associated with each CCP, establishment of procedures to monitor CCPs, establishment of corrective action to be taken when monitoring shows that a critical limit has been exceeded, establishment of an effective record-keeping system that documents the HACCP and establishment of procedures to verify that the HACCP system is working (Stevenson and Combas 1999).

ISO 22000 specifies the requirements of a Food Safety Management System, encompassing all the range of food organisations involved in the food chain from farmers to catering businesses. ISO 22000 creates a uniform and homogeneous platform of requirements, acceptable to all authorities worldwide. The adoption of ISO 22000 was carried out in the year 2005. These food organisations involve the following categories:

Table 20.1 Commonly marketed fresh-cut fruits and vegetables

Commodity	Fresh-cut forms
Fruits	
Apples (*Malus sylvestris* L)	Peeled and cored, cored and sliced
Oranges (*Citrus sinensis* L)	Peeled sections and slices, wedges
Kiwi (*Actinidia chinensis* L.)	Peeled and sliced
Pineapple (*Ananas comosus* L. Merr.)	Peeled and unpeeled slices, chunks, cored, peeled cylinders
Strawberries (*Fragaria X ananassa)*	Washed, destemmed, sliced
Melons (*Cucumis melo* L.)	Balls, chunks, slices with and without rind
Grapefruit (*Citrus paradisi* Macfad)	Peeled and sections
Grapes (*Vitis vinifera* L.)	Washed and destemmed
Mixed wide variety of precut fruit	
Vegetables	
Beets (*Beta vulgaris* L	Peeled, shredded, cut, sliced, julienne
Broccoli (*Brassica oleracea* L)	Individual florets with or without stalk
Carrot (*Daucus carota* L)	Peeled slices and sticks, diced, shredded
Celery (*Apium graveolens* L)	Trimmed sticks, diced, sliced stalks
Cucumber (*Cucumis sativus* L)	Sliced, crinkle cut, wedges
Lettuce (*Lactuca sativa* L)	Cleaned and cored, cored and chopped
Onions (*Allium cepa* L)	Sliced, rings, diced
Spinach (*Spinacia oleracea* L	Cleaned, trimmed individual leaves
Tomatoes (*Lycopersicon lycopersicum* L)	Sliced, diced, wedges
Mixed vegetable salads	

(Schlimme 1995)

Table 20.2 Food group for ready-to-use products

Group	Fruits or vegetables forms
Snack vegetables	Sliced onion, celery strip, cut carrots, sliced cucumbers
Stew vegetables	Cut green beans, sliced onions, diced tomatoes, diced peppers, diced broccoli, diced mushrooms, sliced eggplants,
Salad vegetables	Shredded carrots, shredded cabbage, halved and cored pepper, sliced red cabbage, shredded lettuce, sliced tomatoes, chicory, parsley
Sandwich vegetables	Sliced tomatoes, shredded lettuce
Pizza topping vegetables	Strip-cut peppers, sliced mushrooms, sliced tomatoes
Fruits cocktail	Diced peaches, diced pears, whole seedless grapes, diced pineapples, pitted cherries, diced apples
Fruit salads	Diced peaches, diced pears, seedless grapes, pitted cherry halves, sliced bananas, segmented oranges, segmented mandarins
Fruit cakes	Sliced strawberries, peeled bananas, pitted cherries, sliced apricots
Snack fruits	Sliced melons, pitted plums, peeled oranges

Yildiz 1994

- The directly involved organisations with the food chain, i.e. primary production, food additives manufacturers and raw and auxiliary raw materials for the food industries, food manufacturers, food services, food distributors, pest control companies as well as distribution and warehousing companies
- The indirectly involved such as suppliers of raw materials, equipment, cleaning and disinfectant solutions, packaging materials and other materials that come directly or indirectly into contact with food (Arvanitoyannis and Tzouros 2006)

20.3 Processing of Minimally Processed Fruits and Vegetables: Determination of CCPs

The flow diagram of minimally processed fruits and vegetables is described in Fig. 20.1 where the CCPs of RTU vegetables are shown.

Methods for the preparation of minimally processed fruits and vegetables include: defoliating, peeling, removing parts, cutting, shredding, grating, washing, disinfecting, rinsing, draining, drying and packaging.

Flows of processing operations for leafy vegetables (a) and fruit (b) are shown below:

(a) Raw vegetables selection: first step, cleaning, prewashing and disinfection and peeling-cutting-shredding; second step, washing and sanitation, dipping treatment, drying, dosage and packaging, refrigeration (cold storage), cold transport and distribution
(b) Harvest and handling, reception and storage, cleaning and prewashing, washing and sanitation, cutting (seed removing, peeling, slicing), antioxidant treatment, drying, dosage packaging, cold storage, cold transport and distribution selling

After processing they are kept throughout distribution and retail sale under chilling and modified atmosphere packaging (MAP) which is generated by the use of polymeric films commonly provided of a selective permeability to gas diffusion.

To obtain fresh-cut fruits and vegetables, the basic premise is minimal processing to retain fresh-like texture, colour, flavour and safe quality.

The quality of fresh-cut fruits and vegetables depends directly on the quality of the raw material, the storage conditions before processing and other factors related to processing and distribution (Gorny et al. 1998, Wiley 1994).

Producing fresh-cut fruits and vegetables involves substantial mechanical injury due to peeling, slicing, dicing, shredding or chopping (Portela and Cantwell 2001). Factors affecting the response of tissues to wounding are species and cultivar, stage of physiological maturity, temperature, O2 and CO2 concentrations, water vapour pressure, various inhibitors and the severity of wounding (Cantwell 1992; Brecht 1995).

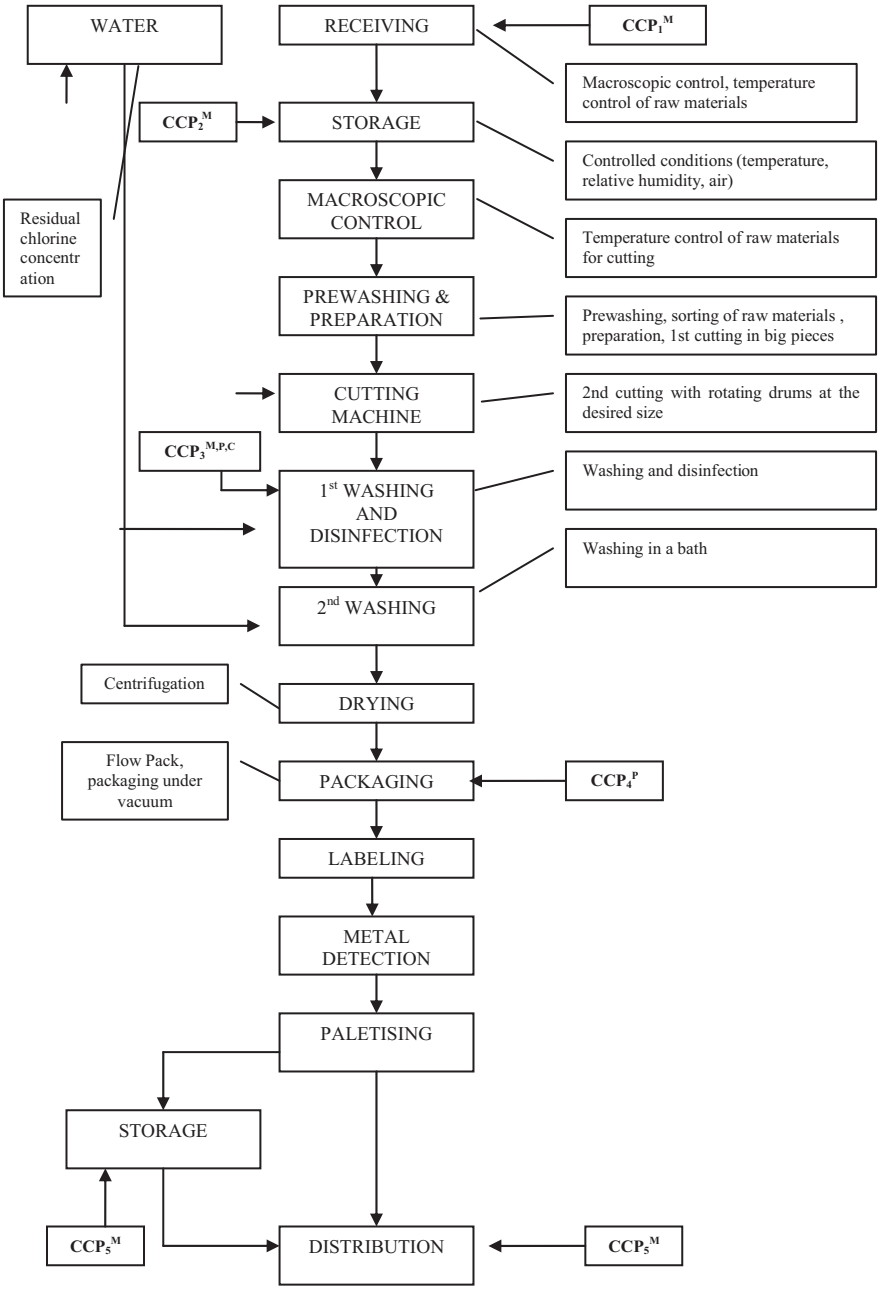

Fig. 20.1 Flow diagram for ready-to-eat fruits and vegetables (Adapted from Varzakas and Arvanitoyannis 2008)

20.3.1 Raw Materials Receiving CCP$_1$

The quality of the raw material is very important to keep the quality of the final product (Wiley 1994). The raw material must be suitable for fresh-cut processing and should be of high quality. They should have good appearance, texture, taste, odour and nutritive attributes, and they must be safe for the consumer. They should be free from mechanical injuries, decay, insects and other damages, and they must resist process operations and further handling and storage procedures.

Cultivar selection is of great importance in fresh-cut fruit processing, because cultivar characteristics, such as flesh texture, skin colour and browning potential, can vary a great deal. There are specific requirements for fresh-cut varieties that need to be met: varieties of lettuce, potato, apples, peaches with low browning potential, fruits with high sugar contents and firm texture and varieties that facilitate cleaning, trimming and cutting operations (Kader 2002a).

For each type of produce, there could be a few or many cultivars in the market, each with particular benefits and limitations for processing. Comparison between cultivars concluded that there exists considerable variation in respiration rates, colour, texture, bruising susceptibility, size, appearance, nutritional value, sensory and other characteristics of many fruits and vegetables (Crisosto et al. 1993; Gorny et al. 2000; Crisosto and Mitchell 2002; Maestrelli and Chourot 2002; Deepa et al. 2007).

Cultivar to be used for the preparation of fresh-cut fruits and vegetables should resist transportation and handling before processing, tolerate the processing operations with minor quality alterations and have a prolonged after-cutting shelf life. Ideal fruits and vegetables for fresh-cut processing are those with the best and homogeneous quality attributes: right stage of development or maturity; high field production and processing yields; availability year-round; free from physical, physiological or pathological disorders; easy to handle; highly resistant to handling and all processing operations and stabilising treatments; and little susceptibility to external conditions, with a prolonged shelf life after processing to maintain quality attributes all the way to the consumer and safe consumption. It should also meet consumer likes and preferences and market requirements (Montero-Calderón and Cerdas-Araya 2011). The stage of maturity of fruit and vegetables destined for fresh-cut processing is a critical factor influencing the eating quality and helps to determine the shelf life of the product. (Gorny et al. 2000; Soliva-Fortuny et al. 2002a). In the case of leafy vegetables, the growth stage at harvest can influence the shelf life of the baby leaves, harvested at an early growth stage due to market demand.

During this stage, macroscopic control is carried out to determine their suitability (presence of foreign matter, soil, stones, etc.). This is the first CCP which is the temperature of raw vegetables that should be lower than 5 °C. It is a microbiological hazard due to the presence of many pathogenic microorganisms such as *Salmonella*, *E. coli* and others.

20.3.2 Storage: CCP_2

Storage is carried out under controlled conditions. Product temperature (<5 °C) and relative humidity (95–100%) should be monitored and kept to the minimum. It is a microbiological danger as described in the previous processing step.

20.3.3 Macroscopic Control

At this stage macroscopic control of fruit and vegetables ready to be cut is carried out so that they do not present any perturbations on their surface, and their temperature is checked, a critical factor for their quality and shelf life.

20.3.4 Prewashing and Preparation

Sorting is carried out at this stage, where dirt and outer layers are removed from the surface of the vegetables. Following that, vegetables are cut into slices or small pieces, and their prewashing follows.

20.3.5 Cleaning: Prewashing

Cleaning and washing may be the only preservation treatments in most of the minimally processed fruits and vegetables. Cleaning refers to the removal of foreign materials. It is clear that if incoming vegetables or fruits are covered with soil, mud or sand, they should be carefully cleaned before processing (Wiley 1994; Ahvenainen and Hurme 1994). Washing treatments make the product ready to eat. A first washing step of whole fruits is generally conducted by rinsing in tap water to eliminate pesticide residues, plant debris and other possible contaminants. This step is followed by a dip in chlorinated water to reduce effectively the microbial loads on the fruit surface. The vegetables should be carefully cleaned before processing, because they are grown mostly in contact with soil (Nicola et al. 2009).

Washing raw materials before cutting is the most effective way of minimising the risk of the presence of pathogens and any residue left on the produce from harvest and handling conditions.

20.3.6 Cutting

Second, cutting is carried out with rotating drums at the desired size. Cutting knives should be adjusted and sharpened regularly and disinfected at every product change. Carbon steel used for scalpel blades is brittle and releases iron ions that may be involved

in brown discolouration. Trimming, limited to broad-leafed and curly chicory, might cause wounding of plant tissue resulting in enzyme leakage. The destruction of cell microstructures leads to biochemical spoilage such as texture breakdown, off-flavour and browning (Varoquaux and Wiley 1997). When trimming, salad leaves must be washed immediately after cutting. Any delay in prewashing will enhance browning.

20.3.7 Peeling-Cutting-Shredding

During the preparation stages, fresh-cut fruits and vegetables are subjected to mechanical injury due to peeling, slicing, shredding or chopping (Portela and Cantwell 2001). These operations are critical to delimit the shelf life of fresh-cut fruits and vegetables because the decompartmentalisation of cellular components and the bruising of tissue near the shear surfaces are provoked. Decompartmentalisation and bruising of tissue lead to oxidative reactions such as the enzymatic browning reaction with the consequence of product darkening. Mechanical injury increases respiration rate, ethylene production and accumulation of secondary metabolites (Varoquaux and Wiley 1997; Rosen and Kader 1989; Rivera-Lopez et al. 2005; Del Aguila et al. 2006). Cut tissues have lower barriers to gas diffusion, and they tolerate higher concentration of CO_2 and lower O_2 levels than intact commodities (Manolopoulou and Varzakas 2015; Toivonen and DeEll 2002).

All unwanted parts of the plant including most of the outer green leaves and core area are removed manually. Peeling should be as gentle as possible and may be done by hand, with steam or boiling water and with lye or alkalis (1–2% NaOH, KOH), by dry caustic peeling with infrared heat, by flame (onions), by mechanical means, by high-pressure steam and by freezing and with acids (Lopez 1987). At industrial scale mechanical, chemical and high-pressure peeling still prevail. Abrasive peeling performed with water vapour or high pressure causes damage that will allow the attack of microorganisms (Wiley 1994).

Some vegetables such as potatoes and carrots need peeling. Automated peelers with abrasive rollers are used. In the case of citrus fruits, enzymatic peeling should be applied in order to minimise wounding. For the preparation of apple slices, FMC has developed a system that automatically peels, cores and slices apples in a high-speed continuous operation (Anon 1998).

The cutting and shredding must be performed with knives or blades as sharp as possible and made from stainless steel (Allende et al. 2006). Slicing with blunt knives impairs quality retention due to the increased breaking of cells and release of tissue fluid (Portela and Cantwell 2001).

Many different solutions have been tested to avoid the acceleration of decay due to peeling, cutting or slicing. “Waterjet cutting” method which is successfully used for, e.g. meat, poultry and vegetables (McGlynn et al. 2003) can also be used in the fresh-cut industry. This is a “noncontact” cutting method (Allende et al. 2006) which slices fresh fruit and vegetables utilising a high-pressure fluid jet (3000 KPa) that minimises bruising in the cut pieces and tissue damage in the vicinity of the

cut surface. In this way the excessive tissue damage caused by compression and tearing the piece along the cut surfaces is reduced. Cell exudates were washed away by the large stream produced. The vegetables particularly adapted to this method are fresh root vegetables, leafy vegetables and fruit and vegetables with firm tissue. Factors which affect the efficiency of the method are orifice size, water pressure and stand-off distance and characteristics of the species and cultivar (Bansal and Walker 1999). Immersion therapy (AR-USDA 2005) is a new method for cutting the fruits while submerged in water. This method controls turgor pressure, due to the formation of a water barrier that prevents movement of fruit fluids while the product is being cut and flushing of potentially damaging enzymes away from plant tissues (Lamikanra and Bett-Garber 2005).

20.3.8 First Washing and Disinfection: CCP_3

RTU vegetables are washed effectively and disinfected with biocides. This process reduces the initial microbial load and minimises the pathogenic microorganisms. Water is used; however, its effectiveness is improved by the addition of biocides (chlorine, citric acid and ascorbic acid). It is a critical control point (chemical, physical and microbiological danger) since water should be free of foreign matter and has a temperature of 1–4 °C, and concentration of disinfectant solution in washing water should be at 100 ppm Cl_2. The minimum chlorine concentration should not drop below 50 ppm. Chlorine may be hypochlorite or chlorine gas. Agitation in the disinfecting tank is insured either by tangential air bubbling or waterjets or mechanically by rotating arms (Varoquaux and Mazollier 2002). The absence of foreign matter from cut vegetables should also be checked.

20.3.9 Second Washing

Following first washing, products go to the second washing machine of the line where they get washed with clean cold water containing less than 0.5 ppm active chlorine, before going to the drying machine. The cold water needs to be continuously renewed to avoid chlorine accumulation from the disinfecting section (Manolopoulou and Varzakas 2015).

Within the processing line, the washing operations are the most important steps affecting the quality and the shelf life of the fresh-cut products. The first wash is done immediately after harvesting in order to remove the non-desirable materials from the surface (soil, insects, pesticides, etc.) and cool the produce. Secondly, a washing step should be performed after peeling and/or cutting to remove microbes and tissue fluids. Washing the product immediately after cutting is one of the most important steps in fresh-cut processing (Gil and Selma 2006), which removes sugars and other nutrients at the cut surfaces that favour microbial growth and tissue discolouration.

Factors which affect the efficiency of washing to remove soil impurities and microbial contaminations are raw material spoilage, the duration of the washing treatment, the washing water temperature, the method of washing (dipping, rinsing, or dipping/blowing), the type and concentration of the sanitiser and the type of fresh-cut fruit or vegetable (Allende et al. 2006; Artés et al. 2007).

The quality of the water used for washing the produce is critical and affects the quality of minimal processing products. The source and quality of water must be considered. Factors affecting the efficacy of washing and which must be controlled are quantity of water used (5–10 L/Kg of product before peeling/cutting and 3 L/Kg after peeling/cutting), the temperature of water (below 5 °C to cool the product) and the concentration of active chlorine (100 mg/L) (Yildiz 1994; Huxsoll and Bolin 1989; Hurme et al. 1994; Ahvenainen et al. 1994; Laurilla and Ahvenianen 2002).

The temperature of water used is low for reducing respiration rate, transpiration and microbial activity. In that case, water is colder than the raw material (warm regions) and the internal gas contracts, thereby creating a partial vacuum that will draw in water through pores, channels or punctures, being sufficient to draw water into the fruit (Bartz 1999; Sapers 2003) and absorbing any chemical contaminants present in water (Hernandez-Brenes 2002). Maintaining the water temperature 5 °C above the internal temperature of the produce can prevent this "suction" effect. One precaution could be an initial air-cooling step before washing, to minimise the temperature gap between the produce and the water temperature (Nicola et al. 2009).

Chlorine may be hypochlorite (sodium or calcium hypochlorite) or chlorine gas. Chlorine gas is slightly more efficient because it decreases the pH of the solution, unlike hypochlorite that increases the pH resulting in bigger dissociation of hypochlorite and decreases in disinfecting efficiency. The sodium hypochlorite is sold as liquid whereas the calcium hypochlorite is sold in powder form. The concentration of organic matter (cellular juices, soil particles, microbes) in washing solution affects the quantity of available (free) chlorine. The difference between total chlorine and available chlorine depends on the amount of organic matter and inorganic compounds that react with the free chlorine (resulting in combined chlorine) during washing (Pirovani et al. 2004). The use of chlorine at a concentration no greater than 200 ppm has been widely reported as an effective sanitation treatment of both whole and fresh-cut fruits (Gorny et al. 2000; Dong et al. 2000; Bett et al. 2001; Soliva-Fortuny et al. 2002b). The effectiveness of NaClO on microbicidal activity is related to the concentration of the sanitiser, the pH and temperature of solution, the type of produce and the diversity of microorganism. According to Adams et al. (1989), washing time has no effect on microbial reduction; however, Kabir (1994) reported that the optimum contact time is 12–13 s if the chlorine concentration is 70 mg/L.

The choice of washing method of fruits is dependent on the purpose of washing and the delicacy of the tissue. Washing systems may be open and closed flume systems, wash tanks and so on. Washing in flowing or air bubbling waters is more preferable than merely dipping into water (Mazollier and Scandella 1997). The fresh-cut products can be single, double or triple washed, or various wash and spray combinations may be implemented (Luo 2007).

Washing and disinfection have economic and environmental implications: The large operational costs of water use have resulted in the industry-wide common practice of reusing or recirculating wash water (Luo 2007). A technique able to disinfect efficiently both the process water and the product would allow a high ratio of recycling and therefore reduction of the waste water rates with less impact on the environment (Olmez and Kretzschmar 2009).

The last step of washing is rinsing with tap water containing less than 0.5 ppm active chlorine. This operation is necessary when the solution used for washing contains chlorine at a concentration higher than 1 ppm (Varoqaux and Mazollier 2002).

Consumers demand more natural food with reduced or eliminated chemical additives. The use of antimicrobial agents from plants and plant products can represent a natural alternative to food additives. These substances, generally recognised as safe (GRAS), are able to inhibit microorganisms and determine flavour and quality because of the presence of some volatile compounds (Utama et al. 2002). Some natural constituents, such as hexanal, hexanol, 2-(E)-hexenal and 3-(Z)-hexenol, responsible for the aroma of some vegetables and fruits, provide protective action against microbial proliferation in wounded areas (Gardini et al. 2002).

Eliminating chlorine from the disinfection process is required due to the concerns about its efficacy on the produce as well as environmental and health risks associated with the formation of carcinogenic halogenated disinfection by-products (Ölmez and Kretzschmar 2009). In some European countries including Germany, the Netherlands, Switzerland and Belgium, the use of chlorine in ready-to-use products is actually prohibited, due to its potential toxicity (Ölmez and Kretzschmar 2009). Other physical methods to remove microorganisms from plant surfaces include: ultrasounds, high pressure (HP), high-density electric field pulses (HELP), ultraviolet radiation (UV), radio frequencies (RF) and ionising radiation (Ölmez and Kretzschmar 2009).

At the moment, the disinfection agents that are used and tested for water sanitation are chlorine, chlorine dioxide, acidified sodium chlorite, ozone, organic acids, hydrogen peroxide, alcohols, phosphoric acids, UVC light radiation, ultrasound electrolysed water (EW) and others, including combinations of some of them for synergistic effects (Weyer et al. 1993; Zhuang and Beuchat 1996; Beuchat et al. 1998; Sapers and Simmons 1998; Day 2001; Seymour et al. 2002; Sapers 2003; Allende et al. 2006; Artés et al. 2007).

Chlorine dioxide (ClO_2) is a water-soluble yellowish green gas with an odour similar to that of chlorine, which is unaffected by changes in pH (6–10) (Dychdala 1991). It does not react with organic matter to form chloroform, but its reactivity was reduced by the presence of organic matter (Beuchat et al. 2004). Chlorine dioxide compared to chlorine produces fewer potentially carcinogenic chlorinated by-products such as trihalomethanes in the presence of organic material (Richardson et al. 1998). Chlorine dioxide cannot be stored commercially or shipped as a gas because it is highly explosive under pressure; therefore, it must be generated at the point of use. It is used in flume waters in fruit and vegetable operations by the US Food and Drug Administration (USFDA). The treatment of produce with chlorine dioxide must be followed by a potable water rinse. The oxidising power of chlorine

dioxide is 2.5 times that of chlorine (Dychdala 1991). The efficacy of chlorine dioxide varies based on concentration, exposure time and temperature and to some extent based on pH, type of fruit or vegetable and microbial species (Zoffoli et al. 2005). Chlorine dioxide is usually used, as other chlorine-based chemicals, at levels of 50–200 ppm free chlorine and with typical contact times of less than 5 min (Gómez-López et al. 2009).

Acidified sodium chlorite is a highly effective antimicrobial agent produced by lowering the pH (2.5–3.2) of a solution of sodium chlorite ($NaClO_2$) with any GRAS acid (Warf 2001). The chemical combination of acidified sodium chlorite, citric acid and sodium chlorite produces active chlorine dioxide (ClO_2) that is more soluble than sodium hypochlorite (NaClO) in water and has about 2.5 times greater oxidising capacity than hypochlorous acid (HClO) (Inatsu et al. 2005). Acidified sodium chlorite is approved by the FDA and Environment Protection Authority (EPA) for application in fresh-cut vegetables as a spray or dip in the range of 500–1200 ppm (Krasaekoopt and Bhandari 2011).

Ozone (O_3) is one of several new sanitising agents for produce, introduced in recent years (Graham 1997; Xu 1999; Kim and Yousef 2000). It demonstrates a broad-spectrum efficacy against virus, bacteria, yeasts and bacterial spores, although it is less active against fungi than bacteria (Block 1991). Ozonated water and gaseous ozone (O_3) are applied to fresh-cut vegetables for sanitation purposes, reducing microbial populations, preventing browning and extending the shelf life of some of these products (Beltran et al. 2005a, b; Selma et al. 2006, 2007, 2008a, b). It is water-soluble gas with broad and rapid biocidal activity and has strong oxidising capacity and high reactivity and penetrability. Ozone is reported to have 1.5 times the oxidising potential of chlorine and 3.000 times the potential of hypochlorous acid. It is, however, unstable under ambient temperature conditions. Ozone rapidly undergoes spontaneous decomposition under conditions of high pH (pH>8) leading to the production of oxygen which is a nontoxic product. Most materials are compatible with ozone at moderate concentrations of 1–3 ppm (Pascual et al. 2007), in the processing industry. However, it is important to keep the applied ozone levels as low as possible because ozone is corrosive to stainless steel, especially when the concentration is above 1 ppm. Ozone reduces the amount of waste water and lowers the refrigeration costs of chilled water because of the less frequent flume water changing, and it can be combined with chlorine, whose use can be reduced by 25% leaving less residual odour on the product (Strickland et al. 2007).

Hydrogen peroxide (H_2O_2) is a strong oxidising agent effective against a wide range of bacteria but less active against fungi (Block 1991). It is listed as GRAS for use in specific food products as a bleaching agent, an oxidising and reducing agent and an antimicrobial agent (Anonymous 2007b). However, it is not specifically approved by the FDA for use on minimally processed fruits and vegetables unless it is used in combination with acetic acid to form peroxyacetic acid (Anonymous 2007a). Diluted hydrogen peroxide solutions are effective in extending the shelf life of fresh-cut vegetables and melons; however, they are phytotoxic to some commodities, causing browning in lettuce and bleaching of anthocyanins in mechanically damaged berries (Sapers and Simmons 1998).

Organic acids naturally present in fruits and vegetables (acetic, citric, malic, tartaric, succinic, benzoic) are capable to reduce the growth of some microorganisms and prevent the growth of others. Citric and acetic acids are often used in the fresh-cut industry to adjust the pH of water in chlorine applications (Herdt and Feng 2009). The action of the acids is attributed to reduction of pH, depression of internal pH of microbial cells or disruption of substrate transport by alteration of cell membrane permeability (Beuchat 2000). Organic acids are very stable in the presence of organic material and generally present no objectionable odour. A major disadvantage of using organic acids is the relatively high cost, because it takes large amounts of acid to adjust the system pH. Additionally, due to the low pH required, they can compromise the organoleptic properties of some produce (Herdt and Feng 2009).

Glycol ethers (butyl) and alcohol type solvents are occasionally used in cleaning products because of their penetration and soil softening properties. They are particularly effective when used in combination with surfactants (IFPA 2001).

20.3.10 Drying-Draining

Excess water is removed from the surface of cut vegetables by centrifugation (special centrifuges are used to achieve optimal draining) or product placement on the air. Excessive free water in packs results in rapid bacterial spoilage mainly at the leaf-film interface. Draining should result in approximately 1% residual moisture compared to the unprocessed salad. Drier should be checked regularly so that no humidity is evident on the surface of cut vegetables. Two methods are used: a spin dryer and an air tunnel. The centrifugation starts with a soft loading of the fragile leaves followed by a smooth acceleration and a careful discharge of the drained products. Air tunnel drying is used in several European and US processing plants and consists of cascade vibrating tables to transport the product and a battery of air-drying units (Manolopoulou and Varzakas 2015; Varoquaux and Mazollier 2002).

The high water content on the surface of minimally processed products serves as a good environment for the propagation of microorganisms; moreover, some enzymatic reactions can be accelerated leading to a rapid degradation of the fruit flavour and/or appearance. Water is gently removed from vegetables through shakers/spinners or centrifuges. Water remaining on the product is a critical issue. The duration and speed of centrifugation need to be adjusted for each product. The combination of the centrifugation speed and operation duration affects the water removal. Drying tunnels with continuous airflows are also used, especially for more delicate vegetables (Donati 2003), in this case. The optimal temperature of the air to avoid possible raw material fading, the thermal difference between airflow and raw material and the residual water on the raw material are factors that could reduce shelf life quality.

20.3.11 Packaging: CCP_4

RTU vegetables are packaged in semipermeable membranes where due to respiration they modify the atmosphere. Modified atmosphere packaging is used and the gases used are oxygen, carbon dioxide or/and nitrogen (O_2, CO_2, N_2). Flow pack machines, vacuum or even vertical line machines could be used. The following should be checked: temperature of semi-processed product so that it does not exceed 5 °C, concentration (%) of O_2, CO_2 inside packaging of final product, resistance in vapour permeability and the ability of thermal welding of packaging. It is a critical control point since there are physical, chemical and microbiological dangers. Foreign matter and chemical residues from packaging materials are physical and chemical dangers, respectively, as well as other microorganisms (microbiological dangers) that might be transferred from elsewhere. Varoquaux and Wiley (1997) reported that injury stress at processing and physiological disorders induced by detrimental packaging conditions along with temperature abuse were the main causes of the premature decay of fresh-cut produce. Moreover, the packing room must be clean and refrigerated at 1–2 °C and must be separated from the washing section. Packing is carried out around a vertical tube at the top of which is the associative weighing machine. Salad bits are poured into the infeed funnel or a vibrating cone designed to distribute the vegetable chunks evenly into feed buckets which release them into the weighing buckets.

Most minimally processed vegetables in France are packed in bags of polypropylene, 25–40 μm thick. In England and Wales, a wider range of vegetables including spinach, broccoli and cauliflower is processed. These highly respiring commodities are packed with microperforated films more permeable to gases than polypropylene. Moreover, oriented polyethylene is preferred to polyethylene due to its brightness, crispness and suitability for machine packing. Pectinolytic bacteria such as *Pseudomonas fluorescens* and *Pseudomonas viridiflava* are responsible for soft rot on stored vegetables (Lund and Snowdon 2000).

Salads that are highly sensitive to oxidation, including butterhead and iceberg lettuces, are flushed with nitrogen so that residual oxygen within the packs ranges from 1 to 3%. The active modified atmosphere will only accelerate the establishment of a protective environment. Some processors introduce CO_2 into the pack to obtain 5–10% of CO_2 after sealing. Shredded carrots and cabbages deteriorate rapidly when stored with excessively high CO_2 and low O_2 partial pressures (Carlin et al. 1990).

The final operation in producing minimally processed fruit and vegetables is packaging. A food package must protect and contain the product from the place and time of manufacture to the point of consumption (IFT 1991). Minimally processed fruits and vegetables have been packaged in polymeric film in an effort to maintain product quality while extending shelf life. After lowering produce temperature, modified atmosphere packaging (MAP) is considered to be the second most effective method for extending the shelf life of fresh and minimally processed produce. Good manufacturing and handling practices along with the appropriate use of MAP

are relatively effective at inhibiting the mechanisms of spoilage and extending shelf life. Shelf life extension also results in the commercial benefits of less wastage in manufacturing and retail display, long distribution channels, improved product image and the ability to sell convenient, added-value, fresh prepared produce items to the consumer with reasonable remaining chilled storage life.

According to O'Beirne (1990), lowering produce temperature reduces respiration rate by a factor of 2–3 (Q10 = 2–3), and use of an appropriate MA can additionally reduce the respiration approximately fourfold. It should be noted that MAP is not a replacement for proper temperature control (Hotchkiss 1988), and temperature modifications are the most important factor in controlling respiration (Shewfell 1986).

A modified atmosphere can be created passively for respiration of fruits and vegetables by using permeable packaging materials (passive MAP) or actively by flushing gas mixture (active MAP) (Kader 2002a). Active MAP is preferred for fresh-cut vegetables, whose shelf life is relatively short.

Various plastic films have been used as packages for fresh fruit and vegetables and minimally processed products such as low-density polyethylene (LDPE), high-density polyethylene (HDPE), thin gauge polypropylene (PP), polystyrene (PS), various grades of polyvinyl chloride (PVC) and rubber hypochlorite (Pliofilm) (Ben Yehoshua et al. 1983; Kader et al. 1989; O'Beirne 1990) blends of PE and ethylene vinyl acetate (EVA) and co-extruded polymers or laminates of several plastics (Kader 2002b). Beneficial modified atmospheres within fresh-cut fruit packages are attained by correctly choosing packaging materials that will provide the appropriate levels of oxygen and carbon dioxide into packets (IFPA 2003; Sandhya 2010). The choice of packaging film depends on the permeability of the film to O_2 and CO_2 that must be adapted to the O_2 consumption rate and CO_2 production rate of the produce. If the permeability for O_2 and CO_2 is perfectly matched to the respiration rate of the produce, an ideal equilibrium modified atmosphere (EMA) can be established inside the package. For most produce this atmosphere is between 1–5% O_2 and 3–10% CO_2, balanced by N_2 (Kader et al. 1989; Day 1993). In many commercial cases, produce is sealed in a packaging film of insufficient permeability resulting in development of undesirable anaerobic conditions (Betts 1996). Exposure to O_2 or CO_2 levels outside the limits of tolerance (e.g. <2% O_2 and>20% CO_2) may lead to anaerobic respiration with the production of undesirable metabolites and other physiological disorders (Soliva-Fortuny et al. 2002a, b; Oms-Oliu et al. 2008; Zagory and Kader 1988). Too low O_2 atmospheres may trigger anaerobic metabolism in fresh-cut fruits and result in an increase in fermentation (Solomos 1997).The factors which influence the creation of EMA (equilibrium modified atmosphere) are product respiration rate, respiring surface area, storage temperature, packaging film permeability, RH, filling weight, pack volume, film surface area, degree and kind of illumination of the display in the retail store as well as the initial microbial load (Artés and Martínez 1996; Jacxsens et al. 1999; Day 2000; Kader 2002a, b). A change in the environmental temperature creates a specific problem in EMA establishment, because the respiration rate is influenced more by temperature changes than film permeability to O_2 and CO_2 (Jacxsens et al. 2002).

Another problem of MAP is the accumulation of water inside the package. This condition enhances condensation on the film and on the package contents. The presence of water may promote the development of spoilage and also block O_2 diffusion into the tissues and through the film causing fermentation (Cameron et al. 1995).

The most difficult task during packaging of fresh-cut produce is to reach the optimal EMA conditions inside the package. Only a few packaging materials present on the market are sufficiently permeable to compensate for produce respiration. Most films are not optimal in O_2 and CO_2 conditions when the produce has a high respiration rate. Recently developed, microperforated films, which have very high gas transmission rates, are now commercially used for maintaining aerobic EMAs (e.g. 5–15% O_2 and 5–15% CO_2) in highly respiring produce items such as broccoli and cauliflower florets, baton carrots, bean sprouts, mushrooms and spinach. However, microperforated films are relatively expensive, permit moisture and odour losses and may allow for the ingress of microorganisms into sealed packs during wet handling situations (Day 1998).

During the retail of modified atmosphere packages, temperature fluctuations are unavoidable. The application of elevated O_2 atmospheres (±70 kPa O_2) has been proposed as an alternative to low O_2 atmospheres to inhibit the growth of naturally occurring spoilage microorganisms, prevent undesired anoxic respirative processes and maintain the fresh-like quality of fresh-cut produce (Amanatidou et al. 1999; Jacxsens et al. 2001; Van der Steen et al. 2002). High levels of oxygen are particularly effective in inhibiting enzymatic discolouration. It has been hypothesised that high oxygen levels may cause substrate inhibition of PPO or, alternatively, that high levels of colourless quinones subsequently formed may cause feedback inhibition of PPO (Mc Evily et al. 1992).

To provide the shelf-life extension and to improve the quality, safety and integrity of the packaged food, innovative, active and intelligent packaging concepts are being developed. Active packaging includes various gas absorbents and emitters and is another interesting packaging method for minimally processed fruit and vegetables (Day 1994). Active packaging systems can be classified into active scavenging systems (absorbers) and active releasing systems (emitters). Examples of active packaging systems are oxygen scavengers, ethylene absorbers, moisture regulators, taint removal systems, ethanol and carbon dioxide emitters and antimicrobial-releasing systems (de Kruijf et al. 2002). Some of the absorbers being used are calcium hydroxide, activated charcoal, zeolite, cellulose, magnesium oxide and sometimes C_2H_4 absorbers like potassium permanganate, to prevent the accelerating respiring effect of ethylene (Dainelli et al. 2008). Intelligent packaging systems monitor the condition of packaged foods to give information about the quality of the packaged food during transport and storage (de Kruijf et al. 2002).

Another possible “packaging” method for extending the shelf life of minimally processed fruits and vegetables is the use of edible coatings. Edible coatings can be applied as either a complement or an alternative to modified atmosphere packaging (MAP) and may help to reduce food deterioration, enhance its quality and improve its safety because of their natural biocide activity or by incorporating antimicrobial compounds (Petersen et al. 1999). Edible coatings are thin layers of material that can be eaten by the consumer as part of the whole food product and can be composed of

one or more ingredients of protein, lipid or polysaccharide nature (Nisperos-Carriedo and Baldwin 1994). Theoretically, edible coatings have the potential to reduce moisture loss, restrict the entrance of oxygen, lower respiration, retard ethylene production, seal in flavour volatiles and carry additives that retard discolouration and microbial growth (Baldwin et al. 1995). The ability of edible coatings to preserve the quality of fresh-cut products may vary depending on the composition and thickness of the coating, type of product, variety and maturity, food surface coverage and storage conditions (Gonzalez-Aguilar et al. 2010).

20.3.12 Labelling-Metal Detection-Palletising

During labelling, production date, expiry date, lot number, name and type of the product as well as nutritional information for the consumers are labelled on the package. Metal detection then follows for the presence of metal pieces (>0.8 mm) and if found they are immediately removed. Casing and palletising then follows and cartons are ready to be loaded.

20.3.13 Storage and Product Distribution: CCP_5

Following palletising, the product is stored in refrigerators (4–6°C), until loading in tracks operating under the same temperature. It is a microbiological danger since temperature should be recorded at both storage and distribution steps.

Low temperatures are necessary to reduce respiration rate, retard microbial growth and retard deterioration such as browning and softening in fresh-cut products. Effective cold chain management is crucial for the preservation of the quality and food safety of fresh-cut produce. The temperature of fresh-cut produce should be maintained below 5 °C to reduce the proliferation of spoilage microorganisms and human pathogens. However, this is difficult to achieve and fresh-cut produce is regularly subjected to abuse temperatures of 8–12 °C (Scandella et al. 1990). For chilling-sensitive commodities in general, low temperatures retard the rate of deterioration of fresh-cut products more than they induce chilling injury.

20.4 Determination of CCPs in RTU Fruits and Vegetables: Decision Tree

The CCP Decision Tree is a tool used to determine the right CCPs for each processing stage (NACMCF 1999; Wedding 1999). However, according to Wedding (1999), it is not the perfect tool and cannot replace common sense and processing knowledge and can sometimes lead to false conclusions. This can be seen in Table 20.3. In Table 20.4 critical control points, critical limits, process control, corrective actions and verification in fresh-cut fruits and vegetables have been described.

Table 20.3 Decision table for critical control point determination during processing of ready-to-eat fruits and vegetables (Adapted from Varzakas and Arvanitoyannis 2008)

	Q1	Q2	Q3	Q4	
Processing stage	Do preventative control measures exist? (Yes/No)	Is the step specifically designed to eliminate or reduce the likely occurrence of hazard to an acceptable level? (Yes/No)	Could contamination with identified hazards(s) or could this increase to unacceptable levels? (Yes/No)	Will a subsequent step eliminate identified hazard(s) or reduce likely occurrence to acceptable levels? *20 (Yes/No)*	Is this step a critical control point? (Yes/No)
1. Receiving of raw materials, fruits and vegetables	μ, C, P: YES	**μ, C, P**: NO	**μ**: YES Pathogenic microorganisms **P**: YES Foreign matter	**μ, P:** NO	CCP_1^{μ}
2. Storage	**μ**: YES Sanitation program Regular maintenance FIFO system	**μ**: NO Storage under controlled conditions	**μ**: NO GMPs Right equipment	**μ**: NO	CCP_2^{μ}
3. Macroscopic control	**μ:** YES Personnel hygiene rules	**μ:** NO	**μ:** NO Personnel training	**μ: -**	–
4. Prewashing and preparation	**μ, P:** YES Cleaning program Right equipment Personnel hygiene rules	**μ, P:** NO	**μ:** NO Personnel training **P**: YES Metal pieces from cutting machine	**μ:** YES **Φ**: YES **Metal detector**	–
5. Slicing	**μ, P:** YES Cleaning program Right equipment Personnel hygiene rules	**μ, P:** NO	**μ:** NO Personnel training **P**: YES Metal pieces from cutting machine	**μ:** YES **P**: YES **-Metal detector**	–
6. First washing, disinfection	**μ, C, P**: YES Cleaning program for equipment Addition of disinfectant solution Control of the mechanism for removal of foreign matter	**μ:** YES Disinfection to reduce microbial load **P, C:** NO	**μ:** - **C:** NO **P:** YES Presence of foreign matter in the product	**μ, C:** NO **P:** YES Second washing follows	CCP_3^{M}

(continued)

Table 20.3 (continued)

Processing stage	Q1 Do preventative control measures exist? (Yes/No)	Q2 Is the step specifically designed to eliminate or reduce the likely occurrence of hazard to an acceptable level? (Yes/No)	Q3 Could contamination with identified hazards(s) or could this increase to unacceptable levels? (Yes/No)	Q4 Will a subsequent step eliminate identified hazard(s) or reduce likely occurrence to acceptable levels? *20 (Yes/No)*	Is this step a critical control point? (Yes/No)
7. Second washing	**μ, C, P**: YES Cleaning program for equipment Control of the mechanism for removal of foreign matter	**P:** YES Foreign matter removal by washing **μ, C:** NO	**μ, C:** NO Operation control of washing machine Water specifications Regular maintenance of equipment **P:** -	**μ, X, Φ**: **YES**	–
8. Drying/ draining	**μ:** YES Regular maintenance of equipment Macroscopic control	**μ:** NO	**μ:** YES Presence of water film on the surface of the food due to inadequate drying	**μ:** NO	
9. Packaging	**μ, C, P**: YES Cleaning program for equipment Quality certificates of packaging materials Personnel hygiene rules	**μ, C, P**: NO	**μ, C, P**: NO Equipment maintenance Macroscopic control	**μ, C, P**: **NO**	$CCP_4^{M, P,C}$
10. Metal detection	**P**: YES Control of the right operation of equipment	**P**: YES Removal of metallic objects	**P**: -	**P**: -	–
11. Labelling	**μ:** YES Macroscopic control	**μ:** NO	**M:** NO -Personnel training	**M: -**	–
12. Palletising	NO	**–**	**–**	**–**	–
13. Storage of final product	**μ:** YES Cleaning program	**μ:** NO	**μ:** YES Growth of microorganisms in case of increase in temperature	**μ:** NO	CCP_5^{μ}
14. Distribution	**μ, P:** YES Right transportation vehicles Cleaning program	**μ, P:** NO	**μ, P:** NO Personnel training Temperature control of transportation vehicle	**μ, P:** NO	CCP_5^{μ}

Table 20.4 Critical control points, critical limits, PrPs, process control, corrective actions and verification in freshly cut fruits and vegetables (Adapted from Varzakas and Arvanitoyannis 2008)

CCP	Critical limits	Process control				Corrective action	Verification
		Way	Frequency	Control sheet	Responsible		
CCP_1^{μ}	Receiving temperature of raw materials lower than 5 °C	Temperature control and recording in the specially designed system	Daily for each receiving of raw materials	Receiving sheet	Warehouse supervisor	Immediate cooling (warehouse supervisor) Return of nonconforming products to the supplier (general manager)	Daily verification Lab analysis of raw materials Vehicles' recording papers
PrP	Concentration of residual chlorine in water: 0.2–0.8 ppm (limit for taking measures, > 1 ppm and < 0.2 ppm)	Control of concentration of residual chlorine in water	Daily – twice	Residual chlorine control sheet	Quality supervisor	Control of dosage pump of chlorine in water Maintenance of waterline (maintenance supervisor)	Daily verification Lab water analysis Microbiological analysis
CCP_2^{μ}	Storage of raw materials at a temperature lower than 5 °C and relative humidity of 95–100%	Control and recording of temperature and relative humidity	Daily every 15 min	Recording meter	Warehouse supervisor Maintenance supervisor	Adjustment of faults (maintenance supervisor) Control Hold suspicious lots (warehouse supervisor)	Monthly verification Lab analysis of raw materials
PrP	Absence of projections on the cut surface of vegetables (smooth cutting)	Macroscopic control of cutting quality	At every product change during cutting	Control sheet of line washing	Production supervisor	Sharpening knives maintenance (maintenance supervisor) Check suspicious lots (production supervisor)	Monthly verification Macroscopic control Microbiological analysis

(continued)

Table 20.4 (continued)

CCP	Critical limits	Process control				Corrective action	Verification
		Way	Frequency	Control sheet	Responsible		
$CCP_3^{\mu,C,P}$	Concentration of disinfectant in washing water at 100 ppm (90–110) Absence of foreign matter in washing water (clean water) Absence of foreign matter from cut fruits and vegetables Water temperature 1–5 °C	Control of the concentration of disinfectant Control of right operation of mechanism of removal of foreign matter (drums, traps) Macroscopic control of purity of washing water Temperature control of washing water	At every product change and during washing	Control sheet of line washers	Production supervisor	Prewashing Check and disinfection of suspicious lots (production supervisor) Adjustment of faults in the temperature of washing water (maintenance supervisor)	Monthly verification Lab analysis of water and final products
PrP	Absence of humidity on the surface of the cut vegetables (removed humidity during drying: 25–30% of the initial weight of the washed product)	Macroscopic control for humidity presence (water film on the surface) Weighing of the removed humidity of the washed product	At every product change and during drying	Control sheet of packaging line	Production supervisor	Adjustment of fault in the drying machine (maintenance supervisor) Control of suspicious lots Repeat drying (production supervisor)	Monthly verification Lab analysis of water and final products

CCP	Critical limits	Process control				Corrective action	Verification
		Way	Frequency	Control sheet	Responsible		
PrP	Temperature of semi-processed product <5 °C Concentration (%) of O_2, inside packaging of final product Operation of vacuum machine	Measurement of the temperature of semi-processed product Control of oxygen percentage Control of operation of vacuum machine	Every 15 min and at every product change	Control sheet of packaging line	Production supervisor	Final products control increase in storage time Control repackaging suspicious lots (production supervisor) Adjustment of faults (maintenance supervisor)	Monthly verification Microbiological analysis of final products Microscopic control
$CCP_4^{P,C,M}$	Right labelling of final products (expiry date, product description, lot number)	Control of labelling (expiry date, product description, lot number)	At every product change	Packaging control sheet	Production supervisor	Control repackaging of lots with wrong labelling (production supervisor)	Monthly verification Macroscopic control
PrP	Absence of metal objects >0.8 mm	Operation control with metal spherical objects with diameter ≤ 1 mm	Three times per shift	Packaging control sheet	Production supervisor	Adjustment of metal detector breakdown Check suspicious products using another detector	Monthly verification
CCP_5^{μ}	Storage and distribution of final products at a temperature lower than 5 °C	Control and recording of temperature	Daily every 15 min At each loading and every half an hour during distribution	Temperature meter Control sheet of final products (loading) Temperature recording meter of transportation vehicle	Storage supervisor -Quality control supervisor. -Vehicle driver	Adjustment of fault (maintenance supervisor) Hold suspicious lots (warehouse supervisor)	Monthly verification Lab analysis of final products

Table 20.5 ISO22000 Analysis Worksheet for determination of the prerequisite programs PrPs (Adapted from Varzakas and Arvanitoyannis 2008)

Processing Step	Are the technical infrastructure and the preventative maintenance program adequate?	Is it feasible to evaluate them?	Do they contribute in the control of recognisable food safety hazards?	Does the effectiveness of the remaining control measures depend on them?	Is it a prerequisite program?
Receiving vegetables	YES	YES	NO	NO	NO
Storage	YES	YES	NO	NO	NO
Prewashing and preparation	YES	YES	NO	YES	YES
Slicing	YES	YES	NO	NO	NO
First washing	YES	YES	NO	NO	NO
Second washing	YES	YES	NO	YES	YES
Drying by centrifugation	YES	YES	NO	YES	YES
Packaging	YES	YES	NO	NO	NO
Metal detection	YES	YES	NO	YES	YES
Labelling	YES	YES	NO	YES	YES
Palletising	YES	YES	NO	YES	YES
Storage of final product	YES	YES	NO	NO	NO
Distribution	YES	YES	NO	NO	NO

As described earlier the requirements for ISO22000 assume the determination of the prerequisite programs (Table 20.5). The questions frequently asked for each processing step involve questions regarding the adequacy of the technical infrastructure and preventative maintenance, the feasibility for their evaluation and their contribution in the control of recognisable food safety hazards, whether the effectiveness of the remaining control measures depends on them. These questions lead to the answer of a program being prerequisite or not.

20.5 FMEA

In FMEA analysis, risk of contamination and its presence at hazardous fraction in the final product are expressed with the Risk Priority Number (RPN) which is defined as follows:

where S is severity of contamination risk, O occurrence of contaminated ingredient and D detection probability of contaminated ingredient.

$$RPN = S \times O \times D \quad (1)$$

where S is severity, O occurrence and D detection.

Corrective action is carried out when RPN is greater than 130.

The classification of hazardous elements occurs according to the RPN assessment as can be seen in Table 20.6, and corrective actions are proposed per identified hazard. Following calculation of the new RPN (the RPN after undertaking corrective actions), a new classification of hazardous elements is shown in Table 20.6.

Analysis is made based both on best expert opinion and product history (epidemiological studies) for similar items of all the ways that each component or subsystem might fail to comply with its intended function (Kumamoto and Henley 1996; James 1998; McDermott et al. 1996; Scipioni et al. 2002).

20.6 Physiology of Fresh-Cut Fruits and Vegetables

Minimally processed tissues have been exposed to substantial injury due to trimming, peeling, washing and cutting operations. Minimally processed fruits and vegetables differ from conventionally processed tissues because they have not blanched and remain in a fresh state and are still living respiring tissues.

Mechanical wounding may induce a diverse array of metabolic pathways and bring about changes in metabolism. These changes include localised increased respiration at the site of injury, stress ethylene production, accumulation of secondary metabolites and enzymatic reactions (Rolle and Chism 1987).

20.6.1 Respiration

Respiration involves the oxidative breakdown of complex substrate molecules normally present in plant cells, such as starch sugars and organic acids, to simpler molecules in the course of which energy, CO_2 and water are given.

Shelf life potential of the fruits and vegetables is associated with the rate of respiration. Higher respiration rates result in more rapid consumption of sugars and other components that influence flavour and nutritive value. Tissues having large energy reserves generally have longer postharvest lives.

The physiology of minimally processed products is essentially that of wounded tissues. The intensity of the wound response is affected by a great number of factors such as species and variety, stage of physiological maturity, temperature, oxygen and carbon dioxide concentration, water vapour pressure, various inhibitors, severity of wounding (Brecht 1995) and initial levels of reduced ascorbic acid and phenolic compounds (Reyes et al. 2007).

Wounding or injury associated with processing and handling of fresh-cut vegetables results in cellular decompartmentalisation or delocalisation of enzymes and substrates and can cause physiological changes which influence respiration rate, ethylene production, discolouration, deterioration of texture, off-flavours and water

Table 20.6 FMEA table of hazardous processing methods for ready-to-eat fruits and vegetables processing (Adapted from Varzakas and Arvanitoyannis 2009)

Defective products								Estimated corrective action result			
Production step CCP	Hazards	Causes	S	O	D	RPN	Corrective actions	S	O	D	RPN
Receiving	Pathogens, parasites, heavy metals, toxins	Unsuitable raw materials	9	5	5	225[a]	Supplier must be reliable (archives confirming handling conditions)	9	2	2	36
Cooling	Heavy metals, pesticide residues	Wrong cooling temperature	9	4	4	144	Check fridges	–	–	–	–
Washing and disinfection	Foreign matter	Wrong handling from the personnel	5	3	4	60	Foreign matter control	–	–	–	–
Packaging	Pathogens, pesticide and aflatoxin residues	Unsuitable packaging	9	4	5	180[a]	Check oxygen concentration, vacuum	9	2	2	36
Storage and distribution	Contamination from wrong temperature or time remaining at the fridge	Improper control of storage conditions and distribution	9	5	5	225[a]	Check storage rooms and distribution vehicles	9	2	2	36

CCP critical control point, *RPN* risk priority number

[a]When RPN is above 130, corrective actions are required

loss, which leads to rapid quality degradation (Varoquaux 1987; Toivonen and DeEll 2002; Soliva-Fortuny and Martin-Belloso 2003).

Fresh-cut products generally have higher respiration rates than the corresponding intact products, indicating a more active metabolism and a faster deterioration rate.

20.6.2 Ethylene Production

Ethylene is a gaseous plant hormone and biologically active at low concentrations (part per billion to parts per million) (Abeles et al. 1992; Saltveit 1999). It is synthesised naturally during plant development, fruit ripening, leaf senescence and responses to stress and pathogens. Synthesis is by reactions involving a cycle in which the amino acid methionine participates in the formation of ACC, ACC synthase being a key enzyme in this pathway (Yang and Hoffman 1984). It is necessary for growth, inhibits longitudinal growth and promotes seed germination, degreening, adventitious root formation, abscission, ripening and senescence (Reid 1985; Baldwin 2004).

Ethylene is considered autocatalytic when ethylene stimulates its own synthesis and autoinhibitory when it turns off continued synthesis (Mattoo and White 1991). It is used commercially to ripen fruits like bananas, mangoes, melons and tomatoes (100–1000 μL/L) and to degreen citrus (Watada 1986).

Wounding increases activity of ACC synthase and results in the accumulation of ACC that is subsequently oxidised to ethylene. Wound ethylene is believed to be involved in the increased respiration of tissues (Yang and Pratt 1978) which differs among fruit products (Toivonen and DeEll 2002). The stimulation of ethylene production by stress typically occurs after a time lag of 10–30 min and subsides later after reaching a peak within several hours (Yang and Pratt 1978).The ethylene production rate is dependent on the type and physiology of the tissue and should be proportional to the injured surface area. Kiwifruit (Agar et al. 1999), tomato (Artés et al. 1999), strawberry (Rosen and Kader 1989) and cantaloupe (Abeles et al. 1992) produce large amount of ethylene as a consequence of cutting. Very small pieces of cantaloupe had a large increase in ethylene production, but large pieces have not presented difference from the intact fruits (Cantwell and Suslow 2002).

Yang and Pratt (1978) concluded that the biosynthetic pathway for ethylene under wound is similar to the pathway for ethylene synthesis in ripening fruit. ACC synthase shows increased activity in tissues following wounding (Hyodo et al. 1985) and may be synthesised de novo in wounded tissue (Hyodo et al. 1983). Increased ACC synthase activity following wounding causes accumulation of ACC (Yu and Yang 1979; Hyodo et al. 1985).

Wound ethylene affects membrane integrity and phospholipids' retailoring and accelerates senescence (Mazliak 1983; Watada et al. 1990).

Wounding effects differ between climacteric and non-climacteric fruit (Rosen and Kader 1989). The ethylene production by the wound usually is greater in preclimacteric and climacteric than postclimacteric tissues (Abeles et al. 1992) and advance ripening only of climacteric fruit.

Inhibition or reduction of ethylene synthesis or action can help to extend the shelf life of fresh-cut products but may differ depending on the type of product. Numerous processing techniques (Artés and Allende 2005; Palumbo et al.2007) used to counteract the reactions of the cut produce to ethylene production and other wound responses are precooling (Vigneault et al. 2008), storage temperature (Wang and Adams 1982), MAP packaging (Bai et al. 2002), coatings, ethanol (Plotto et al. 2006), ethylene absorbents and 1-MCP (Ergun et al. 2007; Saftner et al. 2007).

20.7 Cause and Effect Diagrams

Ishikawa diagram or fishbone diagram was invented by Dr. Kaoru Ishikawa, a Japanese quality control statistician (Ishikawa 1986). The fishbone diagram is an analysis tool that provides a systematic way of looking at effects and the causes that create or contribute to those effects. Because of the function of the fishbone diagram, it may be referred to as a cause and effect diagram. Due to its resemblance to fish skeleton, it is often referred to as the fishbone diagram.

In Fig. 20.2 an Ishikawa diagram is applied to detect the causes due to the bioaccumulation of heavy metals during receiving of fresh fruits and vegetables. The main problems are the extensive use of pesticides and the inadequate control during cultivation as well as low funding, which is a man's problem with secondary problems being the inadequate training. Regarding machines the main problem is

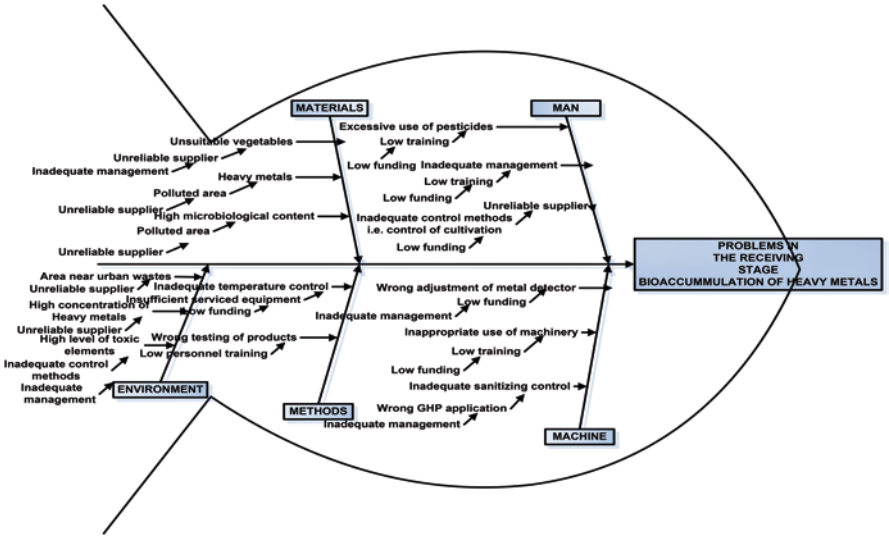

Fig. 20.2 Application of the cause and effect diagram (Ishikawa diagram) to ready-to-eat fruits and vegetables (problems in the receiving stage of fruits and vegetables) (Adapted from Varzakas and Arvanitoyannis 2009)

attributed to the wrong adjustment of the machines in the analysis of pesticides, and the secondary problem is the inadequate training which is due to lack of money. Regarding the environment, the high concentration of heavy metals as well as the area near urban wastes could cause serious problems. Regarding materials unsuitable vegetables, i.e. vegetables polluted with heavy metals or vegetables loaded with microorganisms or unreliable suppliers, could cause problems that cannot be eliminated during processing.

20.8 Conclusions

In this work comparison of ISO22000 analysis with HACCP is carried out over ready-to-eat fruits and vegetables processing and packaging.

Critical control points, critical limits, process control, corrective actions and verification in cut fruits and vegetables have been identified and implemented in the HACCP plan. The decision table for critical control point determination during processing of ready-to-eat fruits and vegetables is shown and compared to the ISO22000 Analysis Worksheet for determination of the prerequisite programs. The prerequisite programs are the main difference between the two systems.

Moreover, comparison of ISO22000 analysis with HACCP is carried out over ready-to-eat salads processing and packaging. However, the main emphasis was put on the quantification of risk assessment by determining the RPN per identified processing hazard. Receiving, storage and distribution, packaging and cooling were the processes identified as the ones with the highest RPN (225, 225, 180 and 144, respectively), and corrective actions were undertaken. Following application of corrective actions, a second calculation of RPN values was carried out leading to considerably lower values (below the upper acceptable limit of 130). It is noteworthy that the application of Ishikawa (cause and effect or tree diagram) has led to converging results, thus corroborating the validity of conclusions derived from risk assessment and FMEA. Therefore, the incorporation of FMEA analysis within the ISO22000 system of a RTU salads processing industry is considered imperative.

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Chapter 21
Bioactive Compounds of Fruits and Vegetables

Hasan Yalcin and Tugba Dursun Çapar

21.1 Introduction

Food provides essential nutrients needed for life as well as other bioactive compounds for health promotion and disease prevention. High amount of plant-based food consumption, at least 400 g of fruits and vegetables, is recommended in dietary guidelines (Agudo 2005). The consumption of fruits and vegetables prevents several diseases, such as hypertension, obesity, coronary heart disease and stroke risk, overall cancer, eye diseases, asthma and osteoporosis (Boeing et al. 2012). Fruits and vegetables are good sources of a wide range of micronutrients and non-nutrient bioactive compounds, including vitamins, phytochemicals such as (poly)phenolic compounds and carotenoids, minerals as potassium, calcium, and magnesium, and dietary fibre. It is estimated that more than 5000 individual phytochemicals have been identified in fruits, vegetables and grains, but a large percentage of them still remains unknown (Liu 2013).

Bioactive compounds, which have pharmacological and toxicological effects in man and animals, are the secondary metabolites of the plants (Bernhoft 2010). Secondary metabolites produced by plants generate important functions in the living cell such as protection against free radicals and prevention of disease as a result of oxidative stress and act as antioxidants (Bernhoft 2010; Kaur and Kapoor 2001).

The bioactive compound biosynthesis is stimulated by light; therefore, they generally accumulate in the skin and leaves of the fruit and vegetables (Bernhoft 2010). The levels of the bioactive compounds in foods differ widely in composition from various fruits, vegetables and genetic factor and environmental conditions such as light, maturity and postharvest treatments. Fruit maturity stage at harvest may be also a main determinant of the levels of bioactive compounds (Deepa et al. 2007; Vallejo et al. 2003).

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Vitamins, minerals and bioactive compounds in fruits and vegetables are in the liquid form which makes them more bioavailable in human diet (Yildiz et al. 2010).

In order to receive the greatest health benefits from fruits and vegetables, it is suggested that a wide variety of plant-based diet should be consumed (Liu 2013). It is widely accepted that diet rich in fruits and vegetables are associated with reduced risk for oxidative stress-related diseases, such as cancer and cardiovascular diseases (Blomhoff 2010). Antioxidants prevent or slow down the oxidative stress that is induced by free radicals. Fruits and vegetables contain a wide range of antioxidant compounds (phytochemicals) that may help the protection of cellular system from oxidative damage and risk of chronic diseases (Liu 2003).

21.2 Phenolics

The phenolic compounds have attracted much interest due to their potential as antioxidants, their great abundance in our diet and their possible role in the prevention of various diseases associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative diseases (Scalbert et al. 2005).

The main dietary sources of phenols are fruits and beverages (fruit juice, tea, coffee) and, to a lesser extent of vegetables, legumes and cereals. Fruits like apple, grape, pear, cherry and various berries contain up to 200–300 mg phenolic compounds per 100 g fresh weight; a glass of red wine or a cup of tea or coffee contains about 100 mg phenolics. The total dietary intake of phenolics is about 1 g/day (Scalbert et al. 2005).

Foods contain complex mixture of phenols. The amount of phenols in the plants is mainly affected by the environmental factors such as soil type, sun exposure, temperature, and rainfall. Phenol concentration varies also genetic and technologic factors, some of which may be controlled by improving growing methods and limiting losses during the course of industrial processing and domestic cooking. The degree of ripeness also affects the amount and ratio of phenols, where phenolic acid concentration decreases during ripening, whereas anthocyanin concentration increases (Manach et al. 2004, 2005; Williamson and Manach 2005).

The amount of phenolic compound classification is proposed by Vasco et al. (2008) using low (<100 mg GAE/100 g), medium (100–500 mg GAE/100 g) and high (>500 mg GAE/100 g). The blackberry and the strawberry can be categorized as having a high concentration of phenols. These fruits are an excellent source of phenols. Red raspberries, blueberries and cherries can be categorized as having average phenol content, and they may also be considered as a good source of phenols.

Several phenols with at least one aromatic (phenolic) ring bearing hydroxyl groups have been characterized (Blomhoff 2010). They are synthesized in large structural varieties belonging to several molecular families such as benzoic acid derivatives, flavonoids, proanthocyanidins, lignans, lignins and tannins. The two main types of phenols are *flavonoids* and *phenolic acids*. Flavonoids are distributed themselves among several classes: flavones, flavonols, flavanones, isoflavones and anthocyanins (Scalbert et al. 2005; Manach et al. 2004).

21.3 Flavonoids

Most of the successful medical treatments in ancient times seem to be due to the employment of flavonoids, which its use has persevered until now. Flavonoids have diverse biological properties such as antioxidative, antimicrobial, anticarcinogenic and cardioprotective. Dietary sources of flavonoids were shown in Table 21.1 according to phenolic classes.

Flavonol Quercetin is a common flavonol-type flavonoid that is present in several foods such as onion, tea and apple and is consumed almost daily. In Western diet, daily intake of quercetin is estimated to be in the range of 0–30 mg (D'Andrea 2015).

Flavanol Catechins (flavan-3-ols) which are diversified as epicatechin, gallocatechin, epicatechin gallate, epigallocatechin, gallocatechin gallate and epigallocatechin gallate are classified under flavanol group. Epicatechin gallate plays the most important role in taste sensation in fruits. Catechins are the major building blocks of tannins and they decrease as grapes mature (Gadkari and Balaraman 2015).

Flavanone Hesperetin, a flavanone present in citrus fruits, has low bioavailability, poor water solubility and short biological life. These restrict its use in medicinal

Table 21.1 Classification and dietary source of phenols (Bernhoft 2010; Dimitrios 2006; Shahidi et al. 1996; Clifford 1999; Macheix and Fleuriet 1990; Dao and Friedman 1992)

Phenolic class	Compound	Dietary sources
Flavonoids		
Flavonols	Quercetin Kaempferol	Cherry tomato, onion, broccoli, tea, red wine, berries
Flavones	Apigenin Luteolin	Cereals, parsley, celery
Flavanones	Hesperetin Naringenin	Citrus fruits
Flavanols	Catechins	Chocolate, beans, apricot, tea, red wine, cherry, apple
Anthocyanidins	Cyanidin	Aubergine, berries, red wine, red cabbage
Isoflavones	Daidzein Genistein Glycitein	Soy products, peas
Phenolic acids		
Hydroxybenzoic acid	Gallic acid Protocatechuic acid p-Hydroxybenzoic acid	Berries, onion, blackberry, raspberry, strawberry
Hydroxycinnamic acid	Caffeic acid Coumaric acid Ferulic acid Sinapic acid	Kiwi, cherry, plum, apple, pear, potato, garlic

Table 21.2 Concentration of anthocyanins in fruits and vegetables

Source	Anthocyanin (mg/100 g)	Reference
Red apple	1.3–12	Wu et al. (2006)
Blackberry	82.5–325.9	Wang and Lin(2000)
Raspberry	20–687	Wang and Lin (2000), Wu et al. (2006)
Strawberry	19–55	Da Silva et al. (2007)
Nectarine	2.4	Koponen et al. (2007)
Grapefruit	5.9	Koponen et al. (2007)
Peach	4.2	Koponen et al. (2007)
Plum	2–25	Wu et al. (2006)
Lettuce	2.2–5.2	Koponen et al. (2007), Wu et al. (2006)
Aubergine	8–85	Koponen et al. (2007), Wu et al. (2006)

application. Instead of using in medicinal application, it should be consumed freshly by citrus fruits (Shen et al. 2016). Naringenin is another natural flavanone, richly found in citrus and grape fruits, which improves brain insulin signaling and cognitive functions (Ghofrani et al. 2015).

Anthocyanins Anthocyanins are water-soluble plant pigments, responsible for the range of colours such as red, blue and purple which were observed in fruits (Rufino et al. 2010). They occur primarily as glycosides or acylglycosides of their respective aglycone anthocyanidins and aglycone forms are rarely found in fresh plant materials. The most common anthocyanidins in higher plants are delphinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin (De Pascual-Teresa and Sanchez-Ballesta 2008). They are abundant in strawberries, raspberries, cherries, berries, red grapes and cranberries (Wu et al. 2006). The anthocyanin concentrations of some foods are listed in Table 21.2. In recent years, there has been an increased attention on anthocyanidins due to their possible health effects (Galvano et al. 2004; Marko et al. 2004). Epidemiologic studies have suggested that the consumption of anthocyanins lowers the risk of cardiovascular disease, diabetes, arthritis and cancer (Prior and Wu 2006). Anthocyanins are unstable, easily oxidized and sensitive to temperature, UV radiation, ascorbic acid and metal ions (Santos-Buelga and Williamson 2003). The anthocyanin composition of fruits and vegetables may vary from fruit to fruit of the same type due to the genetic and agronomic factors, intensity and type of light, temperature, postharvest treatments, processing and storage (De Pascual-Teresa and Sanchez-Ballesta 2008). For example, the anthocyanin concentrations reach values of up to 250 mg/100 g in red grapes, while the concentrations vary in accordance with the varieties of grapes employed as well as with the type of vinification and, specially, with ageing in red wines. However, a medium value could be established of around 500 mg/L anthocyanins in young wines (De Pascual-Teresa and Sanchez-Ballesta 2008). Significant differences have been reported within raspberry cultivars with respect to relative amounts of anthocyanins (Borges et al. 2009; Beekwilder et al. 2005). Moreover, significant year-to-year variations in the contents of anthocyanins have been noted in raspberries by different

authors (Koponen et al. 2007; Kassim et al. 2009). Low and high temperatures have long been considered to promote and reduce, respectively, anthocyanin synthesis in fruits and berries (Bobinaitė and Viškelis 2013). Cyanidin, the widest spread anthocyanin in fruits and vegetables, is responsible for the brilliant colour (red, orange, blue) of many fruits (black currant, raspberry, strawberry, etc.) and flowers. They are of great nutritional interest because of the marked daily intake (180–215 mg/day) in the United States, which is much higher than the intake (0–30 mg/day) estimated for other flavonoids, including quercetin, kaempferol, myricetin, apigenin and luteolin. It's also known as a powerful antioxidant due to their peculiar chemical structure, as they are very reactive because of their electron deficiency (Galvano et al. 2004).

Isoflavone Daidzein, genistein and glycitein are known as isoflavones which are also a group of phytoestrogens. They have considerable oestrogenic activity. These compounds are found in the Leguminosae family, being predominantly present in soybeans and clover (Nemitz et al. 2016).

Proanthocyanidins The last member of the flavonoids is proanthocyanidins, also known as condensed tannins, which are oligomers or polymers of catechins (flavan-3-ol) linked through interflavan bonds. They are formed from the association of several of catechin and epicatechin monomeric units (Auger et al. 2004). They are present in a variety of plant food and beverages such as red wine, fruits (as plum, apples, strawberry, cherry and other berries, peach), beans and grains (particularly broad bean, lentil and chocolate) (Auger et al. 2004). Hammerstone et al. (2000) studied the procyanidin content of red wine, chocolate, cranberry juice and four varieties of apples. They found that chocolate and apples contained the largest procyanidin content per serving (164.7 and 147.1 mg, respectively) compared with red wine and cranberry juice (22.0 and 31.9 mg, respectively). However, the procyanidin content varied greatly between apple samples (12.3–252.4 mg/serving) with the highest amounts on average observed for the Red Delicious (207.7 mg/serving) and Granny Smith (183.3 mg/serving) varieties and the lowest amounts in the Golden Delicious (92.5 mg/serving) and McIntosh (105.0 mg/serving) varieties. Lee et al. (2003) concluded that apple includes an average 9.35 mg/100 g fresh fruit procyanidin. The procyanidin content of fruits and vegetables may change according to origin, stage of ripeness, postharvesting conservation and processing (Auger et al. 2004). The average intake of proanthocyanidins is estimated as 95 mg/day. Proanthocyanidins are more effective than resveratrol or ascorbic acid in scavenging free radicals (Ou and Gu 2014). The procyanidins are of particular interest because recent reports have indicated possible health benefits, especially cardiovascular health (Prior et al. 2001; Corder et al. 2006). They have potential health benefits due to their antioxidant activity and have been found to inhibit low-density lipoprotein (LDL) oxidation in vitro (Da Silva Porto et al. 2003). The amount of procyanidins in fruits and vegetables could help partially explain the correlations observed between fruit and vegetable consumption and the decreased risk of cardiovascular disease (Prior et al. 2001). Proanthocyanidins are also responsible for characteristic astringency or bitterness of the fruits (Scalbert et al. 2005).

21.4 Phenolic Acids

The other type of the phenolics is phenolic acids which are divided into two classes, hydroxybenzoic acids and hydroxycinnamic acids. Gallic acid, protocatechuic acid and p-hydroxybenzoic acid are the derivatives of benzoic acid; caffeic, coumaric, ferulic and sinapic acids are derivatives of the cinnamic acid. While the hydroxybenzoic acid group content of edible plants is generally very low with the exception of certain red fruits and onions, the hydroxycinnamic acids are more common in fruits and vegetables (Clifford 1999). Blueberry, kiwi, plum, cherry and apple have the highest content (0.5–2 g/kg) of hydroxycinnamic acids. Caffeic acid is generally the most abundant phenolic acid and represents most of the total hydroxycinnamic acid content of fruits. Hydroxycinnamic acids are found in all parts of fruit, although the highest concentrations are seen in the outer parts of ripe fruit (Manach et al. 2004). The average amount of some flavonoids and phenolic acids in fruits and vegetables are shown in Table 21.3.

21.5 Phytoestrogens

Phytoestrogens are oestrogenic compounds. They are naturally occurring compounds and found in plants. Four major families of phenolic compounds produced by plants are considered as phytoestrogens: the isoflavonoids, stilbenes, lignans and coumestans (Cornwell et al. 2004). In recent years, phytoestrogens have drawn attention due to their potential protective effects against many diseases and conditions including cancer, cardiovascular disease, osteoporosis and menopausal symptoms (Yildiz and Gultekin 2006; Adlercreutz et al. 2004; Cornwell et al. 2004; Webb and McCullough 2005). Numerous experimental studies have revealed that phytoestrogens may inhibit the growth of both hormone-dependent and hormone-independent cancer cells (Ganry 2005; Peeters et al. 2003).

21.5.1 Isoflavonoid

Phytoestrogens have been mentioned also in flavonoids because isoflavonoids are also accepted as a member of flavonoid group. They show structural similarity to mammalian oestrogens. Genistein, daidzein and glycitein, the most important isoflavones, are present in large amounts in soybeans and soy products such as miso and tofu. Asian people consume large amount of isoflavones that may have a role in the lower incidence of breast and prostate cancer (Ganry 2005; Adlercreutz et al. 2004).

Table 21.3 Average amount of phenols in fruits and vegetables

Phenols	Source	Phenol content (mg/kg or mg/L)
Hydroxycinnamic acids (Clifford 2000; Shahidi 1997; Dao and Friedman 1992)	Blueberry (100 g)	2000–2200
	Kiwi (100 g)	600–1000
	Cherry (200 g)	180–1150
	Plum (200 g)	140–1150
	Aubergine (200 g)	600–660
	Apple (200 g)	50–600
	Pear (200 g)	15–600
	Artichoke (100 g)	450
	Potato (200 g)	100–190
Hydroxybenzoic acids (Sariburun et al. 2010; Lin and Tang 2007; Vinson et al. 2001)	Blackberry (100 g)	227–278
	Raspberry (100 g)	104–206
	Strawberry (200 g)	36–72
Flavonols (Hollman and Arts 2000; Justesen et al. 1998; Crozier et al. 1997)	Yellow onion (100 g)	350–1200
	Curly kale (200 g)	300–600
	Leek (200 g)	30–225
	Cherry tomato (200 g)	15–200
	Broccoli (200 g)	40–100
	Blueberry (100 g)	30–160
	Apricot (200 g)	25–50
	Apple (200 g)	20–40
	Beans, green or white (200 g)	10–50
	Black grape (200 g)	15–40
	Tomato (200 g)	2–15
Flavones (Justesen et al. 1998; Crozier et al. 1997)	Parsley (5 g)	240–1850
	Celery (200 g)	20–140
	Capsicum pepper (100 g)	5–10
Flavanones (Tomás-Barberán and Clifford 2000; Mouly et al. 1994)	Orange juice (200 mL)	215–685
	Grapefruit juice (200 mL)	100–650
	Lemon juice (200 mL)	50–300
Flavanols (de Pascual-Teresa et al. 2000; Arts et al. 2000)	Apricot (200 g)	100–250
	Cherry (200 g)	50–220
	Grape (200 g)	30–175
	Peach (200 g)	50–140
	Blackberry (100 g)	130
	Apple (200 g)	20–120

21.5.2 Coumestans

Coumestans have similar structure and activity with isoflavones and they are often involved in isoflavones (Ganry 2005). There are a wide range of coumestans; only a small number have shown oestrogenic activity, mainly coumestrol 3 and 4-methoxycoumestrol (Cornwell et al. 2004). Legumes, brussel sprouts, spinach, clover and soybean sprouts are the major dietary sources of coumestans (Cornwell et al. 2004; Ganry 2005).

21.5.3 Lignans

Lignans are the secondary metabolites of plants and important source of phytoestrogens. The plant lignans most commonly present in foods are lariciresinol, matairesinol, pinoresinol and secoisolariciresinol. Other lignans are also found in some foods, including medioresinol (in sesame seeds, rye and lemons), syringaresinol (in grains), sesamin and the lignan precursor sesamolin (in sesame seeds) (Smeds et al. 2007; Peñalvo et al. 2005). Lignan contents of some of the foods are presented in Table 21.4. They are present at high concentrations in flaxseed (335 mg/100 g) (Muir and Westcott 2003) and sesame seed (373 mg/100 g) (Milder et al. 2005a; Smeds et al. 2007; Thompson et al. 2006; Peñalvo et al. 2005) and at low concentrations in *Brassica* family vegetables (Milder et al. 2005a), nuts and cereals (Milder et al. 2005a; Smeds et al. 2007; Peñalvo et al. 2007). However, they also found in a wide range of foods consumed daily such as fruits, seeds, vegetables and beverages like juices, beer, coffee and wine (Kuhnle et al. 2008).

The lignan content and composition of foods depend on various factors such as genetic and environmental variations, sampling, storage, drying and extraction method (Smeds et al. 2012). Lignans are generally concentrated in the hull of cereal grains (Smeds et al. 2007), and seeds as well as in the hull of oilseeds (Smeds et al. 2012). Therefore, the lignans may be lost during dehulling of seeds. There is no enough information about the changes of lignan content during processing and storage in most foods (Kuhnle et al. 2009; Peñalvo et al. 2007; Thompson et al. 2006). However, some of the authors reported that the lignan content of flaxseeds (Hyvärinen et al. 2006a, b) and sesame seeds (Moazzami et al. 2007; Wu 2007; Lee et al. 2010) does not changed considerably during processing.

Touillaud et al. (2007) showed that the total lignan intake was ranged from 0 to 5 mg/day. Milder et al. (2005b) observed the total lignan intake did not differ between men and women. However, vegetarians, non-smokers, people with a high socioeconomic status and older people had higher lignan intake than others.

Epidemiological and pharmacological studies showed that lignans are beneficial for preventing atherosclerosis and cancer, reduction of inflammation, risk factors for stroke and oxidative stress (Prasad 2005; Saleem et al. 2005). Johnsen et al. (2010) examined the relationship between plasma lignan concentration and incidence

Table 21.4 Lignan content of fruits and vegetables (μg/100 g)

Source	Lignan content (μg/100 g)	Reference
Flaxseed	301,129	Milder et al. (2005a)
Sesame seed	39,348	Milder et al. (2005a)
Brassica vegetables	126–2321	Peñalvo et al. (2007), Milder et al. (2005a)
Broccoli	93.9–1325	Milder et al. (2005a), Thompson et al. (2006)
Cabbage	79.1	Thompson et al. (2006)
Carrot	171	Milder et al. (2005a)
Corn	103–115	Peñalvo et al. (2007)
Tomatoes	9.1–58	Peñalvo et al. (2007), Thompson et al. (2006), Milder et al. (2005a)
Apricot	450	Milder et al. (2005a)
Raspberries	37.7–1791	Smeds et al. (2012), Valsta et al. (2003), Thompson et al. (2006)
Strawberries	106–334	Peñalvo et al. (2007)
Grapes	42–60	Milder et al. (2005a)
Kiwi	144–175.8	Peñalvo et al. (2007)

of colon and rectal cancer in more than 57,000 participants, aged 50–64. Their results showed that higher lignan concentrations are related with lower risk of colon cancer among women and higher risk of rectal cancer among men. Lemay et al. (2002) concluded that lignans have preventing effects on osteoporosis and menopausal syndrome.

21.6 Stilbenes

Stilbenes are not widespread in foods (Balsano and Alisi 2009). The major dietary sources of stilbenes are grapes, grape juices, wine, peanuts and peanut butter (Cassidy et al. 2000). They are predominantly located in the skin and flesh of the fruits at low amounts and are produced in response to stress (Scalbert and Williamson 2000). One of them, resveratrol, is produced by plants especially in grapevines, berries, pines, peanuts and legumes (Burns et al. 2002) and draw attention as a bioactive ingredient for its various beneficial biological roles. Resveratrol has beneficial effects to human health including chemopreventive, anti-inflammatory, antioxidant, antiproliferative, proapoptotic, cardioprotective and anticancer properties (Gusman et al. 2001; Pervaiz and Holme 2009). It is a polyphenolic natural product and produced as a response to ultraviolet radiation, injury, stress and fungal infection (Kasiotis et al. 2013). They are included into phytoestrogen group due to their ability to interact with oestrogen receptors (Fulda 2010). They are also classified as phytoalexin group (Li et al. 2006). They can be found in the *cis*- or *trans*-configuration. Resveratrol is mainly found in grapes, and the content in grape products has been showed a wide variation. White grape juice is known to contain

lower levels than red grapes. The amount of resveratrol differs widely with different varieties of wines (Nikfardjam et al. 2000). The resveratrol levels of grapes depend on the variety of the plant. Burns et al. (2002) studied on different types of grapes, and they found wide-ranging levels, ranging from 98 to 1.803 μg/100 mL. Red wines contain the highest levels of trans-resveratrol, with approximately 8 mg/L, but levels vary depending on the grape variety. Levels in rose wines range between 1.38 and 2.93 mg/L, while levels in white wines are generally low since during the winemaking process minimal contact is made with the grape skins, which are the main source of resveratrol (Hooper and Cassidy 2006).

Another stilbenes, pterostilbenes, which are structurally similar to resveratrol, occur naturally in foods, especially blueberries (Fulda 2010). Pterostilbenes also have been reported various health beneficial effects, including anticancer, antiproliferative, proapoptotic, antioxidant, anti-inflammatory, anti-invasive and antimetastatic functions (Pervaiz and Holme 2009). Some other stilbenes are trihydroxy stilbenes, 4,4-dihydroxy-*trans*-stilbene (DHS), isorhapontigenin, bridged stilbenes, piceatannol and dihydro-resveratrol. They have antioxidant and cytotoxic activities, which inhibits the growth of breast and prostate cancer cells (Anisimova et al. 2011; Kasiotis et al. 2013).

21.7 Carotenoids

The number of known natural carotenoids is well over 700. β-Carotene, lycopene, lutein, zeaxanthin, flavoxanthin, canthaxanthin, capsanthin, capsorubin and β-cryptoxanthin are the most known carotenoids. Dietary sources of some of them are listed in Table 21.5. Carotenoids are lipophilic, water-insoluble compounds and the structure of these molecules is diverse. The key structural element of carotenoids is the backbone built up of eight isoprene units with conjugated polyene (Rivera and Canela-Garayoa 2012). The basic structure can be modified in many ways such as cyclization, hydrogenation, dehydrogenation, introduction of oxygen functions, chain shortening or combination (Rodriguez-Amaya and Kimura 2004). Carotenoids are a family of compounds, a group of yellow, orange, and red pigments that occur widely in nature. Plants, some microorganisms and carotenogenic macroscopic fungi are able to synthesize them; human and animal organisms can take up these compounds only with diet. Carotenoids are responsible for the colour of many vegetables and fruits, and they always accompany chlorophyll in leaves. These compounds have distinctive physiological effects in plants; they assist photosynthesis and phototaxis (Krinsky and Johnson 2005). Foods contain different carotenoid composition. For example, while green vegetables have lutein, β-carotene, violaxanthin and neoxanthin as principal carotenoids, they contain α-carotene, α- or β-cryptoxanthin, zeaxanthin and antheraxanthin as minor carotenoids. The composition of carotenoids in fruits is much more complex and variable than in vegetables. While some fruits contain insignificant levels of carotenoids (e.g. pear), some fruits contain considerable amounts of carotenoids such as lycopene (e.g. tomato,

Table 21.5 Carotenoids in fruits and vegetables

Carotenoids	Dietary source
Lutein	Spinach, kale, broccoli, brussel sprouts
Zeaxanthin	Egg yolks, maize, spinach
B-Cryptoxanthin	Citrus fruits, avocado, papaya, pepper
Alpha-carotene	Carrots, pumpkin, maize
Beta-carotene	Carrots, spinach, parsley
Lycopene	Tomato and its products, water melon, guava

watermelon, papaya) (Turcsi et al. 2016). Carotenoids are located in two main organelles of the fruits and vegetables: chloroplasts and chromoplasts. In fruits, carotenoids accumulate in chromoplasts, where they are bound to different structures such as proteins (Liu et al. 2015). There is no limit for the production of carotenoids in chromoplasts. For example, lycopene content in tomatoes can be increased by exposure to direct sunlight or increase the storage temperature (Helyes et al. 2007). Carotenoid contents of the orange can be increased by the generation of photooxidative stress in leaves of orange trees (Poiroux-Gonord et al. 2013). Carotenoids are found in chloroplast in a small amount of fruits (e.g. grape). Large amounts of carotenoids are found as epoxide forms (e.g. mango, carambola) (Turcsi et al. 2016). In green leafy vegetables (i.e. spinach, cabbage or lettuce), carotenoids are present in chloroplasts, where they are almost exclusively bound to the proteins that constitute the photosynthetic apparatus (Esteban et al. 2015). Carotenoids, unlike other plant pigments such as anthocyanins or betalains, play multiple roles in plants. They are sources of essential precursors for the biosynthesis of bioactive compounds in plants when oxidative cleavages occur to form carotenoid derivatives. These compounds serve as signalling molecules (Ramel et al. 2012). Plant signals of colour, fragrance and flavour of fruit are also due to molecules derived from carotenoid oxidation. Carotenoids contribute to the sensory appeal of edible fruits, being responsible for most of their colours, and likewise they are the precursors of many important volatile flavour compounds. The colour of fruits apparently can be a signal of ripeness or nutritional value (Schaefer et al. 2004; Walter et al. 2010). They have been implicated in the interactions of plants with their environment (Walter and Strack 2011). Carotenoids have a protective role against reactive oxygen species since they are very efficient physical and chemical quenchers of singlet oxygen and potent scavengers of other free radicals (Stahl and Sies 2003). Carotenoids are essential components of mammalian diets, as the precursors of vitamin A.

Lycopene is a carotenoid and is associated with decreased risk of cardiovascular diseases and preventing prostate cancer in men. Lycopene has a total of 13 unsaturated double bonds with 11 of which are conjugated. Due to the high number of conjugated double bonds, it is considered to be one of the most potent antioxidants among carotenoids. Tomatoes and tomato products are the major dietary sources of lycopene (Omoni and Aluko 2005). Therefore, serum lycopene levels are seen to increase significantly after consumption of tomato products and supplements (Agarwal et al. 2001).

β-Carotene, an important member of the carotenoid family, is a group of compounds widely distributed in nature and is responsible for the yellow, orange and red colours of fruits and vegetables (Kandlakunta et al. 2008). These carotenoids exhibit antioxidant activity by scavenging oxygen radicals and reducing oxidative stress.

21.8 Vitamins

Fruit and vegetable consumption is recommended due to their vitamin C (ascorbic acid) and vitamin E (tocopherols) content as a natural antioxidant. Antioxidants have many health benefits including preventing lipid oxidation, decreasing DNA damage and maintaining immune function (Gropper and Smith 2012).

Most plant and animal can synthesize this water-soluble vitamin from D-glucose or D-galactose. Because of the absence of the enzyme L-gulonolactone oxidase, humans cannot synthesize vitamin C (Szajdek and Borowska 2008). Fruits, especially tropical species and leafy vegetables, are rich in ascorbic acid. Good sources of vitamin C are citrus fruits (like oranges and grapefruit), broccoli, leafy green vegetables, tomatoes, peppers, potatoes, cantaloupe (rock melon) and strawberries.

Vitamin C prevents the formation of nitroso-compounds, which are cancer-causing substances from nitrites and nitrates used for the preservation of meat and meat product (Kaur and Kapoor 2001). Besides the antioxidant properties by preventing the initiation and oxidation process, it also stimulates immunological resistance and facilitates the absorption of nonheme iron (Szajdek and Borowska 2008).

Vitamin C contents of some fruits and vegetables are shown in Table 21.6. The vitamin C concentration of fruits depends on various factors such as species, genetic factors, harvesting time, environmental conditions, maturity stage, postharvest handling, storage conditions and processing (Denardin et al. 2015). Higher amount of ascorbic acid occurs when fruits and vegetables are exposed to sunlight during growth. Whereas sunlight causes ascorbic acid losses in fruits and vegetables in postharvest period (Lee and Kader 2000), loses of ascorbic acid content is accelerated by storage at high temperatures. Vicente et al. (2009) reported that potatoes lose 75–80% of the original levels of their ascorbic acid during 9-month storage. Loss of vitamin C in the same condition varies from one fruit or vegetable to another. Kaur and Kapoor (2001) determined slight decline in ascorbic acid in both carrots and broccoli, but substantial losses were apparent in green beans at the same storage conditions. Wide variations in vitamin C amount also may be seen between cultivars. According to the study of Koh et al. (2012), the levels of vitamin C among 27 spinach cultivars were found between 24.57 and 62.87 mg/100 g of fresh weight. Cooking is also another factor that causes high losses of ascorbic acid. Vegetables may lose 40–80% of their vitamin C content due to leaching and oxidation during cooking (Vicente et al. 2009).

Vitamin E (tocopherol) is one of the well-known bioactive compounds in fruits and vegetables. Tocopherol was firstly isolated from green leafy vegetables in 1922 and has been widely studied since this year (Peh et al. 2015). Vitamin E consists of

Table 21.6 Vitamin C content of various fruits and vegetables (Dasgupta and Klein 2014; Benvenuti et al. 2004)

Source	Weight (g)	Vitamin C (mg)
Orange	131	69.7
Grapefruit	118	39.3
Papaya	304	185.1
Strawberries	166	39.5–97.6
Mango	207	75.3
Kiwi	76	70.5
Raspberries	123	18.9–32.2
Blackberries	144	22.4–30.2
Broccoli	31	27.7
Cabbage	70	27.6
Tomato	240	22.3
Lime juice	38	11.4
Spinach	30	8.4

four tocopherol analogues (α, β, γ, δ) and four tocotrienols (Halliwell et al. 2005). Vitamin E is well known for its potent antioxidant and anticancer activities. It may reduce the risk of cardiovascular diseases and certain types of cancer (Schwartz et al. 2008). In addition, numerous studies have demonstrated the potential health benefits which include hypolipidemic, antiatherogenic, antihypertensive, allergic dermatitis suppressive, neuroprotective and anti-inflammatory activities. It increases the shelf life and the stability of fruits and vegetables (Saini and Keum 2016). Most fruits and vegetables contain relatively low or moderate concentration amount of vitamin E. Tocopherol contents of some fruits and vegetables are presented in Table 21.7. However, they provide proper source of vitamin E with the abundance of plant-derived foods in our diet (Chun et al. 2006). Olives, nuts, peanuts, avocados and almonds are good sources of vitamin E in fruits. Broccoli and leafy vegetables are rich in vitamin E compared to other vegetables (Vicente et al. 2009).

Vitamin E content of fruits and vegetables is affected by many factors: species, variety, maturity, growing conditions (weather, soil type and intensity of sunlight) and harvesting time (Bauernfeind 1980). Storage and processing substantially alter the vitamin E content.

21.9 Glucosinolates

Glucosinolates are sulphur-containing plant secondary metabolites characteristic for the Brassicaceae family. Brussel sprouts, broccoli, cauliflower and cabbage are the most popular members of this family (Verkerk et al. 2009). Glucosinolate contents of some *Brassica* family vegetables are given in Table 21.8. The glucosinolates plays defence roles in vegetable cultivation, as some glucosinolate breakdown products have been found to be toxic or deterrent towards herbivores and pathogens

Table 21.7 Tocopherol content of various fruits and vegetables (mg/100 g edible weight) (Chun et al. 2006; Charoensiri et al. 2009; Saini and Keum 2016)

Source	Total tocopherol content
Apple	0.1–0.43
Avocados	1.52–3.13
Bananas	0.15
Blackberries	3.74
Blueberries	1.05
Cranberries	1.32–1.61
Cherries	0.08–0.24
Coconut	1.04
Date	0.13
Fig	0.76
Grapefruit	0.17
Kiwi	1.45
Olives	1.65–3.81
Orange	0.25
Peaches	0.19–1.23
Pear	0.42
Plum	0.79
Raspberries	3.46
Strawberries	0.27–0.41
Broccoli	1.32–2.43
Brussel sprouts	0.38–0.43
Cabbage	0.12–0.69
Carrots	0.71–0.87
Cauliflower	0.34
Chicory	2.26
Collards	2.26
Coriander leaves	2.5
Cucumber	0.16
Dandelion green	3.44
Lettuce	0.33–1.06
Pepper	0.08–0.16
Spinach	2.11–4.22
Tomatoes	0.66–0.76
Turnip	2.86

(Björkman et al. 2011). The amount of glucosinolate in the plant is affected by genetic and environmental conditions, including plant age, temperature, water stress and soil type (Farnham et al. 2004). Two hundred known glucosinolates are divided into three main groups, aliphatic, aromatic and indolyl glucosinolate. Some of the most abundant of them found in *Brassica* vegetables were shown in Table 21.9. Glucosinolates can be classified by their precursor amino acid and the types of modification to the functional groups. After destruction of plant tissues,

Table 21.8 The glucosinolate content (μg/100 g fresh weight) of *Brassica* family (Song and Thornalley 2007; Kushad et al. 1999)

	Processing	Total glucosinolate content
Broccoli sprouts	Raw	12.8–62.4
Brussels sprouts	Raw	17.2–25.1
Green cabbage	Raw	10.3–10.9
Cauliflower	Raw	13.5–15.1

Table 21.9 Classification of glucosinolates (Ares et al. 2016; Ciska et al. 2016; Rybarczyk-Plonska et al. 2016)

Aliphatic glucosinolates		Indolyl glucosinolates	Aromatic glucosinolates
-Alkyl	-Alkenyl		
Glucoiberin	Sinigrin	4- Hydroxyglucobrassicin	Glucotropaeolin
Glucoraphanin	Gluconapin	Glucobrassicin	Gluconasturtiin
Glucoalyssin	Progoitrin	4- Methoxyglucobrassicin	
Glucoerucin		Neoglucobrassicin	

glucosinolates undergo both enzymatic degradation, and non-enzymatic transformation to a number of biologically active compounds with different influences on human and animal organisms (Agerbirk et al. 2009). Glucosinolates and their breakdown products determine the typical flavour and taste of these vegetables (Bernhoft 2010). The glucosinolates are hydrolyzed by the plant enzyme myrosinase and results in the formation of biologically active compounds, such as indoles, thiocyanates and isothiocyanates (Holst and Williamson 2004). Isothiocyanates released from some of the glucosinolates (e.g. progoitrin, sinigrin, gluconapin) are unstable and may undergo further transformations (Agerbirk et al. 2009). Glucosinolates and their breakdown products are water-soluble compounds, and the loss of glucosinolates during cooking is due to leaching into the cooking water (McNaughton and Marks 2003). Boiling *Brassica* family vegetables for 9–15 min resulted in 18–59% decrease in the total glucosinolate content of *Brassica* family vegetables (McNaughton and Marks 2003). Consumption of *Brassica* vegetables lowers the risk of some types of cancer in humans. Numerous in vivo studies on animals and in vitro studies, using cell cultures, have demonstrated that glucosinolate degradation products such as indoles and isothiocyanates have anticancer effects (Ciska et al. 2016). The National Cancer Institute recommend the consumption of five to nine servings (2–6.5 cups) of fruits and vegetables daily (Institute 2015). Also, the results of some prospective cohort studies suggest that adults should consume at least 5 weekly servings of *Brassica* family vegetables for the prevention of cancer (Giovannucci et al. 2003). Sulforaphane, the isothiocyanate derivate of glucosinolate, found in broccoli, may prevent tumor growth by blocking the cell cycle and promoting apoptosis (Keum et al. 2004; Thornalley 2002). Furthermore, sulforaphane prohibits the gastritis and stomach cancer that is caused by *Helicobacter pylori* (Fahey et al. 2002). On the other hand, a small number of recent studies exhibited that, under certain conditions, glucosinolate degradation products could

promote the process of carcinogenesis (Latté et al. 2011; Wiesner et al. 2014). Hanschen et al. (2012b) reported the formation of nitriles by thermal degradation of sulphur-containing aliphatic glucosinolate in the boiled broccoli sprouts. The formation of nitriles is accelerated by the presence of Fe^{+2} and lower pH (Hanschen et al. 2012a, b) during a longer processing time (Jones et al. 2010).

21.10 Conclusion

Plants produce a wide variety of secondary metabolites which may have health effects in humans. These bioactive compounds include phenolics, phytoestrogens, carotenoids, vitamins and glucosinolates. Fruits and vegetables contain a wide range of micronutrients and non-nutrient bioactive compounds. Many researchers have suggested that a diet rich in fruits and vegetables may prevent several diseases, such as hypertension, obesity, coronary heart disease and stroke risk, overall cancer, eye diseases, asthma and osteoporosis. It is believed that compounds which are largely responsible for those protective effects are bioactive compounds.

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Chapter 22
Environmental Impacts of Minimally Processed Refrigerated Fruits and Vegetables' Industry

Hülya Ölmez

22.1 Introduction

Water is an irreplaceable resource for the food processing industry both as an ingredient and a major processing element. The food industry is one of the most water-intensive industries constituting almost 15% of the total industrial water use (CIAA 2008). Moreover, the food industry has the highest contribution to the total industrial emissions of organic water pollutants with a share of up to 30% (World Bank 2011). The BOD and COD levels in food processing industry wastewaters can be 10–100 times higher than those of the domestic wastewater (Yildiz et al. 1986), FDM-BREF 2006).

The fruit and vegetable processing sector is one of the major water-intensive sectors of the food industry. The life cycle assessment (LCA) studies revealed that the environmental burden associated with the fresh-cut industry is heavily influenced by the processing phase, mainly the washing and packaging operations (Fusi et al. 2016). The water consumption and wastewater volumes are in the range of 2.4–11 m^3 and 11–23 m^3 per tons of product for the fruit and vegetable processing industry (FDM-BREF 2006). Therefore, sustainable use of water is a major environmental and economic challenge for the fruit and vegetable processing industry. There is a need for developing eco-efficient innovative technologies that will aid in reducing water consumption and wastewater generation rates and improving the wastewater quality (Ölmez and Kretzschmar 2009). In this content, washing and sanitation operations are the major concern as they account for almost 70% of the total water use during processing. In some specific cases, like the processing of fresh-cut leafy vegetables, almost 90% of water is used for washing and rinsing, and more than 90% of the organic

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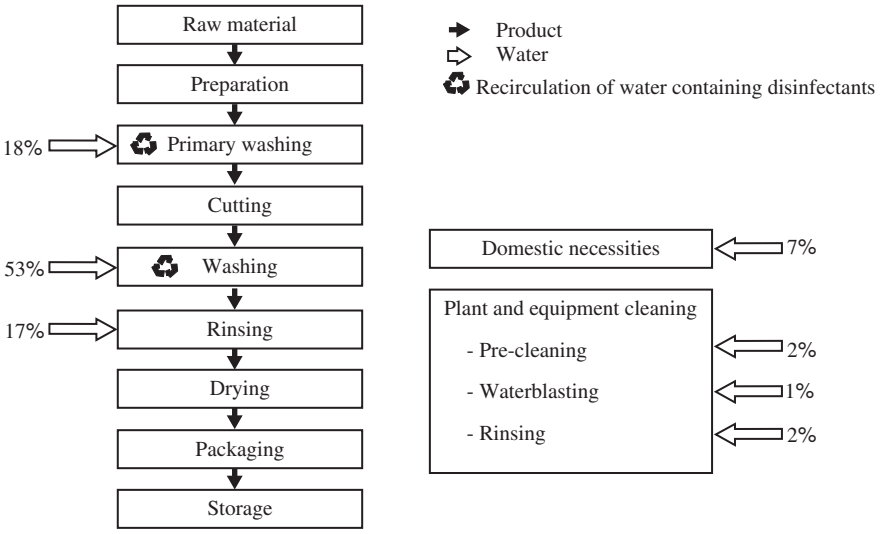

Fig. 22.1 Product flow and water needs in a typical fresh-cut salad production process (Reprinted from Manzocco et al. 2015 with permission)

load of the wastewater is generated from the processing stage (Fig. 22.1) (Lehto et al. 2014; Manzocco et al. 2015). The fruit and vegetable processing sector is one of the sectors of the food industry for which the European Commission (EC) Council Directive 96/61/EC on Integrated Pollution Prevention and Control (IPPC) is laying down measures to reduce emissions in water. The management of wastewater and wastewater quality is at most importance, because the main industrial impact on water resources is due to the highly polluted wastewater discharges rather than the amount of water used. The indicative list of the main pollutants includes the organo-halogens, which are mainly associated with the use of chemical sanitizers, mostly with the chlorine-based sanitizers in the case of fruit and vegetable processing industry. Therefore, there is a great interest in replacing chlorine with more eco-efficient water sanitation technologies during the last two decades. In fact, due to the environmental concerns associated with the formation of toxic disinfection by-products (DBP), the use of chlorine for fruit and vegetable washing has been banned in many European countries (Van Haute et al. 2013).

22.2 Environmental Impact of Water Sanitation Technologies Employed by the Fruit and Vegetable Processing Industry

The employment of the best available technologies together with the implementation of a systematic water management strategy may lead up to 90% reduction in fresh-water demand through the reuse of process water. The current food safety

regulations permit the processors to reuse or recycle water unless the water constitutes a risk to the safety and suitability of the food (Council Directive 98/83/EC). Disinfection is the most critical step in treating water for reuse. Both the microbiological safety of the treated water and the product and the type and level of the DBP formed in the wastewater depend on the disinfection technology applied. A comparison of the most commonly used mature technologies and the emerging disinfection technologies is given in Table 22.1.

Table 22.1 Water disinfection technologies used in the fruit and vegetable processing industry (Modified from Ölmez and Kretzschmar 2009 and Ölmez 2014)

Disinfection method	Advantages	Disadvantages
Chlorination	Low cost Easily available Easy to operate Posttreatment residual effect preventing microbial growth	pH-dependent activity Formation of halogenated DBP, carcinogenic and toxic to aquatic life Requires final dechlorination before final discharge
Electrolyzed water	Simple production On-site generation Cheap and easy-to-find raw materials No need to store toxic chemicals (operation with NaCl and water only) Low operational cost Higher antimicrobial efficacy at lower free chlorine levels	High initial investment cost Formation of halogenated DBP (although at relatively much lower levels)
Ozonation	Higher inactivation efficacy than chlorine Shorter contact times needed Fast inactivation kinetics Reduces COD, turbidity, color, anthropogenic compounds, and UV absorbance in effluent Increases dissolved oxygen levels in effluent No THMs and HAAs formation No harmful residuals Incidental removal of some contaminants	Formation of potentially carcinogenic bromate and other brominated DBP Unstable (requires on-site generation) Relatively difficult to operate Highly influenced by the organic matter Higher investment and operational costs No posttreatment residual effect
Chlorine dioxide	More effective to viruses than chlorine Efficacy less pH dependent than chlorine Less corrosive than chlorine and ozone Residuals degrade more rapidly than chlorine residuals Less impact on aquatic life compared to chlorine Residual effect for preventing posttreatment microbial growth Fewer potentially hazardous DBP formation than chlorine No THM formation in effluent More rapid decomposition of residuals Less impact of aquatic life Low investment cost	Formation of nonhalogenated DBP and organic chlorine compounds Formation of iodinated DBP (more toxic than chlorinated DBP) in the presence of iodide Requires on-site generation and monitoring, relatively difficult to operate Higher operational costs May produce noxious odors Increases TDS and chloride levels in final effluent

(continued)

Table 22.1 (continued)

Disinfection method	Advantages	Disadvantages
Organic acids	Easy to use No toxicity	Very long contact time, not relevant to the industry Effective only at relatively high concentrations Interferes with the sensory quality Relatively lower antimicrobial efficacy
Hydrogen peroxide	No residue problem Easy to use Low cost	Low antimicrobial efficacy Long contact time Slow disinfection kinetics Phytotoxic, negative impact on overall quality Requires the removal of residual H_2O_2 after processing
Peracetic acid	Disinfection efficacy similar to chlorine Few or no harmful DBP formation Not create toxicity in effluents Lower investment cost Some residual effect to control regrowth Principle end products are acetic acid, oxygen, water, and carboxylic acids Reduce odors in wastewater	Disinfection efficacy highly dependent on water quality Less effective against viruses and little effect on spores Slower disinfection kinetics Risk of regrowth Adds organic carbon to effluent May form aldehydes, chlorine, and bromine radicals if applied at high concentrations Unstable, requires on-site generation Relatively difficult to operate Higher operational costs

TDS total dissolved solids, *THM* trihalomethane, *HAA* haloacetic acid

Two important issues regarding the environmental impact of the disinfection techniques are, first, the physiochemical quality of the wastewater effluents and, second, the amount of wastewater generated. The disinfection technique employed actually determines the physicochemical quality of the wastewater effluents, that is, the presence of disinfection residuals, the formation of toxic DBP, and the modification of organic matter, which can harm the environment (Lazarova et al. 1998). For the disinfection of the process water, the product, and the equipment surfaces, more water-efficient disinfection techniques with less environmental impact, such as ozone, electrolyzed water, pulsed UV, or ultrasound, are suggested as an alternative to chlorine. As the amount of wastewater generated per unit mass of product is dependent on the disinfection technique employed, techniques capable of disinfecting efficiently both the process water and the product would allow a high ratio of recycling. This in turn would reduce the wastewater rates and would have less impact on the environment. Moreover the physicochemical wastewater parameters, namely, the biological oxygen demand (BOD), the chemical oxygen demand (COD), the total organic carbon (TOC), and the total suspended solids (TSS), are also of great concern for the fruit and vegetable processing industry as they are the

decisive factors for reusability (FDM-BREF 2006). Therefore, the reuse of wash water should be implemented with special care. It is known that the quality of wash water has an important effect on the final product quality as well as on the effectiveness of the sanitizing treatments.

Actually there is an apparent shift of paradigm on the use of disinfectants in washing water for fruit and vegetable processing during the last decade. This changes the main target of the disinfection operation from the produce itself to the process water (Gil et al. 2009; Ölmez and Kretzschmar 2009). Indeed, this approach made it possible to minimize the level of sanitizing agents used in wash water and thus opened a door for diminishing the environmental impact of fruit and vegetable processing mainly associated with the washing operations. Moreover, this shift of paradigm has also changed the methodologies used in assessing the efficacy of disinfectants toward mimicking the industrial operation conditions with simulated continuous washing operations instead of batch-wise experiments (Gomez-Lopez et al. 2017).

In spite of the well-known formation of toxic DBP, due to the economic accessibility and wide availability, chlorine (sodium hypochlorite) is still the most widely used disinfectant for fruit and vegetable processing. Normally it is used at a concentration of 100–200 mg/L by the industry. However, recent studies revealed that much lower concentrations of chlorine (7–20 mg/L) are sufficient to exhibit similar antimicrobial activities and prevent potential cross contamination associated with the reuse of wash water (Shen et al. 2013; Keskinen et al. 2009; Gomez-Lopez et al. 2014; Zhou et al. 2015). Maintaining a residual free chlorine concentration of lower than 1 mg/L is sufficient to inactivate the suspended pathogenic bacteria in wash water even at high COD levels (1000 mg/L) (Van Haute et al. 2013). Moreover, only 10 mg/L of residual free chlorine is needed to prevent cross contamination during produce wash (Luo et al. 2011). Therefore, it is suggested that hyperchlorination should be avoided and a residual free chlorine level that is sufficient to prevent cross contamination to produce should be maintained throughout the process. It is important to note that even with such lower residual chlorine levels, the trihalomethane level (THM) in water may exceed the allowable limits of 100 mg/L and 80 mg/L of drinking water, respectively, in Europe and in the USA. The formation of toxic DBP associated with the use of chlorine and the factors affecting their formation in fruit and vegetable wash water have been well documented. In general, chloroform (trichloromethane) accounts for the highest portion (>50 to 97%) of the total THM formed in chlorinated wash water followed by bromodichloromethane, dibromochloromethane, and bromoform (trichloromethane) (Gomez-Lopez et al. 2014). The formation of chloroform in wash water depends on the type of fruit or vegetable, the initial concentration and contact time of chlorine, and the pH of the wash water. The repeated use of chlorinated wash water (100 μg/L) up to six batches may lead to the doubling of the level of chloroform formed, increasing from 155 μg/L to 284 μg/L. Luo (2007) reported that washing fresh-cut lettuce using reused water with high COD levels resulted in significantly higher microbial loads, decreased tissue integrity, and increased off-odor development during storage. There is a good correlation between the chlorine demand of wash water and the water quality parameters, namely, the COD and TOC (Weng et al. 2016). Therefore, it is important to minimize the COD

and BOD levels in the wash water, as well as the level of DBP formed, not only from an environmental aspect but also for assuring the quality and safety of the final product. From an engineering point of view, one strategy to achieve this is to keep the chlorine demand of the wash water low through reducing the organic load of the water. Employing a prewash or rinse step for fresh-cut vegetables and having whole-leaf washes at the start are two solutions for keeping a low chlorine demand during washing (Nou and Luo 2010).

The use of chlorine dioxide (ClO_2) leads to negligible levels of chloroform formation compared to the use of chlorine in vegetable wash water. Even at 200 mg/L of ClO_2 levels, only 3 μg/L of chloroform was produced in lettuce wash water, whereas the same level of chlorine resulted in the formation of higher than 40 μg/L of chloroform (Fig. 22.2) (Fan and Sokorai 2015). Similarly Lopez-Galvez et al. (2010)

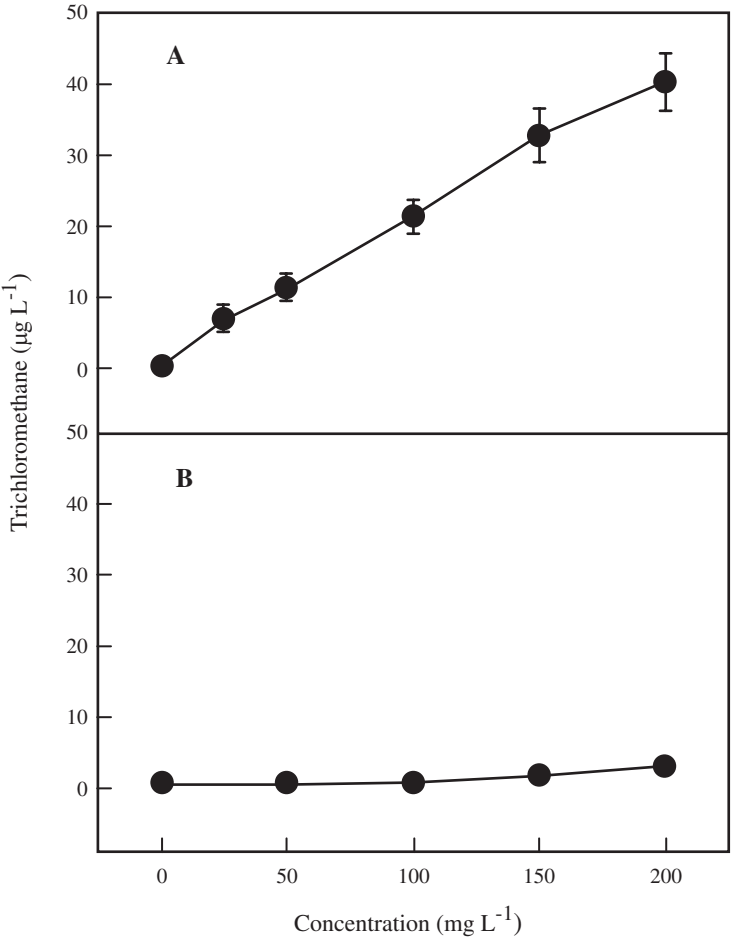

Fig. 22.2 Formation of chloroform (trichloromethane) from chlorine (A) or chlorine dioxide (B) reacting with lettuce juice as a function of chlorine or chlorine dioxide concentration (Reprinted from Fan and Sokorai 2015 with permission)

Table 22.2 Individual and total THM (μg/L) in the process wash treated with different chlorine-based sanitizers

Disinfectant	Chloroform (trichloro-methane)	Dichlorobro-momethane	Chlorodibro-momethane	Tribromo-methane (bromoform)	Total THM	Reference
Chlorine (sodium hypochlorite) (3.8 mg/L, COD =500 mg/L)	119.1	9.9	54.0	11.0	194.0	Gomez-Lopez et al. (2013)
EOW (1.8 mg/L, COD =500 mg/L)	31.5	2.1	13.9	2.7	50.2	Gomez-Lopez et al. (2013)
ClO_2 (3.7 mg/L, COD =700 mg/L)					<5.0	López-Gálvez et al. (2010)

showed that aqueous ClO_2 (3 mg/L) is effective in maintaining the microbial quality of wash water while preventing the formation of high levels of THM (Table 22.2). However, a major disadvantage of ClO_2 is that it forms more iodinated DBP when iodide is present in the source water. Toxicological studies suggest that iodinated DBP may be more toxic than their chlorinated analogs (Plewa et al. 2004). There are no regulations concerning the use of ClO_2 in fruit and vegetable washing in Europe. In the USA, its use is allowed in the wash water of not raw produce at residual ClO_2 levels of less than 3 mg/L provided that a final potable rinse is applied (Banach et al. 2015).

Electrolyzed water (EOW) appears to be a commercially applicable alternative to chlorine, which may have an important effect in diminishing the environmental impact of washing processes. The key advantages of electrolyzed water over chlorine are the simple production based on sodium chloride, low operational costs, and avoiding the need to store toxic chemicals (Gil et al. 2015). Another critical feature of electrolyzed water is its ability to reduce the organic load of process water via the mineralization of organic matter in boron-doped diamond electrodes (Polcaro et al. 2009; Kapalka et al. 2008). Initially the main disadvantage of these systems was the high generator costs, which actually diminished during the last years parallel to the improvements in this technology (Gomez-Lopez et al. (2017). Considerable reduction in the formation of THM can be achieved by the use of EOW in fresh produce wash water. EOW resulted in more than 30% reduction in the individual and total THM generated in the baby spinach wash water compared to the sodium hypochlorite chlorinated wash water at similar levels of free chlorine (2–4 mg/L) (Gomez-Lopez et al. 2013) (Table 22.2).

Ozone is the most powerful oxidant among the listed sanitizing agents in Table 22.1. The main disadvantages of ozone that limits its widespread use in fruit

and vegetable wash waters are its instability which necessitates its on-site production and its low solubility in water. On the other hand, due to the volatile nature, it is well suited for use in plug flow reactors (Banach et al. 2015). Currently, bromate is the only ozone DBP regulated in drinking water in the EU and the USA. The US Environmental Protection Agency (EPA) sets a maximum contaminant level (MCL) of 10 mg/L for bromate (US EPA 1998). The main concern with bromate is that it is not biodegradable and is a possible human carcinogen (Wert et al. 2008).

Peracetic acid (PAA) appears to be an alternative as a wash water sanitizer due to its nontoxic decomposition products and the broad range of pH and temperature usage (Vandekinderen et al. 2009). Moreover, the maintenance and operation of PAA process are relatively simple. However, the relatively higher operational cost and instability at higher concentrations are the limitations of PAA. Unlike the chlorine-based sanitizers, PAA has a much lower potential of producing toxic DBP. However, the disinfection kinetics of PAA is slower than chlorine, and therefore ways of increasing the efficacy need to be developed (Van Haute et al. 2015a).

Hydrogen peroxide is highly affected by the organic matter in the wash water, therefore requires the maintenance of high residual levels to be able to achieve an appropriate disinfection efficacy (Van Haute et al. 2015b).

22.3 Conclusion

The major limitations associated with the reduction in the environmental impact of the fruit and vegetable processing through the efficient use of water include the food safety requirements, regulatory issues, technological constraints, and economic factors. In this content, one of the main strategies for the minimization of water consumption and wastewater discharge rates and improving the wastewater quality in the fruit and vegetable processing industry is to replace or combine the chemical-based water sanitation technologies with chemical-free physical technologies. The use of hybrid technologies employing a chemical-based technology combined with a physical technology is gaining interest in recent years. Ultrasound, pulsed UV, and pulsed electric field (PEF) are examples of potential physical means of wash water disinfection. However, their relatively slower disinfection kinetics, the requirement of long contact times not relevant to industrial processing, high energy demand, and difficulties in the integration of these technologies into the existing processing lines are the major barriers for the practical application of these technologies currently. The reuse and recycling of process water at appropriate points are also efficient strategies for reducing the fresh water demand to a great extent, thereby diminishing the environmental impact of the industry.

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Appendix

Recommended Storage Temperatures for Refrigerated Food Products and Ingredients

Table A.1 Ideal refrigeration temperature for product categories

Commodity	Temperature
Dairy	32–40 ° F (0–4.4 ° C)
Milk	
Eggs	
Butter, margarine	
Sour cream	
Cottage cheese	
Meats, fresh	30–34 ° F (−1.1 to 1.1 ° C)
Roasts	
Steaks	
Chops’	
Hamburger	
Sausage	
Processed meats	
Poultry	30–34 ° F (−1.1 to 1.1 ° C)
Chicken	
Turkey	
Duck	
Goose	
Seafood	30–34 ° F (−1.1 to 1.1 ° C)
Shrimp	
Lobster	
Mollusc	
Fish (fresh and smoked)	
Salads	32–40 ° F (0–4.4 ° C)

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Table A.2 Ideal refrigerated storage temperature for vegetables

Commodity	Temperature	Comments
Artichoke		
Asparagus if stored	32 ° F (0 ° C) 32 ° F (0 ° C) 36 ° F (2.2 ° C)	Chilling injury if held more than 10 days below 36 ° F (2.2 ° C)
Avocado	40–55 ° F (4.4–12.8 °C)	
Fuerte var	45 ° F (7.2 ° C)	
Beans, lima	32–40 ° F (0–4.4 ° C)	
Beans, snap	45–50 ° F (7.2–10 ° C)	
Beets	32 ° F (0 ° C)	
Broccoli	32 ° F (0 ° C)	
Brussels sprouts	32 ° F (0 ° C)	
Cabbage	32″ F (0 ° C)	
Carrots	32 ° F (0 ° C)	
Cauliflower	32 ° F (0 ° C)	
Celery	31–32 °F (−0.6 to 0 ° C)	
Com	32 ° F (0 ° C)	
Cucumber	45–50 °F (7.2–10 °C)	
Eggplant	45–50 °F (7.2–10 °C)	
Greens, leafy	32 ° F (0 ° C)	
Horseradish	30–32 ° F (−1.1 to 0 °C)	
Leeks, green	32 ° F (0 ° C)	
Lettuce	31–33 °F (0–0.6 °C)	
Mushrooms	32 ° F (0 ° C)	
Onion, dry	32 ° F (0 ° C)	
Onion, green	32 ° F (0 ° C)	
Okra	45–50 ° F (7.2–10 ° C)	
Olives	45–50 ° F (7.2–10 °C)	
Peas, green	32 ° F (0 ° C)	
Pepper, sweet	45–50 ° F (7.2–10 ° C)	
Potato	40–70 ° F (4.4–21.1 ° C)	
Early, immature	38–40 °F (3.3–4.4 ° C)	
Late, mature	32 ° F (0 ° C)	
Radish	32 ° F (0 ° C)	
Rutabaga	32 ° F (0 ° C)	
Spinach	50–55 ° F (10–12.8 ° C)	
Squash, winter and pumpkin	32 ° F (0 ° C)	
Squash, summer if stored	45–50 ° F (7.2–10 ° C)	Chilling may occur if held more than 1 week below 45 ° F (7.2 ° C)
Sweet potato tomato	55–60 ° F (12.8–15.6 ° C)	
Mature green Firm ripe	55–70 ° F (12.8–21.1 ° C)	Quality reduced when held below 35 ° F (1.7 °C)
Turnip	32 ° F (0 ° C)	
Turnip greens	32 ° F (0 ° C)	

Adapted from "Commercial Cooling of Fruits and Vegetables," Manual 43, Agricultural Experimental Station Extension Service California, University of California

Table A.3 Ideal refrigerated storage temperature for fruits

Commodity	Temperature
Apple	30–31 ° F (−1.1 to −0.6 ° C)
Chilling sensitive varieties	38–40 ° F (3.3–4.4 ° C)
Apricot	31–32 ° F (−0.6 to 0 ° C)
Banana	56–58 ° F (13.3–14.4 ° C)
Berries:	31–32 ° F (−0.6 to 0 ° C)
(bush-, blue-, straw-)	
Cherry, sour	32 ° F (0 ° C)
Cherry, sweet	30–32 ° F (−1.1 to 0 ° C)
Citrus:	
Grapefruit	58–60 ° F (14.4–15.6 ° C)
Lemon	58–60 ° F (14.4–15.6 ° C)
Limes	45–50 ° F (7.2–10 ° C)
Orange	38–44 ° F (3.3–6.7 ° C)
Tangerine and other mandarins	32 ° F (0 ° C)
Coconuts	32–35 ° F (0–1.7 ° C)
Dates,	32 ° F (0 ° C)
Figs	31–32 ° F (−0.6 to 0 ° C)
Grapes	30–31 ° F (−1.1 to −0.6 ° C)
Mangoes	55 ° F (12.8 ° C)
Melons:	
Honeydew	45–50 ° F (7.2–10 ° C)
Cantaloupe	32–40 ° F (0–4.4 ° C)
Watermelon	40–50 ° F (4.4–10 ° C)
Papayas	31–32 ° F (−0.6 to 0 ° C)
Peach and nectarine	32 ° F (0 ° C)
Pears	29–31 ° F (−1.7 to −0.6 ° C)
Persimmon.	40 ° F (4.4 ° C)
Pineapple	45–47 ° F (7.6–8.3 ° C)
Plums and prunes	31–32 ° F (−0.6 to 0 ° C)
Pomegranate	32 ° F (0 ° C)
Quinces	32 ° F (0 ° C)

Adapted from "The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks" Agriculture Handbook Number 66, United States Department of Agriculture, U.S. Govt. Printing Office, Washington, DC. *Courtesy* National Food Processors Association, Washington, DC

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