

Chapter 5

Biological and Biochemical Changes in Minimally Processed Refrigerated Fruits and Vegetables

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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 compartmentalization 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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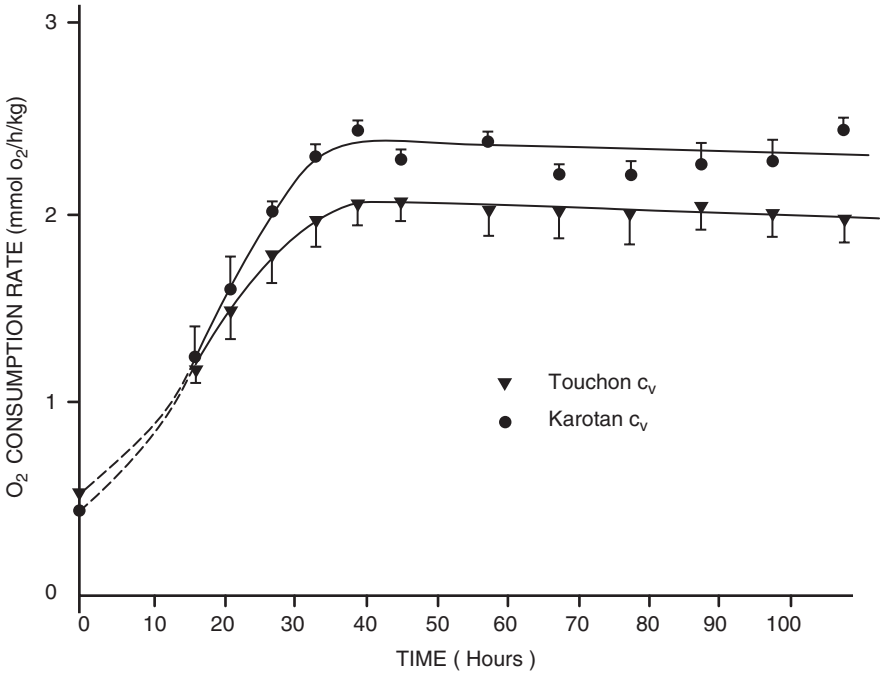


Fig. 5.1 Change in respiratory intensity of fresh grated carrots after standard processing (two cultivars). Grated carrots were stored in air at 10 °C (From Carlin 1989)

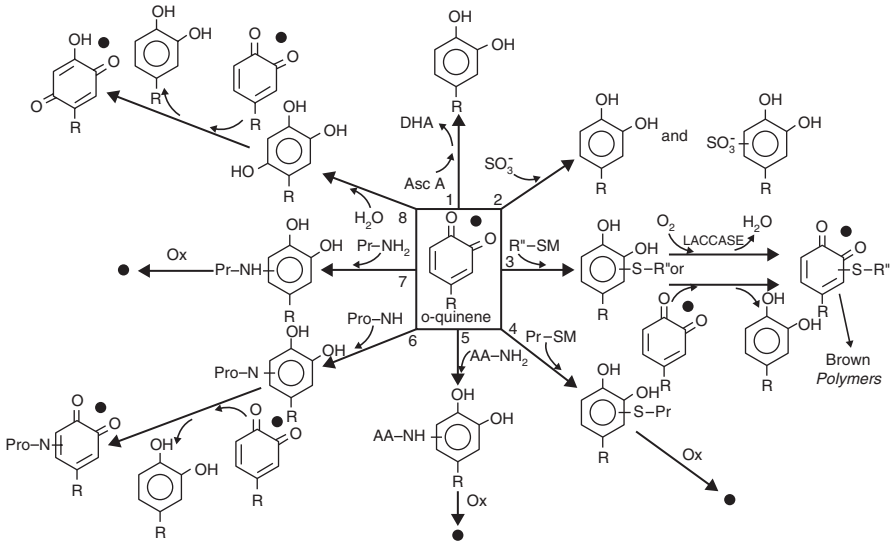


Fig. 5.2 Enzymatic browning; the role of inhibitors on the formation of brown polymers from o-quinone (From Rouet-Mayer et al. 1993)

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 citric 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 CO₂ 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 O₂ 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* (Brocklehurst 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

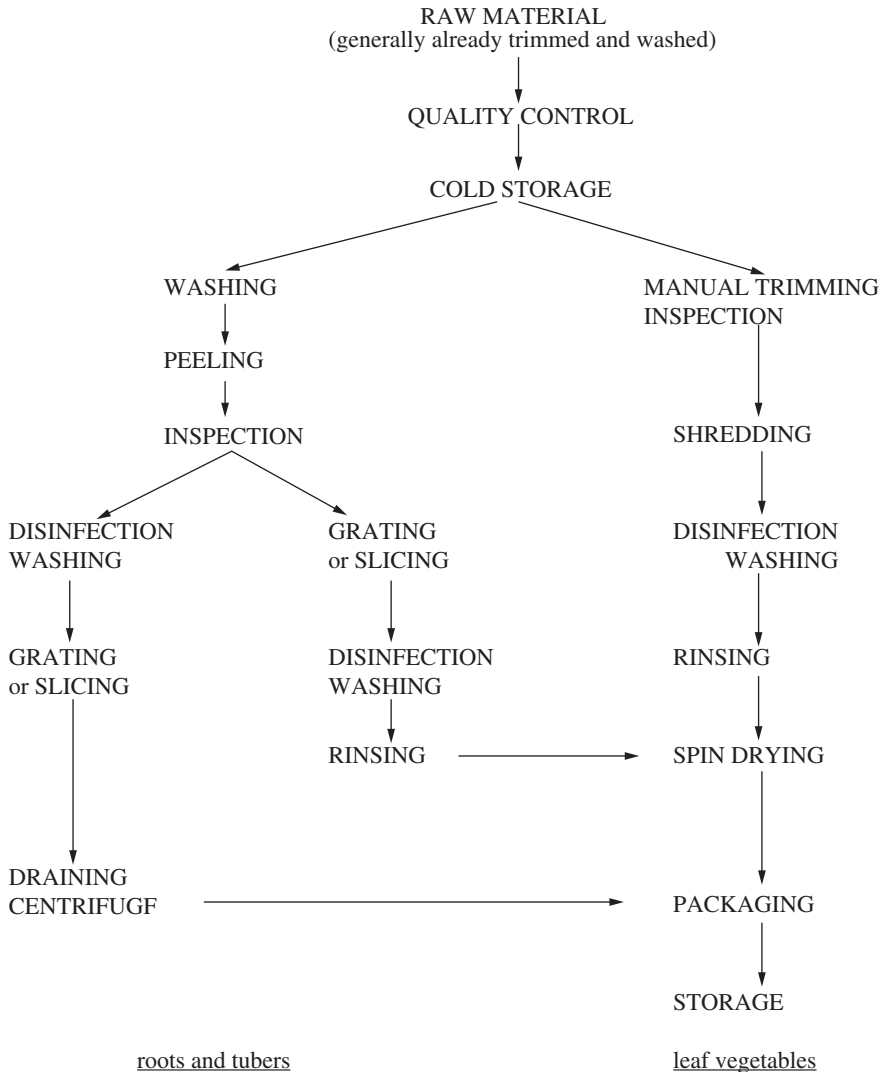


Fig. 5.3 Minimally processed vegetables. Flow diagram of processing lines

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.

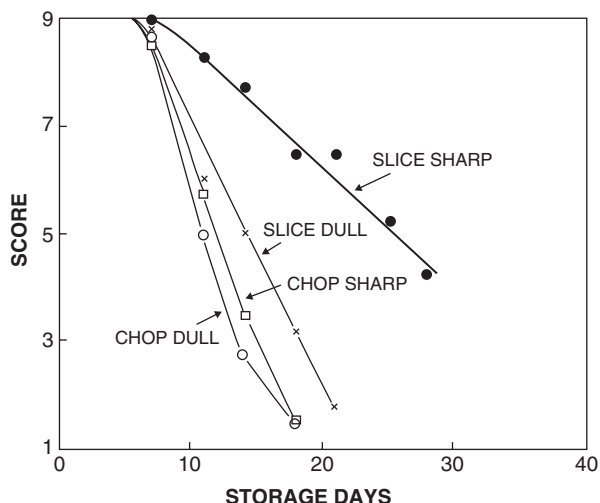
MPR fruit and vegetable production plants must follow the scientific rules which are given below:

- (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)



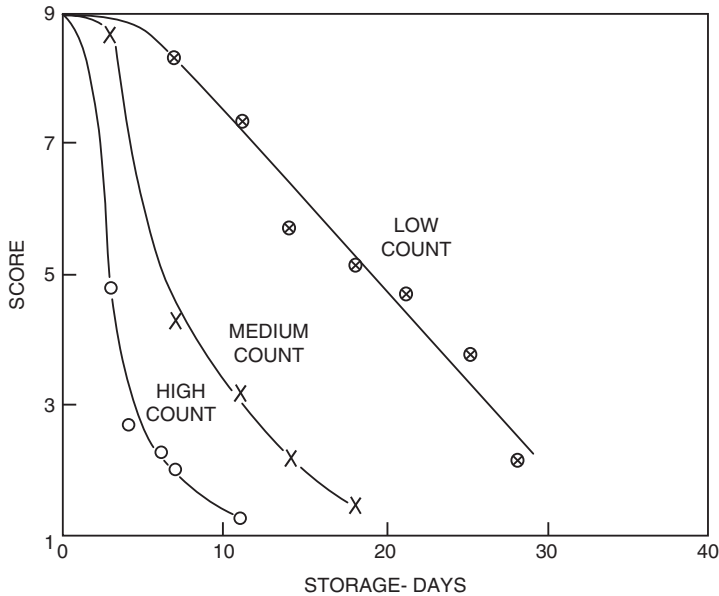


Fig. 5.5 Effect of initial microbial count on storage stability of shredded lettuce (From Bolin et al. 1977)

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.

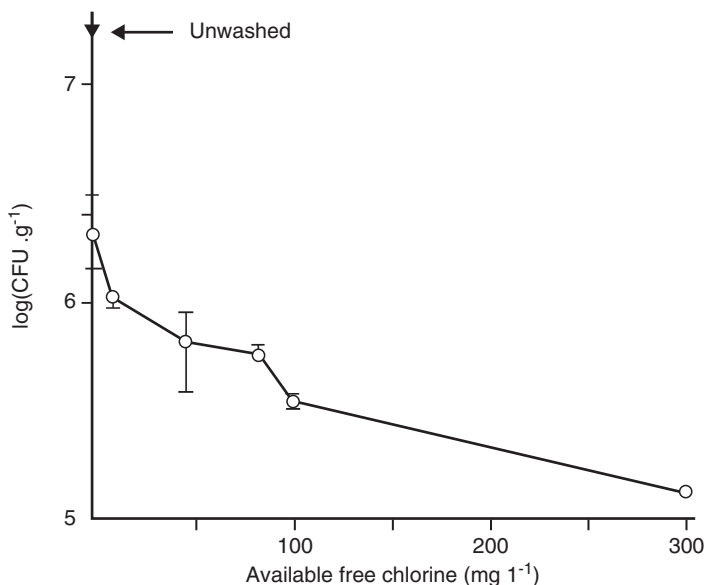


Fig. 5.6 Effect of chlorine concentration on the count of mesophilic aerobic bacteria on washed lettuce leaves. pH increased from 7.3 for unchlorinated water to 9.4 for 300 mg/l of free chlorine indicates ranges in replicate experiments (From Adams et al. 1989)

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 O₂ 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, Q₁₀, averages at 2 for most fruits and vegetables but may range from 1 to 5.

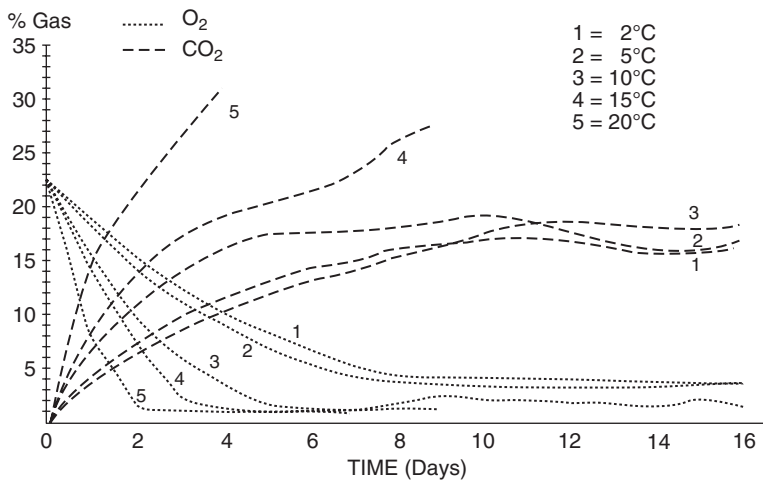


Fig. 5.7 Effect of temperature on O₂ consumption rate of shredded endives in air (From Chambroy et al. 1990)

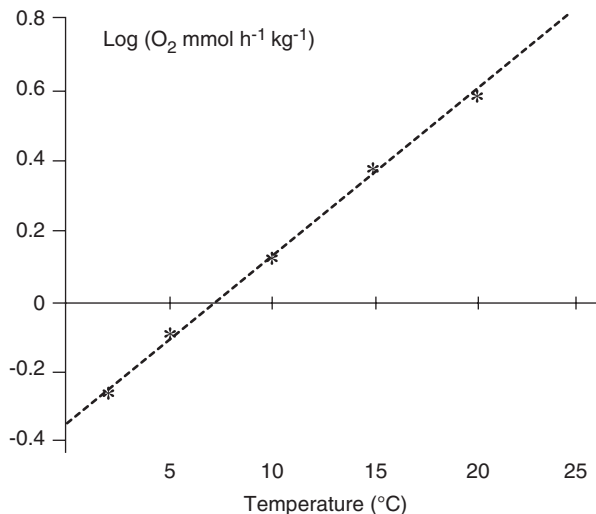


Fig. 5.8 Effect of temperature on atmosphere change within propylene packs of shredded endives versus duration of storage (From Chambroy 1989)

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, CO₂ 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)

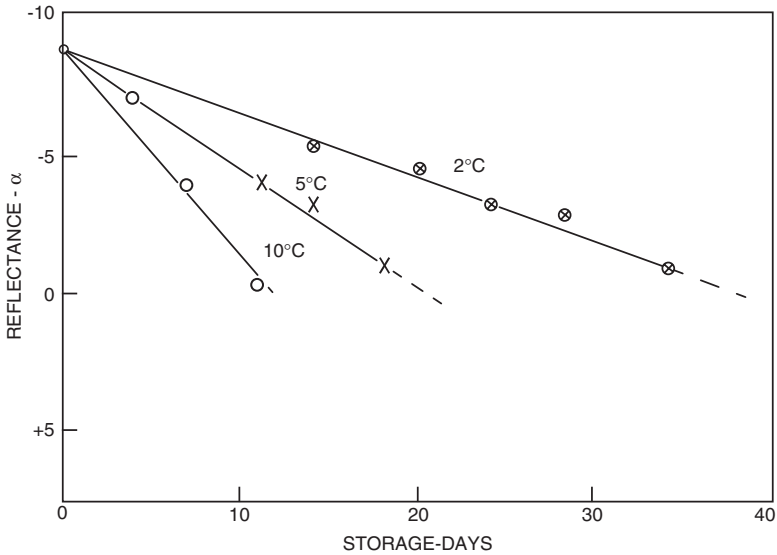
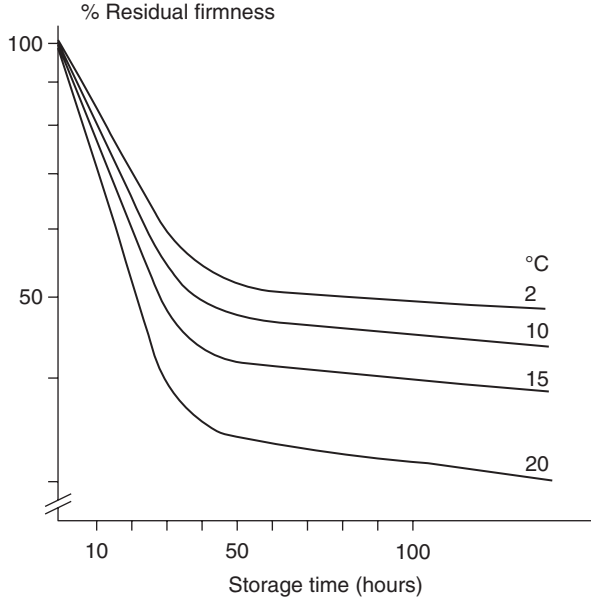


Fig. 5.10 Effect of storage temperature on green color loss in shredded lettuce (From Bolin et al. 1977)

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

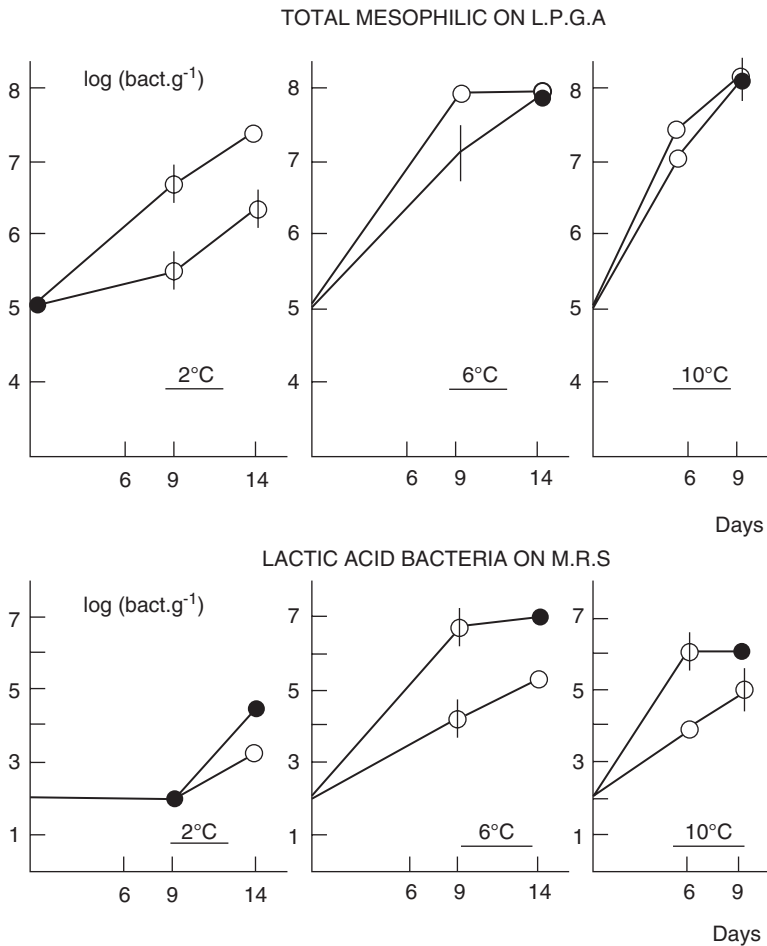


Fig. 5.11 Changes in total flora and lactic acid bacteria in shredded endive packed in polypropylene pouches under air (O—O) or air +20% CO₂ (®—®) as a function of storage duration at 2, 6, and 10 °C (From NGuyen-The and Carlin 1988)

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 CO₂ 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 CO₂ and H₂O with concurrent production of energy. MAP may lower the metabolism and decrease both O₂ consumption and CO₂ production (Laties 1978). The effects of low O₂ and high CO₂ 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 CO₂ concentrations. This phenomenon has been shown for celeriac (Weichmann 1977a), carrots (Weichmann 1977b), and onions (Adamicki 1977). If O₂ is reduced or CO₂ elevated beyond the tolerance levels of the commodity, respiration is then associated with anaerobic metabolism.

It is established that high CO₂ 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 O₂ 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 CO₂, ethanol, aldehydes, and other chemical compounds that produce off-flavors, off-odors, and discoloration. In theory, the O₂ level within the cell which induces anaerobic metabolism is as low as 0.2%, and that outside the product 1–3% (Burton 1974).

The ratio of CO₂ production to O₂ 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). CO₂ levels as low as 5% may induce physiological disorders in common mushroom (Lopez-Briones 1991), and asparagus exhibits surface pitting when stored in >10% CO₂ (Lipton 1977). The average CO₂ toxicity threshold ranges from 10% to 30% depending on plant and storage factors. Crisp head lettuce in storage with elevated CO₂ is strongly affected by O₂ 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 CO₂ injury. Krahn (1977) found that the outer leaves of crisp head lettuce are not injured by 2% CO₂, but the inner leaves and midribs show damage. Also, shredded head lettuce seems to tolerate higher levels of CO₂.

The effect of CO₂ on cell ultra-structures (Frenkel and Patterson 1974) and membranes (Sears and Eisenberg 1961) could account for its toxicity. It can be postulated that CO₂ dissolution, which enhances acidity in the cell medium, may participate in the physiological disorder. Optimum concentrations of O₂ and CO₂ 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 O₂ and elevated CO₂ 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 O₂ requirements. Proper permeabilities to both O₂ and CO₂ 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, Epemnon, 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 10⁵ to 10⁹ 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 CO₂-O₂ 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 O₂ and CO₂ 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 O₂ 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.

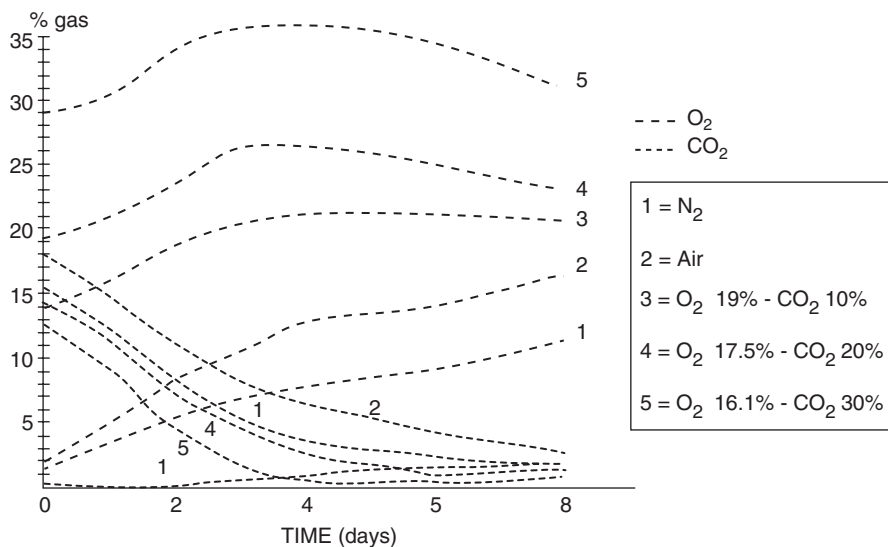


Fig. 5.12 Effect of initial atmosphere composition on gas concentration changes within polypropylene packs of shredded endives versus duration of storage at 10 °C (From Chambroy 1989)

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 CO₂ in the mixture injected at sealing. In this example, O₂ consumption rates are not markedly affected by additional CO₂, but for elevated CO₂ concentrations, diffusion rates exceed CO₂ production through respiration, and therefore the CO₂ concentration decreases. In endives, anaerobic metabolism seems difficult to trigger because injection of pure N₂ in the bags results in a decrease in CO₂ 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 O₂ and CO₂ concentrations on respiration rate
2. The effect of switching, even partially, to anaerobic metabolism
3. The permeability of the film to O₂ and CO₂ (and the effect of moisture on the gas diffusion coefficients)
4. The effect of temperature on film permeability to both O₂ and CO₂ 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 O₂ consumption rate with complete absorption of CO₂ was constant in the range of 11–21% O₂; below this value, O₂ consumption rate decreased linearly with O₂ concentration. Henig and Gilbert (1975) also claimed that when CO₂ accumulation occurred concomitantly with O₂ reduction, there was a significant but surprisingly low reduction in the O₂ consumption rate. They therefore suggested that two straight lines could be used to approximate the relation between O₂ consumption rate and O₂ concentration (Also see Chap. 2.)

This model was dismissed by Cameron et al. (1989) as approximate and not based directly on O₂ 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:

$$X(O_2) = \frac{KSx_0}{KS + \alpha m} + \frac{\alpha mx_0}{KS + \alpha m} e^{-\frac{(KS + \alpha m)t}{V}} \tag{5.1}$$

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

$$\frac{1}{O_2(EMA)} = \frac{\alpha m}{KSx_0} + \frac{1}{x_0} \tag{5.2}$$

where

X = concentration O₂ (%) at time t

x_0 = initial concentration of O₂

K = O₂ diffusivity through the film

S = surface area of the film

V = headspace

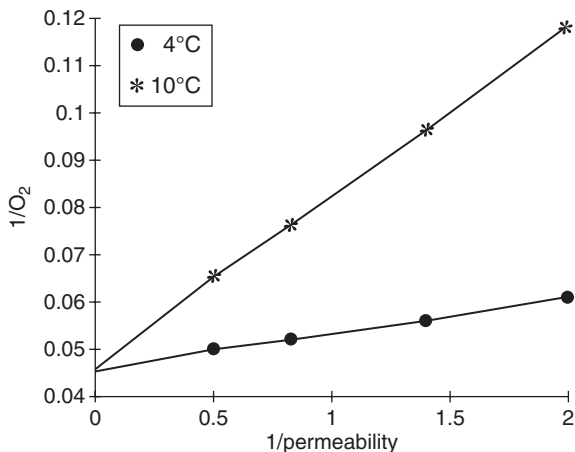
m = weight of plant tissue

t = storage time

α = proportionality between respiration intensity and O₂ concentration

The approximate Eq. (5.1) is not useful in practice, but as time passes, the O₂ 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 ($ml\ O_2/m^{-2} \cdot atm^{-1} \cdot day^{-1}$). 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 O₂ in a storage atmosphere reduces the reaction rate of enzyme-catalyzed oxidations because O₂ is a substrate (Murr and Morris 1974). Polyphenoloxidase and tyrosinase, the enzymes responsible for brown discoloration of plant tissue, have a low affinity for O₂ 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% O₂ and 5–6% CO₂. Color change in broccoli florets is minimal in a 2–3% O₂, 2–3% CO₂ 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 O₂ cannot be separated from the effect of increased CO₂.

CO₂ may inhibit polyphenoloxidase activity (Murr and Morris 1974), but the direct inhibition of this enzyme was not fully demonstrated. Another important effect of CO₂ is increased acidity in plant tissues. Because intracellular pH values are normally regulated within narrow limits, only elevated CO₂ concentrations (as high as 5%) will lower intracellular pH. Bown (1985) proposed that the accumulation of respiratory CO₂ is responsible for the reduction in pH, as dissolved CO₂ diffuses slowly compared to gaseous CO₂. Bertola et al. (1990) determined that the specific resistance to CO₂ 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 CO₂ 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 CO₂ concentrations. Lettuce exposed to air at 20 °C and then stored for 6 days at 0 °C with 16% CO₂ 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 CO₂ 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 CO₂. 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; Loughheed 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 CO₂ levels (over 30%) and low O₂ 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 CO₂ content increased from 10% to 40%, regardless of O₂ 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 O₂ content (Lucey and Condon 1986) or by high CO₂ concentrations (Fig. 5.15). Storing grated carrots in a CO₂-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

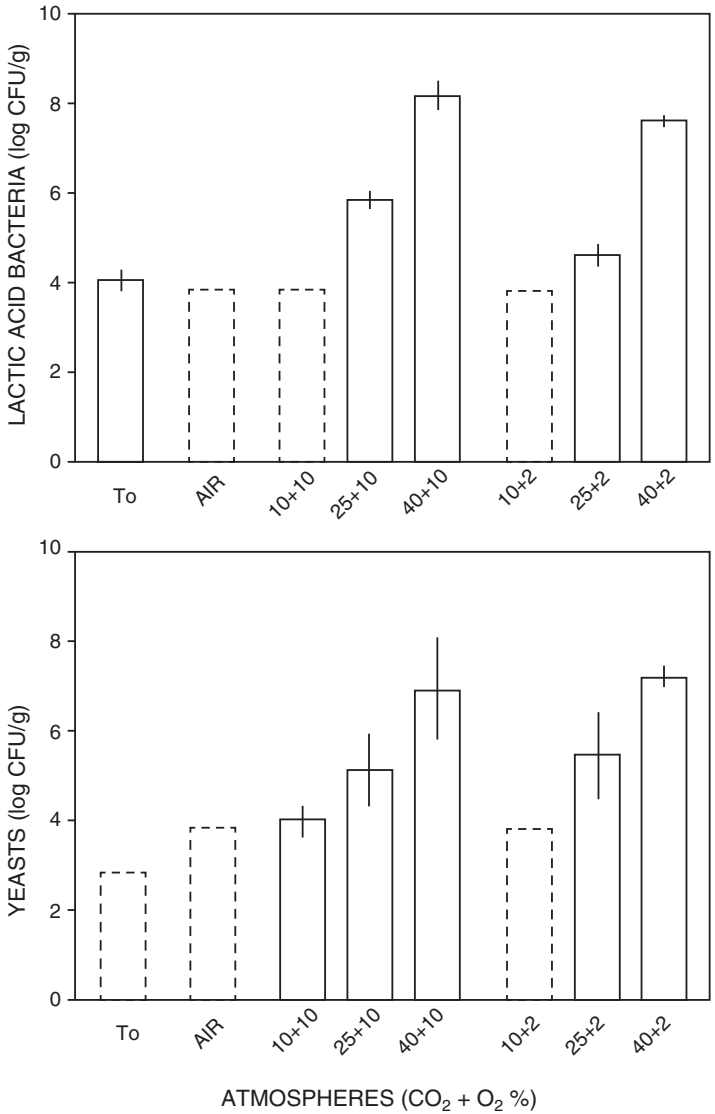


Fig. 5.14 Counts of lactic acid bacteria and yeasts on grated carrots under controlled atmospheres after 10 days of storage at 10 °C. Counts in *dashed lines* were below the detection level, comparing to initial count. *Bars* represent standard deviation (From Carlin et al. (1990b))

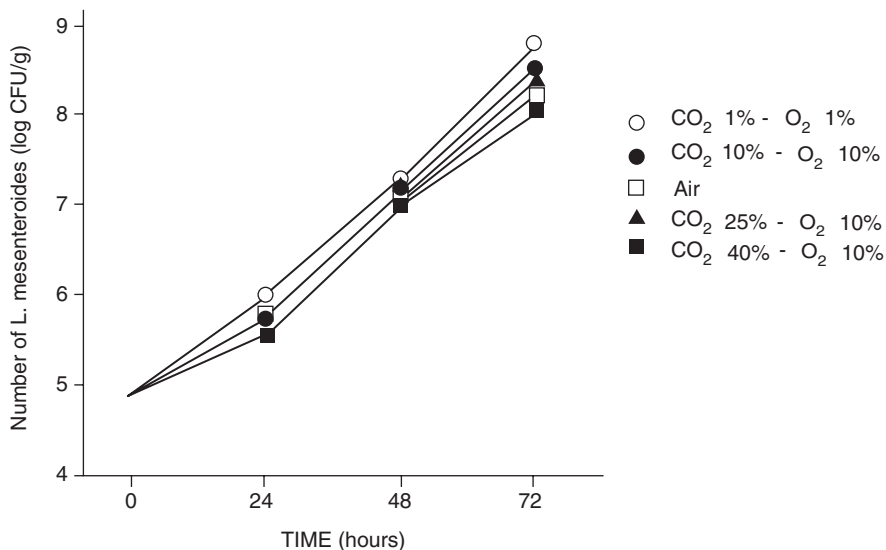


Fig. 5.15 Growth at 10 °C of *Leuconostoc mesenteroides* on a sterile carrot medium under controlled atmospheres (From Carlin et al. (1990c))

under various CAs. They found that O₂ in the CA did not affect leakage, but CO₂ 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% CO₂ and 10% O₂ 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% CO₂, 2% O₂, and in 25% CO₂ and 10% O₂ than in air or in other CAs (Fig. 5.16).

Thus, MAs containing 15–20% CO₂ and 5% O₂ 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 O₂ and CO₂ 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 O₂ < 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)

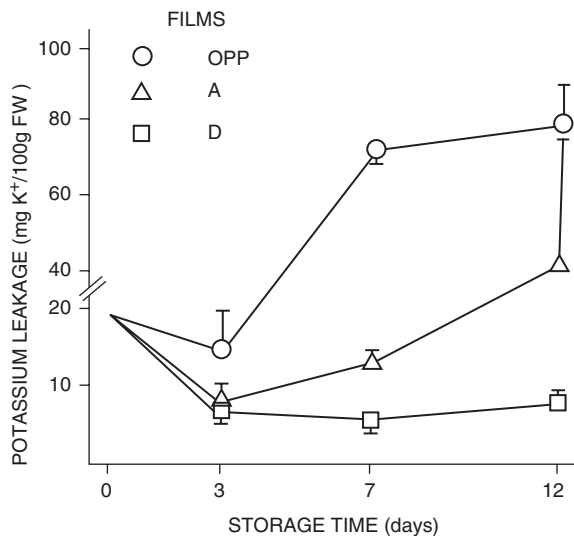


Table 5.2 Effect of film permeability on the quality of minimally processed grated carrots stored at 10 °C

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 O₂ 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		H ₂ O (control)	Inoculum		
% CO ₂	% O ₂		<i>Ps. m</i> (heavy)	Filtrate <i>Ps. m</i>	Filtrate <i>A. niger</i>
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 CO₂ 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% CO₂ 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 CO₂ to 20% reduces the necrosis, and at 40% it prevents any damage.

It is remarkable that MA, with high CO₂ 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 CO₂ on soft rot induced by *P. marginalis* are due to the acidification of cell medium provoked by dissolved CO₂ (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 CO₂ and 1–3% of O₂ 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.

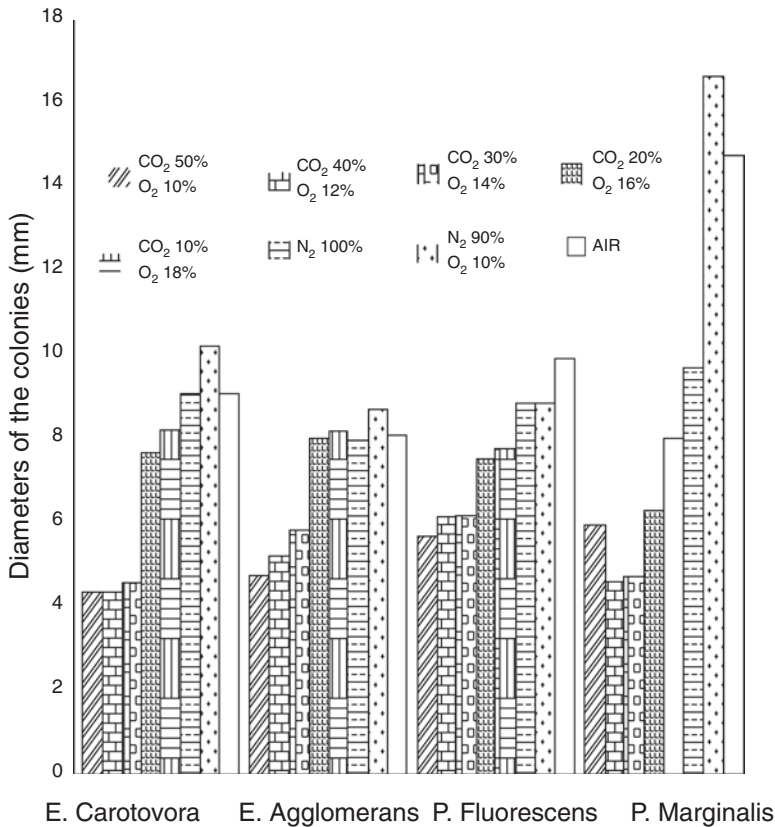


Fig. 5.17 Effect of CO₂ on the in vitro growth of bacteria isolated from minimally processed endive. The percentage of CO₂ varies from 10% to 50% (Colony diameter in mm on LPGA after a 9-day incubation at 10 °C) (From NGuyen-The and Prunier 1989)

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

CO₂ and decreasing O₂ 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 O₂ and CO₂ 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 O₂ and CO₂ 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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