Abdul Malik · Zerrin Erginkaya
Hüseyin Erten *Editors*

Health and Safety Aspects of Food Processing Technologies

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Preface

Food processing techniques date back to prehistoric times, when raw processing techniques included slaughtering, drying, fermentation, preserving with salt, and various types of cooking (such as smoking, roasting, oven baking, and steaming). Food processing can be done at home, by the community in groups, or at a commercial level comprising a higher level of sophistication and investment. The aim of food processing is to ensure microbiological and chemical safety of foods, adequate nutrient content and bioavailability, as well as the acceptability of consumers and caregivers with respect to sensory properties and ease of preparation. The processing can have positive or negative effects on these different properties of food, so each of these factors must be taken into account in the design and preparation of complementary foods.

Nutrient-rich foods, their availability, and food safety are the areas which are becoming more and more worrisome all over the world due to their direct impact on human health. World demand for food depends on processed foods, as processing is expected to affect nutrient content, activity, and bioavailability. Traditional technologies, such as the usage of antimicrobials and thermal processing, increase the nutritional value to some extent, except for the availability of some methods to maintain bioactivity in food. However, they may not be the most effective in improving food safety and in maintaining the structure of food and ingredients properties, in particular its molecular structure. Modern food processing companies also ameliorate the quality of life of people with diabetes and allergies and others who cannot eat some common food ingredients. Processed foods generally are less susceptible to premature deterioration than fresh foods and are more suitable for long-distance transportation from the source to the consumer.

Processed foods often contain food additives, such as flavorings and texture-improving agents, which may have little or no nutritional value or are detrimental to health. Preservatives added or manufactured during processing to extend the shelf life of commercially available products, such as nitrites or sulfites, can have detrimental effects on human health (mutagenic and carcinogenic in humans). It has been shown that the use of inexpensive ingredients that mimic the attributes of natural ingredients (e.g., cheap vegetable oils chemically hardened instead of expensive

natural saturated fats or cold-pressed oils) causes serious health problems. The ingredients for processed foods are often produced in large quantities and are widely distributed among high-value-added food manufacturers; non-compliance with hygiene standards in low-level manufacturing facilities where a common base ingredient is produced can have serious consequences for many final products.

This book offers a unique dealing with the subject; provides not only an update of state-of-the-art techniques in food processing and preservation (i.e, dairy, meat, cereal, vegetables, fruit and juice processing, etc.) but also the health and safety aspects, food technologies in improving the nutritional quality of foods, functional foods, and nanotechnology in food and agriculture industry; and looks into the future by defining current bottlenecks and future research goals. This work will serve as a ready reference of the current subject matter to students and researchers alike.

This book is not intended to serve as an encyclopedic review of the subject. However, the various chapters incorporate both theoretical and practical aspects and may serve as baseline information for future research through which significant development is possible.

The book has 23 chapters with each focusing on a specific topic to cover diverse perspectives. Chapter 1 gives an overview of the management of food safety and hygiene. Other chapters of the book discuss food contamination and food spoilage; microbial escalation in meat and meat products and its consequences; natural microflora of different types of foods; emerging technologies in cereal processing and dairy processing; insect pest infestation in the field and during storage of fruits and vegetables, cereal grains, pulses, and oilseeds; studies on healthy nutrients changing in fruit juices processed with nonthermal technologies; industrial use of compounds from by-products of fruits and vegetables; phytochemicals of whole grains and effects on health; chemical hazards in foods; risk management of chemical hazards arising during food manufacturing; assessment of the risk of probiotics in terms of food safety and human health; beneficial bacterial biofilms in food industry; next-generation probiotics and their molecular taxonomy and health benefits; continuing controversies regarding human health concerns from nitrite and nitrate consumption in the diet; risk of vancomycin-resistant enterococci infections from food industry; new concept in packaging, milk protein edible films; food nanotechnology, an emerging technology in food processing and preservation; nanoparticles in food packaging, opportunities and challenges; ultrasound, food processing and preservation aid; and a natural way of food preservation, bacteriocins and applications.

With great pleasure, we extend our sincere thanks to all our well-qualified and internationally renowned contributors for providing the important, authoritative, and cutting-edge scientific information/technology to make this book a reality. All chapters are well illustrated with appropriately placed tables and figures and enriched with up-to-date information. We are also thankful to the reviewers who carefully and timely reviewed the manuscript.

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We express sincere thanks to our family members for all the support they provided and regret the neglect and loss they suffered during the preparation of this book.

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Management of Food Safety and Hygiene: An Overview

Farhana Masood, Zarreena Siddiqui, Saghir Ahmad, and Abdul Malik

Abstract As all human need food, its safety and nutritional quality is important. According to Codex Alimentarius Commission (CAC), Food hygiene is defined as "all conditions and measures parameter of quality to ensure the safety and suitability of food at all stages of the food chain." Food hygiene includes 2 key points, (i) food safety and (ii) food suitability. CAC defined food safety as "the assurance that food will not cause harm to the consumer, when it is prepared and eaten according to its intended use", while food suitability is defined as "assurance that food is acceptable for human consumption according to its intended use". The fundamental part of any food operation is food safety. In the present era, food safety assurance is different because plethora of chemical, physical and biological agents can contaminate the food source and pose a threat not only to human health but also food business. Today there is sufficient technical and scientific knowledge, and managerial knowledge for ensuring the products safety. Presently concepts like Hazard Analysis and Critical Control Point and risk analysis are developed and incorporated in the food safety management and hygiene both nationally and internationally. In this chapter significance of food hygiene, food safety and various approaches for their management has been reviewed.

Keywords Food safety · Food hygiene · Risk analysis · Risk management · HACCP

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Introduction

The Codex Alimentarius Commission (CAC) is an international organization that sets food standards. According to CAC, the term food hygiene is defined as "all conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain." Food hygiene include 2 key concepts: (i) food safety, and (ii) food suitability (CAC 2009). According to the CAC, food safety is "the assurance that food will not cause harm to the consumer, when prepared and eaten according to its intended use", while the food suitability is "the assurance that food is acceptable for human consumption according to its intended use." Food suitability include quality concepts like:

- Lack of spoilage (microbial or chemical degradation);
- Lack of foreign particles (e.g., hair, insects);
- Authenticity of food (comprising sufficient information about the food and assurance that it is unadulterated); and
- Religious and cultural acceptance.

There was major change in 1990s in the management of food safety and suitability of food. The approach of controlling food hygiene has been shifted from traditional hygienic and empirical methods to scientifically sound risk analysis and control measures. Consequently, resulted in further development of the Hazard Analysis and Critical Control Point (HACCP) system as a method of management of food safety in production of food. In addition, the WTO referred to the standards, practices and other recommendations of the CAC as an international agreement on the health and safety requirements for food. Countries that refused food that met the Codex standards for food safety had to provide scientific evidence that the food in question posed a particular risk to their populations. All the developments at international level and increased public consciousness have led to many changes in food safety management. Implementation and traceability of the HACCP approach have attracted increased consideration and have become mandatory in some countries. Risk analysis developed as a decision making process at government level. In some countries or regions, this has resulted in rearrangement of state institutions.

Basic Principles of Food Hygiene

The implementation of codes of good hygiene practice is the first line of defense. These are general procedures, rules, and practices established on the basis of past experience. To ensure food safety, food suitability and completeness of food these rules offer guidance on general and necessary practices (Holah et al. 2011, 2012; Holah 2014). The important characteristic of these codes is that they are basic requirements and, with some adjustments, they may apply to all groups of food and products, irrespective of location, precise conditions and business types. Depending

on the food chain sector, they are referred to as Codes of Agriculture Practice, Codes of Food Manufacturing Practice, Codes of Good Transport or Storage Practice, etc. Large number of such codes of practice have been developed by the CAC. Such as the recommended International Code of Practice: General Principles of Food Hygiene is commonly used code in the food industry (CAC 2001). For certain food classes some product specific codes are also there, which provide guidance for a specific product group. To ensure both food safety and food suitability these basic measures are called as "prerequisites"; their implementation before applying HACCP is fundamental.

Concepts of Food Safety

Food safety is "the certainty that food will not harm the consumer when it is being prepared/or eaten according to its intended use" (Motarjemi et al. 2014). Food safety contains various significant concepts:

1. The concept of harm, that distinguishes food safety aspects from other aspects of quality that make food unsuitable for human consumption without harming one's health.
2. The assurance concept indicates that food safety must be based on preventive measures. Food safety should be adjudged by the situations under which food is produced and prepared, rather than end-product testing results which is not reliable for many contaminants, especially biological hazards. For biological hazards end-product testing is not a reliable food safety process as it often requires an impracticable number of product tests to achieve a statistical level of safety, when these agents are present in a low number, they are undetectable.
3. The preparation or usage of a food product should be taken into account when designing its safety. "A food product is safe as long as it is prepared and/or used according to its intended use". Therefore, the intended use must be taken into account by the manufacturers, or those selling the product, as well as on the label or warning given to the consumers.

The Control of Food Safety

If hazards are identified in a particular food product or contamination pathways are known, measures to mitigate the risk is to consider the route of contamination. The first step is the control of the basic requirements: the application of Good Hygienic Practice (GHP) and Good Manufacturing Practice (GMP). Second, HACCP system should be used. HACCP first identifies potential hazards prior determining where to control these hazards (critical control points, CCPs,) and what limits should be set. By setting limits; HACCP is still not completed. The performance of the HACCP

system depends on how should these limits be monitored, what measures to be considered during exceeded limits, and the verification of desired measurements. At international level ISO published ISO 15161:2000 (based on ISO 9001), which deals with quality management, rather than food safety. Further in 2005 the ISO published another standard, ISO 22000, which incorporates HACCP into ISO 9001.

Quantitative Risk Analysis (QRA) helps to set limits. Another important aspect is that, in addition to setting up the system, procedures are strictly adhered to, even if changes are required. Certification and the introduction of ISO systems can help in assurance of quality. In addition, it is essential that the staff must be well trained to avoid mistakes. Therefore, further education is a relevant aspect. Risks can be controlled with these structured systems, but a zero risk cannot be achieved. International organizations are increasingly moving towards quantitative risk analysis and assessment.

HACCP System

Before applying the HACCP system in any part of the food chain, this part must have pre-existing programs, e.g., Good hygienic practices in accordance with the Codex General Principles of Food Hygiene, Codex Code of Practice, and adequate food safety requirements. For successful implementation and deployment of the HACCP system, these prerequisites for HACCP must be fully operational and verified. For HACCP system to be efficient all kind of food business activities require responsiveness and management's responsibility. Efficiency also depend on management staff with suitable HACCP information and skills.

It is a system that identifies, assesses and control hazards that are important to food safety. The system checks each step in the food business, identifies the particular hazards and carries out effective control actions and verification methods. It is planned for minimizing risk and thus it is a tool of risk management. The HACCP has distinctive features: it is organized and focuses on scientific risk statements, recognizes specific hazards and responses to them, and focuses on prevention. It is able to take changes into account (e.g., advanced equipment design, technological development). HACCP system can be used throughout the food chain "from farm to the fork". HACCP entails a multidisciplinary approach.

Application of the HACCP system is the second line of defense. It is a scientific, coherent and practical approach to identify, assess and control hazards that are important for safety of product. HACCP system is different from basic good hygiene practice in that HACCP targets certain hazards that are specific for product or process. HACCP system covers seven principles (Fig. 1) as follows:

1. "Identify any hazards that must be prevented, eliminated, or reduced to acceptable levels".
2. "Identify the critical control points (CCPs) at the step or steps at which control is essential to prevent or eliminate a hazard or to reduce it to acceptable levels".

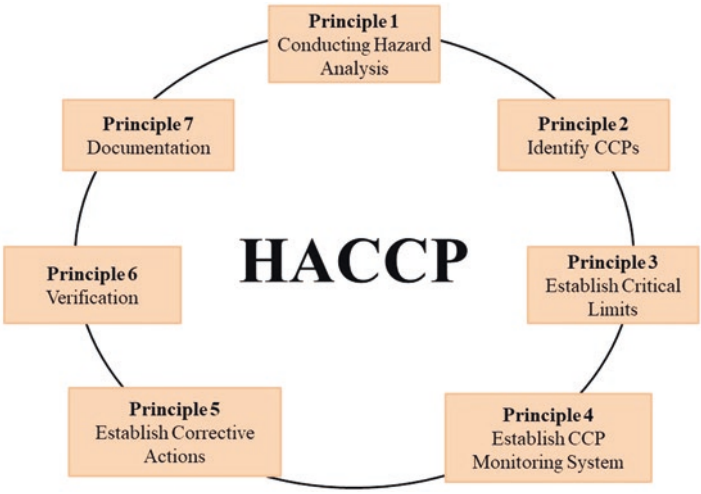

Fig. 1 HACCP basic principles

3. "Establish critical limits at CCPs which separate acceptability from unacceptability for the prevention, elimination or reduction of identified hazards".
4. "Establish and implement effective monitoring procedures at CCPs".
5. "Establish corrective actions when monitoring indicates that a CCPs is not under control".
6. "Establish procedures, which shall be carried out regularly, to verify that the above measures are working effectively".
7. "Establish documents and records commensurate with the nature and size of the food business to demonstrate the effective application of the above measures".

In HACCP research, at each stage of food business, from raw materials to consumption, product and/or specific risk handling and related control measures are defined (Motarjemi 2014). HACCP plan is developed when hazards are considered significant. For this purpose, steps are defined that are considered critical to ensure product safety and therefore need to be monitored. These are called "CCPs". Identifying parameters is crucial for each CCP which identify control actions and set control limits. It is the basic criteria that differentiates acceptance from non-acceptance. Corrective measures are also planned during the HACCP study. When CL is violated, corrective measures have been employed to prevent contaminated product from reaching consumers.

Food Safety and Hygiene Management

Determining responsibility is important to managing any problem. For maintaining consumer confidence in food supply determining and coordinating roles and responsibilities for food hygiene and safety management is crucial. In 1990s WHO created

the concept of shared responsibility. A combined effort is needed from different sectors (government, academics, industries, consumers) for ensuring supply of food is safe, hygienic and balanced nutritionally. Still today the concept of shared responsibility is valid. Recently, NGOs are also involved in promoting progress by asking questions about guidelines and practices. Such organizations and associations, are important forces in opposition to economic power.

Today, decisions on the necessary measures for managing risks are made in accordance with the risk analysis process. Different models are there to illustrate the process of risk analysis. This process comprises of: risk assessment, risk management and risk communication. The risk managers are the regulatory authorities. Their responsibility is (i) promoting the risk analysis process, (ii) establishing health objectives, and (iii) determining risk management priorities.

Risk assessment and risk management process include repeated collaboration between risk managers and risk assessors for adapting mutual understanding and improve assessment of risk. For deciding suitable control actions, risk managers must consider several other factors called as “other legitimate factors”. Based on nature of hazard in question, these factors vary and may consist of costs, viability, benefits, other risks (for example, nutritional or environmental), consumer choices and social values. Sometimes, a risk assessment is mandatory for advising the effectiveness of control measures, and for developing an understanding of public health outcomes rendering to different levels of pollution, to estimate risks of different foods.

In risk management, based on the type and extent of risk and other factors discussed, risk managers have different options available. These options include from making a specified action, such as the actions listed below. Some instances of control measures are:

- Comply with certain standards (setting a standard for chemical hazards or food safety goals or microbiological criteria for microbiological hazards);
- Labeling;

  Food testing and/or certification;

- Inactivating pathogens using particular food processing;
- Apply code;
- Recover products in case of incidents.

For identifying the potential food safety issues and considering the application of risk management conclusions and assess the need to review decisions or implement, the assortment of other types of data each other should be considered.

Some examples:

- Inspection report and assessment of risk management implementation;
- Chemical contaminants monitoring;
- Investigate food-borne diseases;
- Consumer complaints;
- Denial of trade;

- Public withdrawals and/or public incidents;
- Application research based on identified indicators (knowledge, gaps).

In India, food safety issues are gaining importance because of increasing demand of customers for higher food quality. The Food Safety Standards Authority of India (FSSAI) was established under the Food Safety and Standards Act, 2006. FSSAI was established to develop scientific standards for food and regulate producers. Food export, warehousing, distribution channels, domestic sales, import and export, safe and suitable food for people. The acts such as Vegetable Oil Products (Control) Order 1947, Prevention of Food Adulteration Act 1954, Fruit Products Order 1955, Meat Food Products Order 1973, Edible Oils Packaging Regulation 1988 replaced by Food Safety and Standards Act, 2006. The main objective is providing a common reference point for all issues related to food safety and standards.

Food Hygiene Outlook

Risk analysis and HACCP concepts are well established today and implemented into food safety and hygiene management both nationally and internationally. Codes of Good Practices are more robust. Analytical and laboratory approaches are evolved considerably. Communicating with consumers and incorporating their suggestions for making decision is a modern way of government working. In recent years one of the most important development is recognizing the necessity for combined approach for food hygiene and food safety, in particular taking into account industrial practices. Thereafter, private standards, such as ISO 22000, were developed for better and more efficiently control of food businesses. With reference to ISO 22000, prerequisite programmes (PRP) are defined as "the basic conditions and activities necessary to maintain a hygienic environment throughout the food chain suitable for the production, handling and provision of safe end products and food that is safe for human consumption". The management of food safety and hygiene in the food chain has developed but still there are some challenges remaining:

1. Quantitative risk assessment as the foundation for decision-making is still developing. New concepts have been developed, such as food safety targets and performance targets, but in some cases they have not yet well known.
2. Food industry still has difficulty in introducing management systems like HACCP, also because food businesses, big and small, each with its own complexity. The planned use of HACCP encounters enormous difficulties. This is partly due to the time consuming nature of HACCP and many companies' administration does not give the required expertise, time investment and/or human resources.
3. Basic hygiene in some facilities is inferior. A worrying concern is the operation of food services in developing countries. This poses a threat to local people as well as to travelers and international trade.

4. Increased fraud and counterfeiting of food has increased. The unpredictability of such events makes it difficult to prevent and manage them.

Conclusions

Food nutritional quality and safety has a major role in human health. It is not carefully taken as a part of process of food production management. Many biological, physical and chemical substances can contaminate food supply and may endanger human health and food companies. Food safety assurance therefore is the backbone of the food business and consumer confidence. There is now enough scientific and technical knowledge, technological tools and managerial experience for ensuring product safety. It is essential for consumer confidence to have an independent and skilled monitoring basis and oversight by food business supervisors. In order to ensure this, it is necessary for governing authorities to look after the top management of food companies so that they should adhere to their guidelines and investigate managerial failures.

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Food Spoilage and Food Contamination

Z. R. Azaz Ahmad Azad, Mohd. Fahim Ahmad, and Waseem Ahmad Siddiqui

Abstract The interaction of versatile nutrients and enzymes present in food leads to several degradative chemical changes that deteriorate the quality and shelf life of food. The deteriorative changes are enhanced by contamination that occurs at various stages of processing, handling and storage. The undesirable changes include lipid oxidation, enzymatic or non-enzymatic browning, putrefaction and toxicity due to hazardous substances. Contamination occurs from various physical, chemical and biological sources and is affected by external factors such as temperature, poor hygiene and sanitation. The intrinsic factors such as pH, redox potential, water content and the presence of antimicrobial substances in food also affect the degree of contamination and thereby the spoilage. Most of the contaminants occur from natural sources but some are added as a result of human activities. Contamination leads to spoilage of food due to the microorganisms, enzymes, chemical reactions (harmful additives, mycotoxins, bacterial toxins and radiations) and physical changes (caused by freezing, burning, drying, pressure). Spoilage changes the nitrogenous organic compounds in food into alpha-keto acids, ammonia, propionic acid, amides, imides, and urea. The organic acids are oxidized to carbonates causing the medium to become more alkaline and the consumption of contaminated and spoiled food can lead to various food borne illnesses and intoxication. Therefore, it is necessary to implement and maintain proper food hygiene during processing and storage. The various techniques such as biosensors, apt sensors and spectroscopy with chemometrics analysis for the quantitative assessment of food spoilage may be incorporated. The chapter focused on different aspects of food sanitation, microbiology, contamination, personal hygiene, spoilage and the effective measures to increase the shelf-life of the food and food products.

Keywords Contamination · Spoilage · Sanitation · Food safety · Adulteration

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Introduction

Foods are essential and highly complex materials. The properties of constituents and their handling during processing determine the quality and shelf-life of foods. Over the last few decades, the food industry has grown tremendously. The sanitary and hygienic practices have also changed and now become more complex (Banwart 1979). The processing, preparation and preservation of food depends on a more mechanized and large-scale process.

The issue of food scarcity is of higher importance to combat the hunger, improve food security and income of the world's poorest as well as underdeveloped countries. Food losses or wastage have a negative impact on food security, food quality and safety, economic development and on the environment (Bryan 1979). Food losses may be affected by crop production choices and pattern, internal infrastructure and capacity, marketing chains and channels for distribution, and consumer purchasing and food use practices. To ensure the availability of good quality and appropriate quantity of food to every inhabitant of this planet, it is necessary to reduce the postharvest losses. Recently worldwide postharvest vegetables and fruit losses are higher than 30–40% and even much higher in developing countries. The prospects are also that the world population will grow from 5.7 billion inhabitants in 1995 to 8.3 billion in 2025. World production of vegetables amounted to 486 million ton, while that of fruits reached 392 million ton. Reduction of post-harvest losses reduces cost of production, trade and distribution, lowers the price for the consumer and increases the farmer's income. India is the major producer of fruits and vegetables and stands next to Brazil and China in the world. That means it contributes 10% of world fruit production and 14% of total world vegetable production. According to the India Agricultural Research Data Book 2004, the losses in fruits and vegetables are to the tune of 30%.

Foods may be contaminated due to biological contaminations, physical contamination, chemical contamination and cross-contamination. Food deterioration is caused by the growth of microorganisms, the chemical reaction with their environment, or the presence of foreign bodies in foods. Food spoilage is a complex process and even with modern conservation and preservation techniques, large amount of food is spoiled due to microbes. Despite the heterogeneity of raw materials and processing conditions, the microbial flora produced during food storage and deterioration may be based on source, matrices and conservation parameters. Often foods contain a variety of organisms, most of them being saprophytes. Their existence is inevitable because they mainly come from the environment in which food is prepared or processed and it is difficult to eliminate them completely (Fields 1979; Marriott 1994; Frazier and Westoff 1996; Berthiller et al. 2013). However, by changing the environmental conditions, their numbers or activity can be reduced. Therefore, understanding the factors that promote or inhibit their growth is essential to understand the principles of food preservation. Most foods are an excellent medium for the rapid growth of microorganisms for being rich in organic matter, sufficient water content with a neutral or slightly acidic pH (Felix 1992) (Fig. 1).

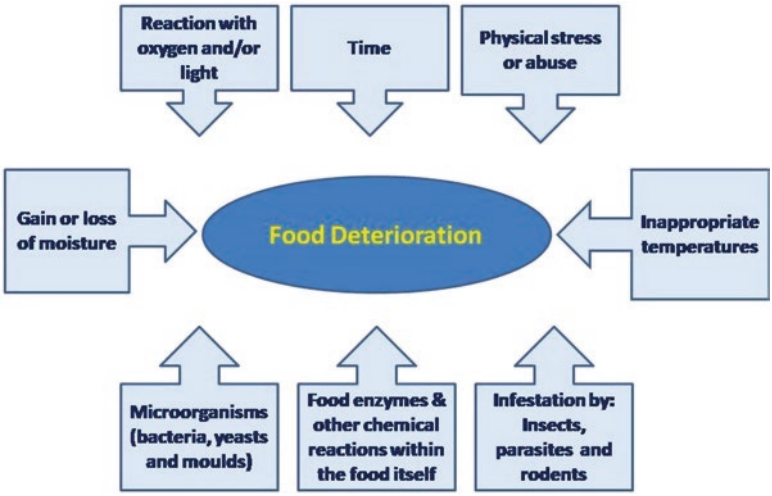


Fig. 1 Factors causing food deterioration

Foods consumed by humans and animals are ideal ecosystems where bacteria and fungi can multiply. The presence of micro-organisms in foods in small numbers may not be harmful, but their unrestricted growth makes food unfit for consumption due to damage or degradation. Many foodborne microbes are found in healthy animals (in the intestine) that are consumed as food. Meat and poultry carcasses can be contaminated during slaughter by human touch or unsanitary conditions (Niven Jr 1987). Salmonella species can infect chicken ovary so that the internal contents of normal eggs can be contaminated with the bacterium (Lechowich 1980). The shellfish and oysters can concentrate Vibrio bacteria that are found naturally in seawater. Similarly, fresh fruits and vegetables may be contaminated if they are washed or irrigated with water contaminated with animal manure or human sewage (Fig. 2).

At a later stage in food processing, food-laden microbes can be introduced from an infected person or by mutual contamination from other raw agricultural products. For example, *Shigella*, Hepatitis A virus (HAV), and Norwalk virus can be introduced from unwashed hands of infected food connoisseur themselves. In the kitchen, microbes can move from one food to another using the same knife, kitchen/cutting board or other tools to prepare food without washing the cutting surface or cooking pots. Even fully cooked food can be infected if raw or cold food is touched or mixed that contain microbial pathogens. Bacteria need to multiply in large numbers in food to cause disease. Due to the warm and wet conditions and adequate supply of nutrients, the bacteria multiply every half an hour and produce millions of strains within 12 h. As a result, the food can be contaminated overnight and become highly contagious the next day. If food is cooled, the bacteria multiply at a slower rate. In general, freezing prevents bacteria from growing and multiplying and generally maintains them in the state of suspended animation. However, *Listeria monocytogenes* and *Yersinia enterocolitica* can actually grow at refrigeration temperatures.

Fig. 2 Spoiled food and food products

Classification on the Basis of Stability of Foods

Foods are often classified based on being non-perishable, semi-perishable or perishable. The heat-treated and canned foods are often listed as non-perishable. However, canned food may become susceptible to damage under certain circumstances, when chance exists to re-pollinate due to damaged cans, rust or others so that it is not tightly closed. The semi-perishable foods are usually dry goods, such as flour, legumes, hard cheese, dried fruits, vegetables, and even tarpaulins. Most of the food items fall in the category of perishable. The perishable foods include fruits and vegetables, eggs and poultry, meat and fish, in addition to all cooked food. Frozen foods, although primarily perishable, can be classified as semi-perishable provided they are stored properly. However, it is important to note that almost any type of food will spoil if it is wet and unfrozen and the damage would occur faster at warmer temperature (Table 1).

Table 1 Spoiled food and food products

Non-perishable	Semi-perishable	Perishable
• Stay good for a year or more without freezing or refrigeration. • Examples: Sugar, legumes, cereals, oils, pickles, etc. • Storage in a cool and dry place is however, necessary	• Stay good for weeks to months, if stored properly. • Examples: Flours, fruits such as apples, vegetables such as potatoes and onions, frozen foods, etc.	• Spoil within a few days. • Examples: Milk, eggs, meat, fish, poultry, and most fruits and vegetables, especially green leafy vegetables.

Food Spoilage

Food spoilage may be defined as deterioration of food to the point in which it is not edible to human or its quality of edibility reduces. Such changes can be detected by smell, taste, touch or sight. These changes can be caused due to air and oxygen, moisture, light, temperature, chemical and biological means. The latter includes the action of enzymes, microorganisms, insects and contamination by Trichinella (Moral et al. 2017; Manisha 2018). It is estimated that spoilage due to microorganisms alone causes the loss of almost one quarter of the world's food supply. Many foods may not be degraded but contain certain types of bacteria or toxins that make it unfit for human consumption.

The criteria of a particular food suitable for human consumption are:

(a) It should be in the desired condition and maturity.
(b) It should be free from contamination throughout production and handling procedures.
(c) It should be free from chemical and physical changes caused due to pressure, freezing, heating, drying, etc. during processing, handling and storage, and because of the action of enzymes, microorganisms, parasites, insects and rodents.

Types of Spoilage

The food may become unsuitable or unacceptable for human consumption due to the following reasons:

(a) Growth of micro-organisms such as bacteria, yeasts and molds; the most common and most important cause of food spoilage.
(b) Activity of enzymes within the food (e.g., enzymatic browning).
(c) Infestation of pests such as insects and rodents.
(d) Non-enzymatic chemical changes in the food e.g., chemical oxidation of fats producing rancidity and browning due to Maillard reaction.
(e) Physical damage caused by drying (caking), freezing (freezer burn), etc.

Microorganisms and Food

The microbial damage to food is the beginning of a complex natural process of degradation that recycles the elements in animal or plant tissues into the environment. Microorganisms that may cause food damage include molds, yeast and bacteria. The general sources of food spoilage microorganisms are the air, soil, sewage, and animal wastes. Pollution with molds is, as a general rule, easily detected by the presence of fictitious strands or string-like structures which, in many cases, are colored. They often contribute a musty smell and flavor to the food in which they are found. However, some molds, are not completely harmful. Semi-humid or low-water foods are partially dry and the water is sufficient to maintain bacterial growth which is ideal for pollination with mold and yeast. Yeasts are small single-celled organisms which multiply by budding. In general, sugars are the best source of energy for yeast and carbon dioxide and alcohol are the end products of fermentation. Yeast damage is usually identified by bubbles, aroma and alcoholic taste (Pretorius 2000). Bacteria destroy food in many ways and it is not always possible to identify damage through sight, smell or taste. Unfortunately, some bacteria that cause suffering from a health point of view may multiply without altering the appearance, smell or taste of food (Nesse and Williams 2012). Microorganisms may include the original flora of food, as well as the added contaminants during handling, processing, transport, storage, preparation and presentation.

Microorganisms Present in Food

(a) **Bacteria**: Being prokaryotes, bacteria are much smaller than the eukaryotic microbes like yeasts. They come in different sizes and shapes and usually possess a cell wall. Bacteria can bring about a variety of changes in the food, most of which may be undesirable. The changes include pigment production, lysis of complex nutrients, production of foul smelling volatile substances and production of toxins. Many bacteria can form endospores which may survive harsh treatments like chemicals, radiation and heat.

(b) **Yeasts**: Yeast cells are larger in size and grow at a slower rate than bacteria. Most of the yeasts prefer high water activity (a_w) and mild acidic conditions. Yeasts are more likely to grow in packaged, low-pH foods such as fruit juices. Growth of yeast may make the foods smelly, feel slippery, and turbid (in case of clear juices).

(c) **Molds**: molds may have different colors but usually have cotton-like texture and appearance. They are spread as tiny spores that germinate wherever nutrient source is available. Due to the formation of spores, molds can survive wide ranges of temperature and pH. Molds prefer near neutral pH, room temperature and high a_w, but can grow in low or high pH as well as very low a_w conditions. In fact, under low a_w conditions, they can outgrow bacteria and yeasts.

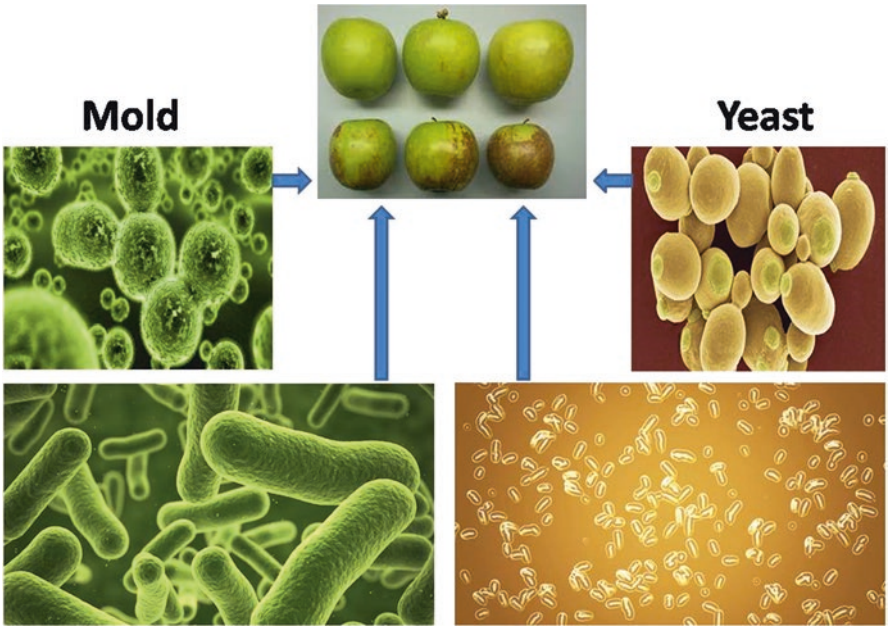


Fig. 3 Microbes causing deterioration in foods

(d) **Viruses**: Although not considered as true living organisms, viruses are important to discuss as they also affect the quality of food. Viruses do not consume or degrade the food nutrients but may be carried by contaminated food to cause various food-borne viral infections (Fig. 3).

Microbial Growth

The mode of reproduction in bacteria is asexual, in which a bacterium divided into two daughter cells, by the process called binary fission. If the number of dividing cells exceeds unity on average, the bacterial population undergoes exponential growth. The measurement of bacterial exponential growth curve in batch culture requires bacterial enumeration (cell counting) by direct and individual (microscopic, flow cytometry), direct and bulk (biomass), indirect and individual (colony counting), or indirect and bulk (most probable number, turbidity, nutrient uptake) methods (Skarstad et al. 1983). The growth of spoilage microorganisms in food depends on many factors e.g., type of organism, ability to extract nutrients from food, competition with other microorganisms, microbial load and environmental conditions. There are four distinct phases of the growth curve of bacteria and other microorganisms:

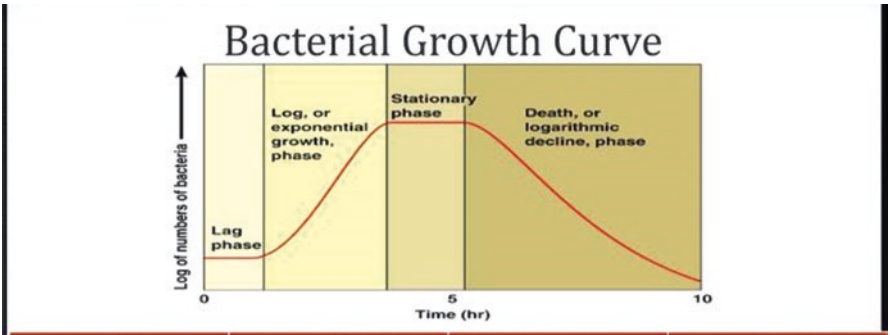
(a) **Lag phase**: This is the phase during which the bacteria adapt themselves to the new environment. During this phase, there is no or little increase in the number of bacteria. During this phase, the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs. The duration of lag phase depends on several factors including the type of bacteria, the initial number and condition of cells, the type and concentration of nutrients in the new environment, temperature, etc. (Robinson et al. 1998). This period of little to no cell division and can last for 1 to several hours. Good hygiene would lower the initial number of bacteria and hence, would increase the lag phase.

(b) **Logarithmic or exponential phase (Log phase)**: During this phase, the bacterial population grows exponentially and doubles at an interval equal to their generation time (or doubling time, the time of bacterial reproduction). If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period. For such type of exponential growth, plotting the natural logarithm of cells against time gives a straight line. The slope of this line is the specific growth rate of the organism, which is a measure of a number of divisions per cell per unit time. This phase may last up to several hours under optimal nutrient conditions. The duration of this phase is also dependent on several factors including availability of nutrients (Rauprich et al. 1996).

(c) **Stationary phase**: This phase is the result of depletion of growth limiting factors such as the essential nutrients, and/or formation of inhibitory products such as organic acid. As the nutrient concentration depletes the bacteria enter the stationary phase. During this phase, the net growth is zero, i.e., growth rate is equal to death rate. As a result, a smooth horizontal linear part of the curve during the stationary phase. However, bacteria still remain metabolically active. Many microorganisms produce secondary metabolites during this stage which affect the chemical composition of the medium in which they are growing. Many of these secondary metabolites are moderately to highly toxic (Ayoola 2007). The duration of this phase may vary from several hours to several days depending on the type of microorganism and the availability of nutrients.

(d) **Death or decline phase**: Once the nutrients further deplete and toxic waste further accumulate, the death phase commences. This phase, at least, in early stage, is also logarithmic. Several factors determine the time of commencement and duration of this phase. Once the population is drastically reduced by the early death phase, a slowdown in the death rate is observed (Fig. 4).

Bacteria need about four hours to adapt to the new environmental conditions before they begin to grow rapidly which means that there are less than four hours to decide whether one has to cool, heat or eat the food. With the growth of microorganisms, they tend to form colonies and the secretions from this large population of cells become toxic. This is the fixed stage in which some cells begin to die. If the growth of bacteria is controlled, then the main cause of food damage is limited (Sallam 2007). The proliferation of perishable organisms depends on the nutrients



Lag phase	Log phase	Stationary phase	Death phase
• Little to no growth • Pop. doesn't increase • Bacteria acclimate to new environment • Intense metabolic activity= growth in size	• Period of exponential growth with constant generation time • Period of most rapid growth • Cells are more susceptible to adverse environmental factors • Example: antibiotics	• Cell growth= cell death, stability • Slow microbial growth influenced by limited nutrients • Low O_2 • Accumulation of toxic waste	• Cell death exceeds cell growth

Fig. 4 Bacterial growth curve (Adapted from https://slideplayer.com/slide/6092622/)

and many other factors—the type of organisms involved, competition from other microorganisms, environmental conditions etc.

Factors Affecting Food Spoilage

Food spoilage and deterioration is a naturally occurring process. The microorganisms grow best at room temperatures (60–90 °F), but most do not grow well at refrigerator or freezer temperatures. The major reason for food spoilage is caused by the increase in the number of microorganisms, utilization of nutrients, causing enzymatic changes results in bad flavors due to breakdown of some food components or synthesis of new compounds. Because of these microbial activities food becomes unfit for human consumption. These microbes can oxidize reduced carbon; nitrogen and sulfur compounds present in dead plants and animals and can contribute the minerals to the biogeochemical cycle. Most foods contain enough nutrients to support microbial growth, promote serial factors, inhibit or limit the growth of microorganisms in food (Zottola 1972). Factors influencing microbial growth are divided into two types—intrinsic and external parameters. Microflora components compete with one another over the available nutrients and fastest

growth under a given set of conditions becomes dominant causing septic symptoms. The component of microorganisms that become dominant is determined by the complex interaction between contaminated microflora components (implicit factors), the storage environment (external factors) and the physico-chemical properties of food (internal factors) (Lemus-Mondaca et al. 2012; Perhar and Arhonditsis 2014). The knowledge of the intrinsic and extrinsic parameters should be able to determine the broad range of organisms which may be present in a particular type of food, for example, whether the food may be destroyed by bacteria, yeast or mold. The foods with high water content and a pH above 5.0 are likely to be destroyed by bacteria because they grow fastest under these conditions (Pelczar et al. 1993). Even if the water activity is high, foods with a pH lower than 4.2 may be destroyed by yeast and mold.

Extrinsic Factors

These are the external environment factors that affect the growth of micro-organisms. Some important extrinsic factors are:

Temperature

Microorganisms can grow over a wide range of temperatures. The environmental temperature not only determines the propagation but also the species of microorganisms that will flourish and the extent their activity. For example, changing the temperature to just a few degrees may favor the growth of a new organism and a different type of food damage and poisoning. The lowest temperature at which microorganism has been reported to grow is −34 °C; the highest is somewhere in excess of 100 °C. But some spore producing bacteria such as *Bacillus stearothermophilus*, *Clostridium tetani* and *Clostridium perfringens* can grow above 100 °C. Based on temperature ranges upon which microbes grow, microorganisms are classified as three groups-

1. Psychrotrophs (cold temperature tolerant microorganisms) or Psychrophiles (cold temperature loving microorganisms) can tolerate temperatures as low as freezing temperatures. These microbes can grow between the temperature ranges of 2 °C to 20–30 °C. The most important psychrotrophs include *Alcaligenes, Shewanella, Brochothrix, Corynebacterium, Flavobacterium, Lactobacillus, Micrococcus, Pectobacterium, Pseudomonas, Psychrobacter, Enterococcus* and others. The psychrotrophs found most common on foods are those that belong to the genera *Pseudomonas* and *Enterococcus.* These organisms grow well at refrigerator temperature and cause spoilage at 5–7 °C of meats, fish, poultry, eggs and other foods normally held at this temperature.

2. Mesophiles (microorganisms that require close to room temperature condition), which grow best between 20 and 45 °C (e.g., *Bactobacilli, Staphylococci*). Mesophilic species and strains are known bacteria among all genera and may be found on food held at refrigerator temperatures.
3. Thermopiles (microorganisms that grow well at high temperatures), which have growth optima at temperatures ranges of 55 °C–65 °C (e.g., *Bacillus, Lactobacillus*). Most important thermophiles in food belong to the genera *Bacillus, Paenibacillus, Clostridium, Geobacillus, Alicyclobacillus,* and *Thermoanaerobacter.*

Like bacteria fungi are also able to grow over wide ranges of temperature. Many molds are able to grow at refrigerator temperatures, especially some strains of *Aspergillus, Cladosporium* and *Thamnidium*, which may be found growing on eggs, sides of beef and fruits (Jay et al. 2005). Yeasts prefer psychrotrophic and mesophilic temperature ranges but generally not within the thermophilic range.

Atmospheric Gases

Several studies showed that the antimicrobial activity of gases at ambient and subambient pressures on microorganisms important in foods (Loss and Hotchkiss 2002). Gases inhibit microorganisms by two ways. (1) They can have direct toxic effect that can inhibit the growth and proliferation. Carbon dioxide (CO_2), ozone (O_3) and oxygen (O_2) are gases that are toxic to certain microbes. It is dependent upon the physical and chemical properties of gases and its interaction with aqueous and lipid phases of foods. Oxidizing radicals generated by O_3 and O_2 are highly toxic to anaerobic bacteria and can having an inhibitory effect on aerobic depending on their concentration. CO_2 is effective against obligate aerobes and at high level can deter other microorganisms. (2) Second inhibitory mechanism is achieved by modifying the gas composition, which has indirect inhibitory effects by altering the ecology of the microbial environment. Atmospheres that have a negative effect on the growth of one particular microorganism may promote the growth of another. This effect may have positive or negative consequences depending upon the native pathogenic micro flora and their substrate. Nitrogen replacement of oxygen is an example of this indirect antimicrobial activity (Moral et al. 2017). As with temperature, the availability of oxygen determines which microorganisms will be active. Some microorganisms have an absolute demand for oxygen, while other organisms grow in the total absence of oxygen. However, other microorganisms can grow either with or without available oxygen. Microorganisms that require free oxygen are called aerobic microorganisms (e.g., *Pseudomonas* sp.). Organisms that grow in the absence of oxygen are called anaerobic microorganisms (e.g., *Clostridium* sp.). Microorganisms that can grow with or without free oxygen are called optional microorganisms (e.g., *Lactobacillus* sp.).

Relative Humidity

The relative humidity (RH) of the storage environment is important extrinsic parameter both from the standard point of a_w within foods and the growth of microorganisms at the surfaces. When food with low aw contents are placed in high RH environments, the foods takes up more moisture until equilibrium has been established. Similarly foods with a high a_w lose moisture when placed in an environment of low RH. There is a relationship between RH and temperature that should be borne in mind in selecting proper storage environment for foods. Generally, if the temperature is high then the RH low and vice versa. All microorganisms have high water requirements to support their growth and activity. High relative humidity can cause moisture condensation on food, equipment, walls and ceilings. Condensation causes wet surfaces, which lead to microbial growth and damage. Also, microbial growth is inhibited by low relative humidity (Wihan 2007). Microbial bacteria require higher relative humidity than different organisms. The optimal relative humidity of bacteria is 92% or higher, while yeast needs 90% or higher, and for molds, the relative humidity should be 85–90%.

Intrinsic Factors

These are the internal factors related to the properties of the substrates (food or debris) that affect the type and growth of microorganisms. The more important intrinsic factors are:

Water Activity (a_w)

Water is an excellent solvent for all life processes in every living organism for biocatalytic activity. The amount of water required varies for different organisms. Water is required by micro-organisms, and a reduction of water availability constitutes a method of food preservation through reduction of microbial proliferation. It is important to recognize that it is not the total amount of moisture present that determines the limit of microbial growth, but the mount of moisture which is readily available for metabolic activity. The unit of measurement for water requirement of microorganism is usually expressed as water activity (a_w). It is defined as the vapor pressure of the solution divided by the vapor pressure of the pure solvent: $a_w = p/p_o$, where p is the vapor pressure of the solution and p_o is the vapor pressure of pure water. The optimal a_w for the growth of micro-organisms is 0.99, and most microbes require an a_w higher than 0.91 for growth. The relationship between relative humidity (RH^W) and a_w is RH = $a_w \times 100$. Therefore an a_w of 0.95 is equivalent to an RH of 95% generally. Pure water has an a_w of 1.00, a 22% NaCl solution (w/v) has an a_w of 0.86, and a saturated solution of NaCl has an a_w of 0.75. The water activity (a_w) of most fresh foods is above 0.99. In general bacteria require more water activity

Table 2 Lowest tolerable a_w values for different types of microorganisms involved in food spoilage

S. No.	Group of microorganisms	Minimal (a_w) value
1.	Bacteria	0.91
2.	Yeasts	0.88
3.	Molds	0.80
4.	Halophilic bacteria	0.75
5.	Xerophilic fungi	0.65
6.	Osmophilic yeasts	0.60

than molds and yeasts. Gram negative bacteria have higher water requirement than gram positive bacteria (Grant 2004). Molds normally have the lowest a_w requirements, with yeasts being intermediate. Most spoilage bacteria do not grow at an a_w below 0.91, but molds and yeasts can grow at an a_w of 0.80 or lower. Molds and yeasts are more likely to grow in partially dehydrated surfaces (including food), whereas bacterial growth is retarded (Table 2).

The effect of lowering a_w below optimum is to increase the length of the lag phase of growth and to decrease the growth rate and size of final population of microorganisms. This is due to adverse influences of lowered water on all metabolic activities in microorganisms since all chemical reactions in cell require an aqueous environment.

pH

All the microorganisms have a minimal, maximal and optimal pH for their growth, survival and activity of their enzymes. Influence of pH of food not only has effect on growth of microorganisms but also on processing conditions. Food having acidic contents promotes growth of acid loving microorganisms such as yeasts, molds and some acidophilic bacteria. Molds can grow over a wider range of acidic pH than bacteria and yeast. Most of the fermentative yeasts can grow at pH of about 4.0–4.5, as in fruit juices and acid food such as sauerkraut and pickles (Seideman et al. 1976). A food with an acid pH would tend to be more microbiologically stable than neutral or alkaline food. Because of this restrictive pH the food such as fruits, soft drinks, fermented milks, sauerkraut and pickles and stable against bacterial spoilage. Most of the bacteria, except acid fermenters are favored alkaline or neutral pH. Most of the bacteria preferred a pH range between 7.0 and 7.5 but some proteolytic bacteria can grow on food substrate with high pH. The buffer content in the food is important to maintain the stability against microbial spoilage. Buffers permit an acid (or alkali) fermentation to go on longer with a great yield of products and organisms (Heller 2001). Vegetable juices have low buffering capacity permitting a decrease in pH with the production of only small amount of acid by the lactic acid bacteria during the early stage of sauerkraut and pickle fermentation. This helps to inhibit the growth of pectin hydrolyzing and proteolytic competing bacteria in food.

The pH for optimal growth of most micro-organisms is near neutrality (7.0). Yeasts can grow in an acid environment, but grow best in the intermediate acidic 4.0–4.5 range. Molds tolerate a wider range of pH (2.0–8.0), although their growth is generally greater in acid pH. Molds can thrive in a medium that is too acid for either bacteria or yeasts (Fleet 2011). The bacterial growth is usually favored at near-neutral pH values. However, acidophilic (acid-loving) bacteria will grow on food or debris down to a pH value of approximately 5.2. Below pH 5.2, microbial growth is dramatically reduced when compared to growth in the normal pH range.

Oxidation-Reduction Potential

The potential of reducing oxidation is an indicator of oxidizing strength and the ability to reduce the substrate. The reducing and oxidizing power of the food will influence the type of organism and chemical changes produced in the food. The concentration of oxygen in food, chemical composition and type of microorganisms associated contribute to the oxidation-reduction (O-R) potential of food and affect growth of microorganisms in them (Lobo et al. 2010). The redox potential of food is determined by characters such as- (a) Oxygen tension of atmosphere above the food, (b) access of atmosphere to the food, (c) resistance of food to the changes occurring and (d) O-R state of materials present in food. Aerobic microorganisms grow more easily with high potential to reduce oxidation (oxidizing reaction). Microorganisms can alter the redox potential of food to the extent that the growth of other microorganisms is restricted. For example, many organisms can reduce the availability of oxygen to a level so that the growth of other aerobes is discouraged.

The O-R potential is written as Eh and measured and expressed as millivolts (mV). If the substrate is highly oxidized would have a positive Eh and substrate is reduced is a negative Eh. Aerobic microorganisms such as bacilli, cocci, micrococci, pseudomonas, acinetobacters require and grow at positive O-R potential and anaerobic such as clostridia and bacteroides require negative O-R potential for their growth.

Most of the fresh plant and animal food have low redox potential because of reducing substances present in them. Fresh vegetables and fruits contain reducing substances such as ascorbic acid, reducing sugars and animal tissues have sulfhydral (-SH) and other reducing group compounds considered as antioxidants.

Fresh vegetables, fruits and meat promote growth of aerobic microorganisms in the surface region because of positive redox potential (Petersen et al. 1999). However the anaerobic microorganisms grow in inner parts of vegetables, fruits and meat because of negative redox potential. Most of processed plant and animal food gain positive redox potential therefore promote growth of aerobic microbes.

Types of Nutrients

Nutrients are one of the most important compounds for the growth and functioning of all living beings. Nutritional quality of food depends on the chemical composition, nutritive value or nutrients, their proportion and growth promoting ability to the microorganisms.

The most important factors which have to be considered are the energy substances in food, nitrogen substances, growth promoting substances, accessory food substances or vitamins, minerals and water content which all are essential for growth or energy production of organisms. The common energy sources of organisms are carbohydrates. Complex carbohydrates such as cellulose, hemicelluloses, starch, pectin etc. can be utilized by various types of microorganisms. At the same time other carbon compounds such as esters, alcohols, peptides, amino acids, organic acids and their salt also serve as energy sources for many organisms (Longree and Armbuster 1996). Bacteria are identified and classified based on their ability to utilize various sugars and alcohols. Most organisms can hydrolyses complex carbohydrates and can use glucose as energy source. Some microorganisms can hydrolyze triglycerides and other types of fats by microbial lipases and produce glycerol and smaller fatty acids. Hydrolytic products of proteins and peptides serve as sources of nitrogen for many proteolytic bacteria such as *Pseudomonas* spp. The primary nitrogen source utilized by heterotrophic microorganisms is amino acids. Some microbes are able to utilize nucleotides and free amino acids, whereas others are able to utilize peptides and proteins (Thomas and Surdin-Kerjan 1997). Molds are most effective in using proteins, complex carbohydrates and fats because they contain enzymes capable of decomposing these molecules into less complex components. Many bacteria have similar capacity, but yeast requires simple compounds. Some microorganisms require vitamins and other growth factors for their growth and development such microbes are called fastidious organisms. Some microbes produce vitamins and other growth factors which support growth of other organisms present in food. Each kind of microorganisms has a range of food requirement.

Inhibitory Substances

Inhibitory substances are present in the food as its own origin, or added purpose fully for preventing and inhibiting the growth of microbes. The stability of certain foods against attack by microbes is due to the presence of certain naturally occurring substances that possess and express antimicrobial activity. Some plant species are known to contain essential oils that possess antimicrobial activity. Eugenol in cloves, allicin in garlic, cinnamic aldehyde and eugenol in cinnamon, allylisothiocyanate in mustard, eugenol and thymol in sage and carvacrol (isothymol) and thymol in oregano are some of the best studied examples. Milk contains several antimicrobial substances, including lactoferrin, conglutinin and the lactoperoxidase

system. Lactoferrin is an iron-binding glycoprotein that is inhibitory to a number of foodborne bacteria and its use as a microbial blocking agent on beef carcasses (Gillespie 1981; Gravani 1987). Eggs contain lysozyme; ovotransferrin and conalbumin having antimicrobial properties.

Deteriorative Changes Brought About by Microorganisms

Growth of microorganisms brings about rot and decay in the food. Food that has undergone microbial decay is considered unfit for human consumption. Some of the most common physical and chemical changes caused by microorganisms have been discussed below:

Physical Changes in Foods

Physical damage brought about by microorganisms is usually more pronounced than chemical changes. The microbial damage usually results in a considerable change in the physical properties of food such as flavor, odor, and color. Food damage can either be categorized as aerobic or anaerobic depending upon the type of process, or on the basis of the causative agent such as bacteria, mold or yeast. Aerobic damage is usually caused by bacteria and yeast in the formation of mud such as odors and unwanted taint, color change and flavor of fat breakdown (Garbutt 1998). The formation of mud by certain types of bacteria or yeast depends on environmental conditions, especially temperature change and the oxidation dye—lead, grey, brown or green color. The physical deterioration by molds leads to a viscous surface of foods, the appearance often referred as "whiskers". Mold deterioration can affect the appearance of fat in food similar to those found in bacteria and yeast, and produce unpleasant odor and alcohol flavor.

Aerobic damage to food from molds is usually restricted to the surface of the food which can be trimmed in foods such as meat and cheese, and the rest is generally acceptable for consumption (Marsh and Bugusu 2007). The surface beneath the molded growth is usually limited in microbial activity. However, if the growth on the surface is followed by penetration within the surface of the food it may contain toxins. Anaerobic damage occurs within the food products or in a closed container where oxygen is either absent or limited. Therefore, the damage caused by bacteria is through disintegration, decay or contamination. Further degradation occurs due to the accumulation of organic acids after the enzymatic degradation of complex molecules (carbohydrates). Also, proteins without rot can contribute to stress accompanied by the production of different gases.

Chemical Changes in Foods

Chemical changes occur due to the activity of enzymes present in the food, and enzymes produced by microorganisms. Due to different intrinsic and microbial enzymes, proteins, fats, carbohydrates and other complex molecules decompose into smaller and simpler compounds. With increased load and microbial activity, the rate of the process of degradation is enhanced. The availability of oxygen determines the end products of the bacterial action such as the normal hydrolysis of proteins and carbohydrates into final products amino acids and glucose. Under anaerobic conditions, proteins decompose into a variety of sulfur-containing compounds, which generally have offensive odor and taste (Thomas and Surdin-Kerjan 1997). Other chemical changes include the action of lipases, produced by microorganisms that hydrolyze the triglycerides and phospholipids into glycerol and fatty acids. Most microorganisms use carbohydrates as preferential energy source leading to the formation of a variety of end products such as alcohol and organic acids. However, in sausage and cultured dairy products, a controlled microbial fermentation of organic acid (lactic acid) contributes to its distinctive and unique flavor.

Food Contamination

Food contamination is the introduction or occurrence of contaminants in food. Contaminant refers to any biological or chemical agent, foreign body or other substance that is unintentionally added to food which may endanger food safety. Chemically contaminated food is a global health issue and a major cause of international trade concern. Contamination can occur through environmental pollutants such as toxic heavy metals, polychlorinated biphenyls (PCBs) and dioxins or through deliberate use of chemicals such as pesticides, animal drugs and agrochemicals. Food additives and contaminants from food production and processing can also adversely affect health. When food is contaminated with pathogens, chemical contaminants or heavy metals, they can pose serious health risk to consumers and impose a severe financial burden on individual or communities. Cross-contamination of food is a common cause of foodborne illness (Guthrie 1988). During food preparation and storage, food may be contaminated by microorganisms (bacteria and viruses) from different sources.

There are three main ways of cross-contamination.

(a) Food to food
(b) People to food
(c) Equipment to food

Contamination of Plants

The internal cells and tissues of healthy plants are mainly sterile. The invasion of healthy tissues and subsequent growth of microorganisms can be prevented by:

(a) Outer mechanical barrier e.g., epidermis with outer waxy and corky layers.
(b) Chemical constituents that are anti-microbial e.g., tannins, organic acids and essential oils.
(c) Inert wall in tissues that is difficult to penetrate.
(d) Active cells with intact membranes.

Plant material is harvested in healthy condition and as long as the mechanical barrier is intact they can be stored at low temperature for several months without damage.

Contamination of Animals

In animals, skin, intestine, mouth, lymph nodes and liver can also be invaded by microorganisms. The invasion of healthy tissue and subsequent growth of microorganisms can be prevented by:

(a) Epithelial barriers e.g., stratified skin epithelium (epidermis) and intestinal mucosa.
(b) The immune system consisting of lymphatic system, white blood cells and antibodies.
(c) Active cells with intact membranes.
(d) Presence of natural antimicrobials e.g., lysozyme in tears, saliva.
(e) Voiding mechanism such as vomiting.

Sources of Food Contamination

There are five major events that can cause food contamination:

(a) **Food production:** The use of chemicals, fertilizers and manures has the potential to contaminate food as it is being grown.
(b) **Food processing:** The food processing area can be a major source of contamination. Areas used for processing need to be kept clean or cross-contamination can occur especially with meat products (natural bacteria residing in the intestines of animals are a major source of cross-contamination).
(c) **Food storage:** Food that is not properly stored, for instance, uncooked chicken resting next to a bunch of fruits can be a source of bacteria and other contaminants from one food to another.

(d) **Food preparation:** A great deal of food contamination occurs during the preparation stage. A chopping board used for meat that is not washed and used for vegetables is another source of possible contamination. Unwashed hands, dirty kitchen space, insects and rodents in the kitchen etc. are all possible sources of food contamination.
(e) **Environmental factors:** Bacterial parasites, fungal spores, etc. travel in the wind, float on water, deposited with dust and reside in the soil. They are a part of nature and will always be a possible source of contamination if not dealt appropriately as part of a consistent and dedicated approach to food hygiene.

Conclusion

Food contaminations and spoilage make food and food products unsuitable for consumption. The possibility and extent of spoilage is influenced by various external and internal factors, the type of food, packaging and storage conditions. It is estimated that one-third of the worlds' food produced is lost every year due to contamination and spoilage. Bacteria, fungi, improper packaging, mishandling and improper storage are the causes of spoilage that can create serious consequences, but there are preventative measures that can be taken to avoid or delay contamination and spoilage.

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Microbial Escalation in Meat and Meat Products and Its Consequences

Mohammad Anas, Saghir Ahmad, and Abdul Malik

Abstract Microorganisms are present everywhere, they play serviceable and detrimental role in various ways; deteriorate the basic needs of human life, escalation of their population in foods hamper the shelf-life of food and cause illness in humans. Among all foods, meat and meat products are staple food which are used worldwide. Meat and meat products are well known for their high nutritional values as they contain good amount of proteins which comprise of essential amino acids with high biological value. Meat is also rich in vitamin B complex and several important minerals like iron, magnesium and zinc. Being an excellent medium; meat and meat products favour the growth of a variety of microbial flora such as bacteria yeast and molds, few of them are pathogens. Under aerobic condition bacterial population grow and spoil the meat. Meat spoilage is commonly caused by the bacteria like *Pseudomonas* spp., *Brochothrix thermosphacta*, members of Enterobacteriaceae family, and Lactic acid bacteria etc., which alter the component of meat and meat products continuously, thus leading to adverse changes in sensory characteristics such as appearance, odour, texture and flavour as well as acceptable qualities. This chapter summarizes cause of spoilage of meat and meat products in order to develop optimum preservation techniques to keep up the freshness of meat and meat products. The chapter also gives insights about how microbes deteriorate the nutritional, sensory and textural qualities of meat and hamper the shelf life.

Keywords Meat and meat products · Microbial escalation · Spoilage · Preservation · Sensory properties

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Introduction

Meat and meat products are well known for their high nutritional values as they contain good amount of proteins, fat, vitamins and minerals. Global meat production is high and expected to be increased by 16% by the year of 2025 compare to the base time period between 2013 and 2015 and it is expected that the meat consumption per capita will reach 35.3 kg retail weight equivalent (r.w.e) (OECD/FAO 2016). A significant portion of sustainable meat is lost by microbial spoilage since favourable pH, high moisture content and abundance of nutrients support the growth of microorganisms in general and bacteria in particular. Thus, meat becomes an excellent medium for the growth of bacteria, yeast, and molds. Under certain condition microbial population proliferate, which lead to undesirable modification in nutritional constituents, sensory and textural properties hence meat and meat products get spoiled, resulting in a big economic loss. Food borne pathogens are a major concern that causes illness and death; raw retail meats have been recognized as a potential factor for spreading food borne diseases. About 90% food borne illnesses are caused by contaminated raw meat (Arul and Saravanan 2011). Various meat samples have been analysed and found to be contaminated with drug resistant bacteria, which are transferred to the human being. Bacteria such as *Escherichia coli*, *Salmonella* and *Campylobacter* which causes food borne illnesses were previously found to be multi drug resistant (Dan et al. 2015; Maka and Ppowska 2016).

Source of Microbial Contamination in Meat and Meat Products

Muscles of healthy living cattle are sterile while their outer surface like skin, hide and hairs are naturally contaminated with a variety of microorganisms. According to Featherstone (2003), live healthy animal's hide contains 10^7 organisms on 1 cm^2 area of hide. The internal organs like the digestive tract (potential source of faecal contamination and pathogenic bacteria) and respiratory tract are held by various microbiota (Okonko et al. 2010). The animal faeces contain about 10^8 coliforms bacteria per gram of faeces and act as potential source of meat contamination (Unc and Goss 2004). Faecal matter could be a major source of carcasses contamination which could reach through direct contact as well as by indirect contact through contaminated and uncleaned slaughtering equipment (saws, knives and cleavers), surface contact, labours, installations, air (aerosol) and liquids of slaughterhouses (Omuruyi et al. 2011). Bacteriological quality of meat and meat products is strongly influenced by the prevailing hygienic condition during their production and handling (Osama and Gehan 2011). Contamination subsequently occurs by the introduction of microorganisms on the meat surfaces in operations performed during cutting, processing, storage, and distribution of meat (Clarence et al. 2009), hence maintaining the cold chain as well as hygiene during the shipping of meat is of

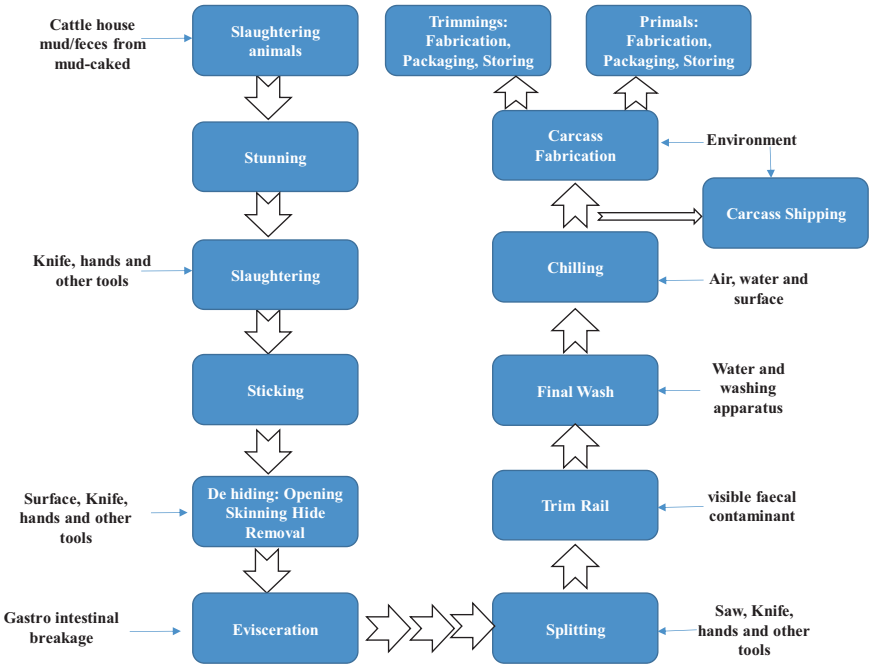

Fig. 1 Schematic representation of successive steps from cattle slaughtering to meat production and possible ways of microbial contamination

extreme importance (Adzitey et al. 2011). Carcass contamination not removed by trimming or washing and it act as contaminant for newly exposed surfaces (Stivarius et al. 2002; Marriot 2004). Therefore, in the prevention of meat contamination, individual hygiene plays an important role (Featherstone 2003). A schematic representation of successive steps from cattle slaughtering to meat production and possible ways of microbial contamination is presented in the Fig. 1.

Factors Influencing the Microbial Growth in Meat and Meat Products

Both intrinsic and extrinsic factors influence the microbial growth which is responsible for spoilage and shelf life of meat and meat products. Intrinsic factor includes physical and chemical properties like types and age of slaughtering animals, initial microflora, nutrient content, water activity and pH; extrinsic factor includes storage condition like temperature and atmospheric composition, other factors like processing and implicit include cooking methods and synergistic or antagonistic reflection of microorganism respectively (Bruckner et al. 2012).

Intrinsic Factors

Composition of Meat and Meat Products

Microorganisms require energy for their growth and metabolism, the key materials they are unable to synthesize; obtained from the surrounding food environment which permit the effective existence of food borne bacterial strains (CenciGoga 2012). Meat act as a natural ecosystem in which beneficial or detrimental situations govern the survival and growth of a particular strain. Poor carbohydrate (1%) and abundance of protein (21%), lipids (5%), vitamins and minerals offers an opportunity of few species instead of others with different nutritional necessities. Food ingredient, natural or chemical additives such as nitrite, and the existence of growth factor further select the survival of specific bacterial strains (Ray and Bhunia 2013).

pH and Buffer Capacity

pH of meat also plays a crucial role for selection and survival of bacterial species, each species has an optimum pH for their growth and metabolic activity. After slaughtering of animals, enzymatic activity ceases that also inhibit the glycolysis, and muscle pH normally decreases to 5.4–5.8, while dark firm dry meat (obtained from stressed animals) and cooked meat products such as sliced ham, pH have been found to be >6 (Aymerich et al. 2002). The presence of adipose tissue and a high pH of meat not only determine the rapid bacterial growth but also responsible for quick spoilage (Ray and Bhunia 2013).

Water Activity (a_w)

Water activity (a_w) is the degree of water quantity present in food accessible for microbial growth, enzymatic reaction and other biochemical processes. It directly affects the microbial safety of food. Water activity in meat and meat products represent the relative humidity of air in equilibrium with respect to the products (Comaposada et al. 2000). On the basis of moisture availability, food can be categorised as under:

- Moist foods have $a_w > 0.85$ and require refrigeration or other methods to regulate pathogens growth
- Semi moist food which has a_w 0.60–0.85 with limited shelf life, are more prone to yeast and molds attack
- Low moist foods which have $a_w < 0.60$ with extended shelf life are stored normally.

Moist food category such as fresh meat, vegetables and fruits have $a_w > 0.85$ hence more prone to bacterial proliferation with some other pathogens. Every

microorganism has a minimum (Fig. 2), maximum and optimum a_w and normally grow best between a value of a_w 0.98–0.99 while their growth stops at $a_w < 0.90$, but yeasts and molds are able to grow at lower a_w of 0.60. Growth of toxin producing pathogenic bacteria such as *Clostridium botulinum* and *Staphylococcus aureus* will avert at or below a_w of 0.85 (USDA 2005). Dried food is commonly considered as self-stable and less prone to bacterial attack hence, scientifically it is accomplished that drying is the best procedure to reduce water activity which prevent pathogens growth and toxin production (Leonard 2011). In meat and meat products water activity can be controlled by drying, refrigeration, adding salt and sugar. Addition of salts or sugars bind the water molecules in food thereby making them unavailable for the rapid proliferation of microorganisms.

Extrinsic Factors

Storage Condition and Atmospheric Composition

Microbial spoilage flora significantly influenced by the storage condition of meat and surrounding atmospheric conditions (Sechi et al. 2014; Rossaint et al. 2015). Aerobic storage stimulates the growth of *Pseudomona*s spp., *Acinetobacter* spp. and *Moraxella* spp. which are considered to be main cause of meat deterioration stored aerobically at various temperature ranging from 1–25 °C. *P. fluorescens,* psychrotrophic *P. fragi*, *P. lundensis* and *P. putida* are commonly isolated from spoiled meat that has been packaged in the presence of air (Ercolini et al. 2010). The genus

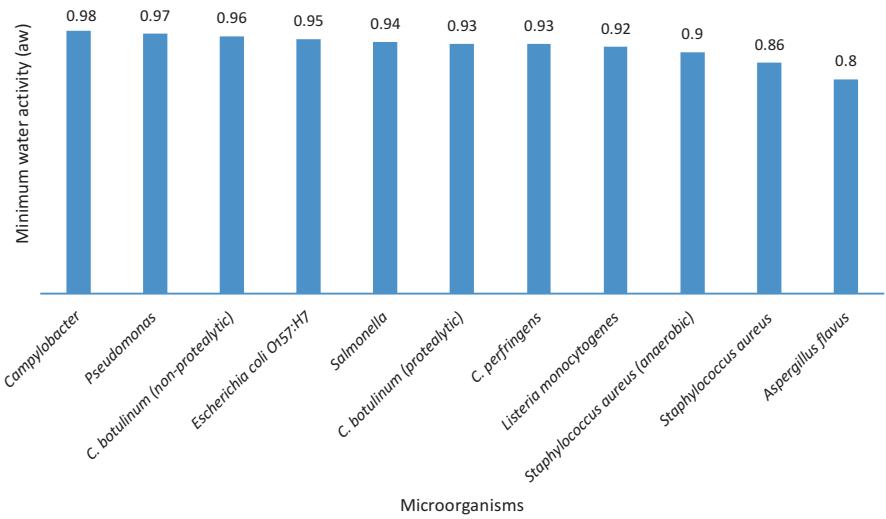

Fig. 2 The minimum water activity (a_w) requirement for the growth of most common microorganisms related with dried meat products

Shewanella, *S. putrefaciensis* are predominant and causing spoilage of meat packaged under vacuum and high vacuum pH (Doulgeraki et al. 2012). Atmosphere containing higher concentration of carbon dioxide (CO_2) or packaging in vacuum significantly enhance the shelf life of meat and meat products over the conventional packaging methods (Yost and Nattress 2002). Nitrogen (N_2) and CO_2 are used in food packaging; though prolong the lag phase of aerobically growing microorganisms and stimulate the growth of facultative and strict anaerobes. The determination of bacterial shift from aerobic (*Pseudomonas* spp.) to lactic acid bacteria as well as facultative anaerobe (*Brochothrix thermosphacta*) is depend on the alternation in the packaging conditions and their trends (Nychas et al. 2008; Doulgeraki et al. 2012). Lactic acid bacteria (LAB) prevail in vacuum or CO_2 modified atmospheric packed food products and causing spoilage (Arvanitoyannis and Stratakos 2012). Microaerophilic condition with decreased water activity prevent the growth of Gram negative spoilage microbes and promote the escalation of LAB (Samelis et al. 2000; Audenaert et al. 2010). Following factors contribute to the efficacy of Modified Atmosphere Packaging (MAP) of meats:

- changes in the composition of headspace (the remaining space inside packed food) gases concentration of CO_2 during storage
- temperature
- packaging configuration and
- meat characteristics

CO_2 in head space is adsorbed by fat and muscles tissues till it saturates, resulting in decrease of headspace volume and ultimately packages collapse (Ercolini et al. 2006a, b). *Serratia* spp. is the most commonly isolated genus of Enterobacteriaceae family from MAP meat (Doulgeraki et al. 2012).

Storage Temperature

Storage temperature influences the quality of meat and meat products by altering the duration of lag phase, the specific growth rate and finally cell numbers of the spoilage bacteria. Different microbial groups require an optimum temperature for their growth. The temperature range for psychrophilic bacteria is −5 to 20 °C, optimal temperature for their growth ranges between 5 and 15 °C, while for mesophilic optimum range is between 20 and 25 °C, and thermophiles optimal range varies from 45 to 60 °C, for extreme thermophiles the optimal temperature is between 85 and 90 °C. Lower storage temperature reduces the microbial growth and promote the proliferation of both Gram-positive and Gram-negative psychrotrophic bacteria such as LAB and *Pseudomonas* spp. during refrigeration storage (Doulgeraki et al. 2012). In MAP and Vacuum Packed (VP) meat items, the predominance of LAB is additionally kept up under refrigerated conditions. *Carnobacterium* spp. dominates in a vacuum at 1.5 °C, though homo-fermentative *Lactobacillus* spp. dominate at 4 °C and 7 °C. *Serratia liquefaciens* and *Hafnia alvei* are the two bacterial species among Enterobacteriaceae family which dominate and prevail at 1.5 °C and 4 °C

respectively. Psychrophilic *Clostridium* spp. could be identified in VP, chilled meat and meat products (Ray and Bhunia 2013).

Microbial Flora Associated with Spoilage of Meat

Spoilage is a multifarious incident involving a number of biological and chemical changes by rendering a product unacceptable for human consumption (Gram et al. 2002). The major changes caused during spoilage is microbial growth, oxidation of lipids and autolytic enzymatic reactions. Meat and meat products give a remarkable natural condition to the development of an assortment of microbial flora (bacteria, yeast and molds). The microbial flora that inhabit the fresh meat extremely depend on the type and the method by which it was prepared and processed (Nychas et al. 2008). The psychrotrophic microbes which develops in meat and meat products at low temperature includes *Acinetobacter*, *Brochothrix*, *Clostridium*, *Flavobacterium*, lactic acid bacteria, *Moraxella*, *Micrococcus*, *Psychrobacter*, *Pseudomonas, Staphylococcus* and diverse genera of the Enterobacteriaceae family (Doulgeraki et al. 2012). Occurrence of different bacterial species at specific storage condition of meat is given in Table 1. In oxygenic condition at low temperatures various *Pseudomonas* species such as *P. fragi*, *P. lundensis* and *P. fluorescens* have often been isolated from ruined meat and meat products (Ercolini et al. 2006a, b, 2009, 2010, 2011; Doulgeraki et al. 2010, 2011; Pennacchia et al. 2011). *P. fragi* is also found at lower degree in VP and MAP stored meat and its spoilage activity is because of the generation of numerous volatile compounds potentially responsible for off smelling (Ercolini et al. 2007, 2009, 2010; Pennacchia et al. 2011). Additionally, other Gram-negative bacteria of family Enterobacteriaceae and *Aeromonas* can also support to meat deterioration. Primarily, *Serratia liquefaciens* is the most widely recognized individual from the Enterobacteriaceae family in meat stored in various environments and *Hafnia alvei* is exceptionally as often as possible found in Hamburger stored under MAP or VP (Ercolini et al. 2006a, b, 2009; Doulgeraki et al. 2011), *Enterobacter agglomerans* is commonly isolated from aerobically stored meat and in MAP (Samelis 2006) though *Rahnella* spp. is commonly isolated from beef stored in MAP and VP (Ercolini et al. 2006a, b; Pennacchia et al. 2011). *Shewanella putrefaciens* has the capability to produce hydrogen sulphide (H_2S) gas therefore distinguished as one of the significant spoilage microorganism in meat and meat products (Gram and Daglaard 2002; Nychas et al. 2008).

LAB for example, *Lactobacillus* spp., *Carnobacterium* spp. and *Leuconostoc* spp. are also associated with meat spoilage stored under MAP or VP and some time also in aerobic conditions (Nychas and Skandamis 2005). The species principally found in meat are *Lactobacillus curvatus*, *Leuconostoc* spp. and *Lactobacillus sakei* (Yost and Nattress 2002; Fontana et al. 2006; Pennacchia et al. 2011). Additionally, the predominance of *Leuconostoc* spp. has been reported in aerobically stored, MAP and VP packaged meat. Among the LAB, *Carnobacterium maltaromaticum*,

Table 1 Occurrence of different bacterial species under specific storage condition of meat

Gram-positive bacteria	Storage condition			Gram-negative bacteria	Storage condition		
	AIR	MAP	VP		AIR	MAP	VP
Bacillus spp.	+		+	*Achromobacter*	+		
B. gaviniae			+	*Acinetobacter*	+	+	+
B. noackiae			+	*Aeromonas*	+		+
Brochothrix thermosphacta	+	+	+	*Alcaligenes*	+	+	+
Buttiauxella agrestis			+	*Alteromonas*	+	+	+
Carnobacterium divergens	+	+	+	*Campylobacter*	+		
C. maltaromaticum	+	+	+	*Chromobacterium* spp.	+		
Corynebacterium	+			*Citrobacter freundii*	+	+	
Clostridium spp.			+	*Enterobacter cloacae*	+		
Cl. Algidicarnis			+	*E. agglomerans*	+	+	
Cl. Estertheticum			+	*Flavobacterium*	+		
Cl. gasigenes			+	*Hafnia alvei*	+	+	+
Cl. Putrefaciens			+	*Klebsiella* spp.	+		
Lactobacillus spp.			+	*Kluyvera* spp.	+		
Lb. kimchi			+	*Moraxella* spp.	+		
Lb. curvatus	+			*Pantoea* spp.	+		+
Lb. sakei	+	+	+	*P. agglomerans*	+		
Lb. algidus			+	*P. anantis*		+	
Lb. oligofermentans		+		*Photobacterium* spp.	+		+
Lb. graminis		+		*P. kishitaniiclade*			+
Lactococcus piscium	+	+	+	*Proteus vulgaris*	+	+	
Leuconostoc spp.			+	*Providencia* spp.	+	+	+
L. gelidum		+	+	*Pseudomonas* spp.	+		+
L. mesenteroides	+		+	*Ps. fragi*	+	+	+
L. pseudomesenteroides	+	+		*Ps. fluorescens*	+		
L. gasicomitatum		+		*Ps. lundensis*	+		
L. carnosum	+	+		*Ps. migulae*	+		
Kurthia spp.	+			*Ps. Putida*	+	+	
Listeria	+	+		*Psychrobacter* spp.	+		
Microbacterium spp.	+	+	+	*Rahnella* spp.	+	+	+
Micrococcus spp.	+	+		*R. aquatilis*	+	+	+
Paenibacillus spp.	+			*Ralstonia* spp.			+
Staphylococcus spp.	+	+	+	*Serratia* spp.		+	
Staph. pasteuri			+	*S. grimesii*	+	+	+
Staph. saprophiticus	+		+	*S. liquefaciens*	+	+	+
Staph. xylosus		+	+	*S. marcescens*	+		+
Strep. parauberis			+	*S. proteamaculans*	+	+	+
Kocuria spp.	+			*Shewanella putrefaciens*	+		
				Stenotrophomonas maltophilia			+
				Vibrio	+		

(continued)

Table 1 (continued)

Gram-positive bacteria	Storage condition			Gram-negative bacteria	Storage condition		
	AIR	MAP	VP		AIR	MAP	VP
				Yersinia	+		+

+ sign indicates the incidence of the bacterial species under specific storage condition (The species are listed as per literature search; they were recognized at any rate once in the particular conditions irrespective of storage time)

and *C. divergens* are the most common species reported to be associated with meat. *Br. thermosphacta* is also recognized to be one of the important bacteria causing spoilage in aerobically stored, MAP and VP packaged meat and it is perceived to add off -order (Russo et al. 2006; Samelis 2006; Axelsson 2008; Ercolini et al. 2010, 2011; Pennacchia et al. 2011). Anaerobic psychrotrophic members of Clostridia such as *Clostridium algidicarnis*, *C. putrefaciens*, *C. algidixylanolyticum*, *C. estertheticum*, *C. frigidicarnis* and *C. gasigenes* have been isolated from VP stored meat (Brightwell et al. 2007; Adam et al. 2010; Silva et al. 2011).

Microbial Degradation and Alteration Associated with Sensory and Textural Properties

Since microbial survival pursues distinctive pathways relying upon the numerous elements and the noticeable impacts are different like distinctive growth, textural changes (degradation of polymers), off odour and off flavors. The rate and kind of spoilage appear to rely among other factors i.e. concentration of sugars, presence and concentration of lactic acid, nitrogenous compounds and free amino acids present in meat. These molecules are attacked in different ways by a number of microorganisms to generate diverse metabolic by-products. The metabolic end products thus generated greatly influence the sensory and textural properties of both fresh and cooked meat. The microbial degradation of carbohydrates, fat and proteins leads to various undesirable changes in the odour, flavour, texture, aroma and may also lead to the formation of slime thereby rendering the meat product unfit for human consumption (Nychas et al. 2008; Casaburi et al. 2015).

Microbial Degradation of Meat Carbohydrates

Glucose is one of the most preferable substrate for microorganisms which is generally found in muscles tissues, it is even preferred by most of the bacteria growing at chilled storage of meat packed at any conditions. In aerobically stored meat, where Pseudomonads are the predominant members and catabolises D-glucose, L-glucose leading to the accumulation of fermentative metabolites like lactate, pyruvate and

gluconate, which are utilized by *Pseudomonas* spp. under aerobic conditions while as the by-products like amino acids and acetate are consumed by the same Pseudomonads under anaerobic conditions. Glucose has been observed to be the precursor of numerous off odour producing compounds for example, acetate, acetoin, di-acetyl, acetic acid, isovaleric acid, isobutyric acid, 2-methyl butyric acid, 3-mthyl butanol, 2-methyl ethanol and propanol in stored meat, and the concentration of these precursors may influence the growth rate and type of spoilage bacteria (Nychas et al. 2008).

Sugar uptake is highly important for LAB escalating in meat and for the related kind of spoilage. Both obligatory and facultative heterofermentative LAB grow by producing lactate, acetic acid, CO_2 and ethanol from glucose. In the scarcity of glucose, ribose fermenting Lactobacilli switch their sugar uptake from homofermentative to hetero-fermentative pathway influencing a significant release of acetic acid. Lactate along with its precursor pyruvate are oxidized into acetate by the activity of various LAB associated with meat spoilage. This enhancement in the concentration of acetate imparts a characteristic smell (like that of vinegar) and therefore negatively effects the sensory quality of meat and meat products. In vitro studies demonstrated that *L. sakei* can hydrolyse arginine to promote their growth at the scarcity of glucose producing ammonia (NH_3) and biogenic amines, for example, putrescin and spermin. *Br. thermosphacta* has dual nature, it acts as heterofermentative and homofermentative, in anoxygenic condition it uses glucose as a substrate while in oxygenic conditions it can utilize additionally ribose, glycerol and amino acids generating numerous volatile compounds which affect the sensory quality of meat and meat products (Pin et al. 2002; Nychas et al. 2008). Some of the psychrotrophic clostridia related with spoilage of VP stored meat particularly utilise glucose as a substrate with butyric fermentation generating butyric acid ($C_4H_8O_2$), carbon dioxide (CO_2), hydrogen (H_2) resulting into discolouration of meat. Glycogen is found in animal muscles which is anaerobically break down and producing lactic acid which alter the pH of meat (Diwan 2007).

Meat Proteins

Proteins play a major role to maintain the structural and functional properties of muscles food. Based on their solvency attribute muscle proteins can be classified in to three groups, such as, sarcoplasmic proteins (SP). It is the metabolic protein which is soluble in water or diluted salt solutions, representing 30–35% of the complete muscle proteins. Due to its weak water holding property it forms weak and fragile gels affecting the textural properties of muscles food such as sausages and meat patties (Miyaguchi et al. 2000), myofibrillar proteins is the contractile proteins soluble at high salt concentration. It includes about 50–60% of the total skeletal muscle proteins, and remaining is connective tissue proteins which is neither soluble in water nor in salt solution.

At the point when bacterial contamination is about 10^8 CFU g^{-1}, the carbohydrates are exhausted and *Pseudomonas* spp., in relation with some other Gram-negative psychrotrophic bacteria for example, *Alcaligenes* spp., *Aeromonas* spp., *Moraxella* spp., *Pantoea* spp. furthermore, *Serratia* spp. can use amino acids coming about into development of volatiles malodorous sulphides, esters and amines (Samelis 2006) Fig. 3.

Meat Fat

Animal fat consists of mainly neutral fats and phospholipids. Meat fat has high amount of free fatty acids; oleic acid is the most abundant pursed by palmitic and stearic acids. Fat plays a significant role in preparing some stable meat emulsion type products and its textural properties are highly affected by the consistency of fat. Reduction in fat percentage resulting in cooking loss and decline in hardness (Youssef and Barbut 2011a, b; Alvarez and Barbut 2013). Autoxidation influences fatty acids and stimulate oxidative disintegration of meat and off-flavors development. In meat, lipid hydrolysis can take place both enzymatically and non-enzymatically. The enzymatic hydrolysis of fat is named as lipolysis and is regulated by specific enzymes i.e. lipases, esterase and phospholipase. Animal tissues, blood and skin are the main source of lipase enzyme; which splits glycerides producing free fatty acids responsible for off flavor commonly described as rancidity. Lipid oxidase produces oxygenated compounds like aldehydes and ketones and these

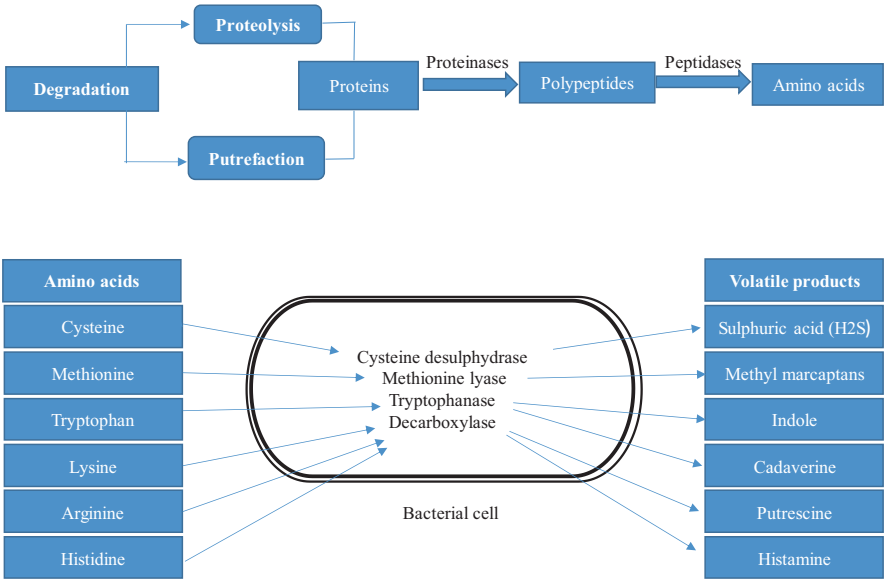

Fig. 3 Microbial degradation of amino acids and formation of volatile products

auxiliary products can cause loss of sensory, textural and nutritive values of meat and meat products.

Hydrolysis of phospholipids and triglycerides may produce volatile fatty acids in meat which act as a primary substrate for oxidation of numerous odorous substances. Fatty acids having two to six carbon atoms originated from the amino acids degradation and also emerge from ketones, esters and aldehyde by oxidation process. Volatile fatty acids are significantly produced in the storage of fresh meat by the activity of *Br. thermosphacta* and *Carnobacterium* spp. Butanoic acids and hexanoic acids are long chain fatty acids mainly found in meat stored under various conditions, while 2 and 3-methylbutanoic acids (considered as branched chain fatty acids) were found only in aerobically stored meat. Butanoic acid can get from the microbial utilization of free amino acids by means of the Stickland process as well as butyric fermentative metabolism of *Clostridia* in VP stored meat and meat products (Martin et al. 2010). All the volatile fatty acids produced during meat storage under various conditions affect the sensory property of meat and meat products by means of rancidity, pungent smelling and sour taste.

Tainted Odours and Flavors

The microbial catabolism produces various type of volatile compounds which include aldehydes, ketones, ethyl esters, alcohols, organic acids, volatile fatty acids, ammonia, sulphur containing compounds and some other metabolites. These catabolic products influence the sensory quality of fresh as well as cooked meat depending on the olfactory thresholds (Casaburi et al. 2015). Tainted odours are noticeable to costumer when the TPC is in the range of 10^{7}–$10^{7.5}$ CFU g^{-1}. Under aerobic condition, *B. thermosphacta* metabolises glucose and generate obscene odour due to the production of acetic acid and acetoin. *Pseudomonas* spp. and some members of Enterobacteriaceae family produce dimethyl sulphide and hydrogen sulphide (H_2S) which give the sulphuric odour to meat. *Shewanella* spp. releases malodorant compounds such as H_2S in VP meat (Doulgeraki et al. 2012). Members of the family Enterobacteriaceae, homofermentative *Lactobacillus* spp. and *Br. thermosphacta* generated acetoin or diacetyl and 3-methylbutanol gave cheesy odour (Casaburi et al. 2015). Lactic acid bacteria produce lactic acid which give the sour and acid aroma to the spoiled meat. The rate of glucose utilization of *Br. thermosphacta* is affected by CO_2 and O_2 ratio, as a result, anaerobic metabolism releases low intense odour while it may be high in aerobic metabolism; therefore, low O_2 concentration in MAP is effective for keeping the acceptable properties of meat and meat products (Pin et al. 2002).

Colour Alteration

Colour alteration of fresh meat results from such factors that cause a shift in the state of the iron particle in the heme of myoglobin (Fe^{+2}/Fe^{+3}) or a shift in the molecule at the free binding site of the heme. The colour of the interior part of fresh meat is devoid of O_2 which is dark purple and characteristics of myoglobin in its reduced state; the predominant form present in meat. Reduced myoglobin is oxygenated to oxymyoglobin on exposure to O_2 which appears red in colour; the typical red colour of meat. The pigment associated with the undesirable brownish colour of meat is known as meta myoglobin, its occurrence result discolouration problem which is due to the oxidation of iron from ferrous (Fe^{+2}) to ferric (Fe^{+3}) state. Under aerobic condition *H. alvei*, *S. putrefaciens* and *L. sakei* utilise glucose and produces H_2S which change the muscles pigment to green sulphomyoglobin. Formation of hydrogen peroxides (H_2O_2) by the *Leuconostoc* spp. and *Weissella viridescens* oxidizes nitrosomyochromogen which may cause meat products to turn green (Doulgeraki et al. 2012).

Microbial Control in Meat and Quality Assurance

Increasing world population and demands of global shipping of meat in supermarkets, microbial control became essential to provide safe meat and meat products without altering its textural, nutritional and sensory properties to the consumers. The purpose of microbial control by means of various preservation techniques is to prevent and reduce the spoilage of meat and meat products caused by microorganisms, oxidation and enzymatic reactions. Several traditional techniques for examples, canning, smoking, brining and drying were employed for preservation of meat and meat products. Currently microbial control in meat and products is carried out with new techniques based on controlling temperature, water activity and use of chemical and biochemical preservatives. These methods are broadly categorised as follows:

Low Temperature Storage

The fundamental point of cooling system is to slow down the rate of spoilage as temperatures below the optimum range may significantly affect the cell growth and therefore limit the rapid multiplication of spoilage microorganisms. Low temperature strategies commonly utilized to inhibit microbial growth in food are:

Chilling Storage

Chilling is one of the important meat preservation method in which carcasses are kept at 4 °C immediate after slaughtering. This method is used in slaughter houses and during meat shipping. Chilling is basically applied for safety, hygiene and prevents protein denaturation (denatured proteins are more prone to microbial attack).

Freezing Storage

It is an excellent method for keeping the real qualities and extending the shelf life of fresh meat. Freezing changes most of the meat water into ice (Heinz and Hautzinger 2007). The rate of ice formation is depending on applying temperature i.e. at −5 °C almost 75%, at −20 °C 98% tissue fluid freezes, and at −65 °C complete crystal formation occurs while chemically bound water do not freeze at these temperatures (Rosmini et al. 2004). Microbial growth generally halted at −12 °C, while complete cessation of cellular metabolism may be achieved at temperatures below −18 °C (Perez-Chabela and Mateo-Oyague 2004). Rahman (1999b) reported that about 60% of total viable count killed during freezing while the remaining population proliferates during frozen storage. At −55 °C meat quality can be prevented completely but enzymatic process, oxidative rancidity and ice crystal formation remain continue which play crucial role in meat spoilage (Hansen et al. 2004; Zhou et al. 2010).

Super Chilling

This procedure is generally used for preservation of fish and poultry. In this process muscle foods are kept below its preliminary freezing point (1–2 °C) however ice crystal doesn't seem to be generated. In this procedure, rather than adding outside ice to the food item, some portion of the inside water is frozen and act as a refrigeration reservoir, ensuring its refrigeration throughout shipping and distribution of food items (Bahuaud et al. 2008). During super chilling storage the activity of cell remains while their respiratory metabolism and ageing process inhibited (Ando et al. 2005). The principal benefit of this procedure is that it expands the shelf life of meat and meat products several times and inhibits the most microbial activity in the meat system (Magnussen et al. 2008).

Chemical Methods

Antimicrobial agents are those materials which are used to diminish the microbial escalation in meat and meat products during each steps of slaughtering, shipping, processing and storage. It can extend the shelf life of meat and meat products.

Antimicrobial agents combined with low temperatures offer a decent insurance for meat against the spoilage microorganisms. Commonly used antimicrobial agents are:

Sodium Chloride (NaCl)

It represses the microbial development by expanding osmotic pressure and diminishing the water activity in the micro habitat. A concentration of 2% or less of sodium chloride can inhibit some bacteria and yeasts excluding some genera of salt tolerating *Micrococci* and *Bacillus*. The combination of two or more antimicrobial agents like sodium lactate with NaCl prolonged the multiplication of aerobic, psychrotrophic and lactic acid bacterial counts resulting in to an increase in shelf life of meat and meat products.

Nitrites of Sodium and Potassium

Sodium and potassium nitrite are used in meat industries as preservatives and act as antimicrobial agent inhibiting the development of toxin producing bacteria such as, *Yersinia enterocolitica*, *Clostridium botulinum* and *S. aureus* under anaerobic condition and VP meat. In addition to controlling the toxin producing bacteria it is effective to control lipid oxidation, colour and odour of meat. It influences the growth of microorganisms in muscles food through a few responses:

- At low pH value it reacts with alpha (α) amino groups of amino acids
- It binds iron (Fe) making it unavailable for the growth and multiplication of microorganisms, and
- Restricts the transport of nutrients across the cell membrane by affecting its permeability

Sodium Sulphite and Organic Acids

Sodium sulphite is mainly used to control Gram negative aerobic bacteria including pathogens, yeasts and molds, and commonly used as preservative in meat and meat products. The antimicrobial property of sulphites is due to sulphurous acid which penetrates the cell and interferes with thiol groups of proteins and co-factor of enzymes. In yeast sulphites stop the cysteine disulphide linkage by reacting with cellular ATP (Adenosine Triphosphate) (Davidson et al. 2005). Organic acids such as lactic acid, sorbic acid, ascorbic acid and benzoic acid are known to their antimicrobial properties. These are used globally as a preservative in meat industries inhibiting the growth of bacteria, yeasts, molds and extending the shelf life of meat and meat products.

Lactoferrin (LF)

It is a natural protein having broad spectrum antimicrobial property against microorganisms such as bacteria, fungi, protozoa and viruses (Elbarbary et al. 2010). The antibacterial activity of LF has been demonstrated against pathogenic *E. coli* O157:H7, *Salmonella* spp., *Campylobacter*, *Listeria monocytogenes* and meat spoilage bacteria *Pseudomonas* spp., and *Klebsiella* spp. (Naidu 2000).

Phenolic Derivative Antioxidants

Low temperature method cannot counteract oxidative and enzymatic spoilage therefore, combination of chemical preservatives (antioxidants) and low temperature methods are relatively useful in order to optimize stability, products quality, sensory and nutritional values of food. Phenolic derivatives antioxidants are globally used in meat as inhibitory additives for lipid oxidation also termed as primary antioxidants which act as a chain reaction inhibitors or hydrogen donors as well as scavengers. Propyl Gallates (PG), Butylated Hydroxytoluene (BHT), Tertiary Butylhydroquinone (TBHQ) and Butylated Hydroxyanisole (BHA) are phenolic derivatives and sometime also known as synthetic antioxidants. The aim of applying these phenolic derivatives in meat is to delay or prevent the negative impacts of lipid peroxidation by decreasing the development of primary and secondary radicals.

Phosphates

Phosphates are one of the primary recognized food additives with potential antioxidant properties used in meat and meat products. A scope of functionalities has been given by the phosphates to improve muscle foods and the functionalities of phosphates depends on kind of its salts (ICLPP 2006). The functional properties of phosphate salts include:

- It reduces oxidation process by binding iron into the system
- It acts as a chelating agent for divalent cations and decreasing the rancidity which extend the shelf life of meat and meat products
- Keeping up the constancy of protein-fat-water system
- Improve the emulsification property of fat by co-operating with muscle fibres, and
- It affects the pH and enhance the water holding capability of muscle proteins

Other ways of meat preservation and shelf life extension is the use of plant based natural antimicrobials and essential oils which are effective against foodborne pathogens and meat spoilage bacteria. It has increased much consideration by the consumers and food industries due to its beneficial effect over synthetic preservatives and reduces the misuse of antibiotics which is the major cause of antibiotic resistance in bacteria (Domíngueza et al. 2018). Essential oils extracted from

different sources such as clove, cinnamon, coriander, cumin, eucalyptus, lime grass and rosemary have been demonstrated to be good antimicrobial agents and effective against a number of foodborne pathogenic bacteria including *Campylobacter* spp., *S. aureus*, *E. coil* O157:H7, *L. monocytogenes* and *Salmonella* etc. (Calo et al. 2015). Therefore, plant derived natural substances can be used in food industries due to their safety and broad spectrum antimicrobial activity against foodborne pathogens and food spoilage bacteria.

For the quality assurance, microbial testing of meat and meat products is one of the potential tools that can be used to evaluate safety and risk management. The important parameters of microbial testing for industrial significance and consumer interest are Standard Plate Count (SPC) (also referred as aerobic plate count or the total viable count), coliform, faecal coliform, faecal streptococci, *Salmonella*, *Shigella* and Staphylococci count these are potential biological hazards and directly related to the quality and shelf-life. Ranken and Kill (1993) described the microbial criteria which relate the quality (bacteria) of meat as 10^2 CFU g^{-1} excellent, 10^4 CFU g^{-1} good for commercial, 10^6 CFU g^{-1} rejection limit in many commercial conditions, 10^8 CFU g^{-1} meat and meat products smell and at 10^9 CFU g^{-1} meat become slimy. According to the guide line of FDA (2014) and FSANZ (2014) any pathogenic microorganisms should not be detected in in 25 g of meat samples.

Food safety protection can be enhanced by the control of biological hazards through the use of precautionary methods such as good sanitation, Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Point (HACCP) system through the production, processing and distribution in the food chain.

Conclusion

Due to higher nutritive values and abundance of protein, meat and meat products are globally consumed by the human being. Since microbial spoilage of meat is a problem for producers, retailers and consumers equally. The microflora on meat forms an ecosystem that is affected by large number of variables, such as packaging, atmospheric composition, temperature, water activity, pH and buffer capacity of meat. Microbial escalation, lipids oxidation and autolytic enzymatic activity are the three major cause of meat spoilage. Microbial escalation results in degradation of structural components of meat (carbohydrates, fat and proteins), discolouration, gas formation, alteration in pH and slime formation which alter the sensory (off-odour, off-flavor, sour taste) and textural properties of meat and meat products. Due to its perishability, increasing world population and global meat shipping, regulation of meat spoilage become crucial in order to enhance the shelf life and retain its sensory, textural and nutritional values. Proper sanitation, good management and appropriate preservation techniques can enhance both qualities as well as the shelf life of meat and meat products. Modern meat preservation techniques such as, controlling water activities, temperature and use of chemicals and biochemicals are

preferred over conventional procedures like drying, smoking, brining and canning. However, more works are needed to know the preliminary microbial load, nature of bacteria and their relations with respect to improve the shelf life of meat and meat products. Present information allows us to maintain hygiene during meat handling and new forms of edible packaging are needed that dramatically limit the microbial growth and extend the shelf life of meat and meat derived products.

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Natural Microflora of Different Types of Foods

Hüseyin Erten, Bilal Agirman, Cennet Pelin Boyaci-Gunduz, Erdem Carsanba, and Sezgi Leventdurur

Abstract The sources of thousands of different microorganisms in foods could be either natural or external. The microorganisms in foods are divided into three groups as molds, yeast and bacteria. Natural microflora of foods influences the quality, availability and quantity of products. Molds are generally known as spoilage microorganisms in foods. Therefore, usage of them in food processing is limited (i.e. mold ripened cheese, soybean fermented foods). Conversely, yeasts and bacteria are widely used as important microorganisms in food industry. They have ability to ferment sugars to some industrially important compounds such as ethanol, lactic acid, acetic acid and carbon dioxide. In addition, they can contribute to the texture, flavor and safety of foods. The microorganisms intrinsically existing in food play a key role in processing of numerous foods especially plant based fermented foods, milk and meat based fermented products. The aim of the present chapter is to discuss the natural microflora belonging to different foods and also to explain their functions during food processing.

Keywords Microflora · Fermentation · Milk · Fruits · Vegetables · Cereals

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Introduction

Many foods exist in shelves as fresh or even processed contain various types of microorganisms, including yeast, bacteria and molds are responsible from development of organoleptic and nutritional properties as well as spoilage of foods depending on type of process or storage conditions applied. During fermentation process, several chemical compounds found in food sources (such as glucose, amino acids and fatty acids) are converted also into secondary metabolites by metabolic activities of relevant microorganisms (mainly lactic acid bacteria, yeasts and molds). As a result, sensorial properties of foods in terms of flavor and texture are enhanced, bioavailability of the nutrients are enriched, food safety are controlled due to the preservative effects, bioactive compounds are produced, anti-nutritive compounds are degraded and probiotic potential is increased.

However, diverse or even same type of microorganisms can also play detrimental roles in foods in regard to spoilage. Mainly, aerobic psychotropic Gram-negative, heterofermentative *Lactobacilli*, spore forming bacteria, molds, and yeasts can be involved in spoilage of many foods. Off-flavors and odors, change in colors and texture, production of excess gas as well as high acidity are generally observed as a consequence of spoilage of foods. These undesirable circumstances in many fresh or processed food products can be prevented by reducing the pH via lactic acid fermentation or addition of acids and other conservatives, reducing water activity by adding salt or sugar, forming desired microflora to control the growth of undesired microorganisms, packaging techniques to restrict oxygen as well as keeping in low temperatures.

This chapter primarily focuses on the fundamental information about microorganisms such as morphological, physiological properties and factors influencing microbial growth and then demonstrates comprehensive data regarding distinctive microflora of common raw and processed foods. Moreover, the developments of functional properties as well as spoilage in food products by related microorganisms are exemplified and reviewed in details.

Main Group of Microorganisms in Foods

Bacteria

Bacteria are the most important microorganisms for the food processor. They are unicellular organisms and one of the smallest living creatures known. Bacteria are classified as prokaryotic organisms and they have not got any organelles and nucleus. There are several types of bacteria that are divided into several categories depending on various characteristics (Anonymous 1999, 2019) (Table 1).

Most bacteria have protective cell wall which is made up of a peptidoglycan (murein) and some of them have capsule, which is a protection layer. Bacteria also have a circular DNA called plasmid, the plasmids make some strains resistant to antibiotics.

Table 1 Classification of bacteria

Type of classification	Sub-classification
Shape	Bacillus (rod-shaped)
	Spirilla
	Coccus
	Vibrio
Composition of the cell wall	Peptidoglycan cell wall
	Lipopolysaccharide cell wall
Mode of nutrition	Autotrophic
	Heterotrophic
Type of respiration	Anaerobic
	Aerobic

Bacteria reproduce as asexual, which is called binary fission. Some rod-shaped bacteria in same cases can form endospores which resist them to temperatures and unfavorable conditions. When conditions become favorable, the spores germinate and become a vegetative cell with reproduction ability (Anonymous 1999, 2019).

In addition to bacteria that cause spoilage and diseases, most are highly beneficial. Some of these bacteria are quite important as they provide various functions. Beneficial such as probiotics are increasingly important for food processor. At the same time, bacteria such as lactic acid bacteria are essential in the production and maturation of fermented foods and beverages (Ray and Bhunia 2013; Erten et al. 2014; Agirman and Erten 2018).

Fungi

Fungi are a broad group of eukaryotes and mushrooms, molds and yeasts belong to this big taxonomical group. Fungi exhibit remarkable diversity and are predicted to have more than five million species. *Zygomycota* and the Ascomycota phyla are important for the food industry. Fungi are non-photosynthetic, heterotrophic organisms and have a cell wall. Yeasts are unicellular fungi, while mushrooms and molds are multicellular (Yu 2015). Some of these microorganisms may produce toxins (mycotoxins). However, they are used in the production of some foods (some special cheeses) and some of fungi types are edible (mushrooms) (Anonymous 2019).

Molds

Mold is a visible group of fungi that live on plant or animal matter. Molds can grow in many types of food environments. The presence of molds in many plants and their tolerance to acidic and dry conditions make them particularly problematic for food producers (Yu 2015).

Molds have both beneficial and detrimental effects on the foods. Some molds are safe to eat and some of them are used in the manufacture of different foods and are ingredients of some foods (Lasztity 2004). On the other hand, some can be quite toxic and may cause allergic reactions, or produce poisonous substances called mycotoxins. Mycotoxins are toxic secondary metabolites, are not readily destroyed by cooking, and their toxicities are not often treatable (Yu 2015).

Under a microscope, most are filamentous (hypha) organism, have branches and roots that are like very thin threads. And when hyphae come together, micelles are formed. Molds can grow rapidly, reproducing mainly asexually via spores. In general, molds require less moisture than bacteria and yeasts. Most molds grow well at ordinary room temperatures, require free oxygen for growth and grow over a wide range of pH. Therefore, molds grow easily on the surface of contaminated food (Lasztity 2004). *Aspergillu*s (*A.*) and *Penicillium* (*P.*) molds are common storage molds that can survive at low water activities (Yu 2015; Campbell 2016).

Yeasts

Yeasts are non-photosynthetic, single-cell, usually oval-shaped, living fungi. They are smaller than molds, but larger than bacteria. They reproduce mainly by budding, some species reproduce by forming spores within a special cell (Erten et al. 2014; Moral et al. 2017).

Yeasts are important for preservation and spoilage of foods and some of them can be pathogenic. They become a classic food contaminant because of their ability to grow at unfavorable conditions and even in the presence of some chemical preservatives. *Zygosaccharomyces* (*Z.*) *rouxii*, *Z. bailii*, *Saccharomyces (S.) cerevisiae*, *Debaryomyces hansenii* (*D.*), *Brettanomyces bruxellensis* are some important food spoilage species (Tournas et al. 2000). On the other hand, they are largely beneficial to human culture and are widely used in the food industry. They have been accepted as the preferred organism for the production of alcoholic beverages, industrial alcohols, baker's yeast, meat and dairy products, enzymes and yeast-derived flavour products (Walker 1999; Moral et al. 2017). The genus mainly present in fermentations are the species of *Saccharomyces* (especially *S. cerevisiae*), *Pichia* (*P.*), *Debaryomyces*, *Candida* (*C.*) and *Kluyveromyces* (*K.*) and *Yarrowia* (Erten et al. 2014).

Yeasts grow more slowly than bacteria depending on conditions. On the other hand, they are quite adaptive to adverse conditions such as acidity and dehydration. Therefore, they can be more dominant than bacteria in such environments. Like molds, yeasts are more tolerant to cold than heat. Yeasts and their spores possess little resistance to heat and most of the yeast forms are destroyed when heating to about 80 °C (Moral et al. 2017).

Microbial Growth

The factors related to the environment and the conditions which food stored are effective on the growth of microorganisms. These factors divided into two groups.

Intrinsic Factors

pH and Buffering Capacity

All organisms have a characteristic pH range where growth is possible. pH is important because of its effect on proteins and plasma membrane. A small number of organisms can grow at pH <2 and >10, most microorganisms grow best at pH 7 and poorly below pH 4. Compared to bacteria, generally yeasts and molds are more acid tolerant (Tortora et al. 2010a, b). However, some bacteria such as acetic or lactic acid bacteria, who produce acid during their metabolism, can grow at low pH (Wareing et al. 2011). In general, bacteria grow fastly in the pH range 6.0–8.0, yeasts 4.5–6.0 and filamentous fungi 3.5–10.0 (Doyle and Beuchat 2007).

The level of pH affects the growth of microorganisms. It is also effective on the survival rate during storage and various conservation treatments (Anonymous 2019). Buffering capacity is another important control mechanism of foods on microorganisms. It means ability of foods to resist changes according to the pH value. Foods with a low buffering capacity will rapidly change pH in reply to acidic or alkaline compounds produced by microorganisms (Anonymous 2003).

Redox Potential

Redox potential (Eh) is a measure of the tendency of a chemical species to gains or loses electrons. Different foods have different redox potentials and this is effective on the growth of microorganisms in food matrices (Moral et al. 2017). Aerobic organisms require an environment to have a high redox potential while anaerobes require a low redox potential for growth (Wareing et al. 2011; Moral et al. 2017). Salt and other nutrient components affect the relationship between Eh and growth of microorganisms. The measured Eh values vary according to features of food such as pH, microbial growth, packaging, the partial pressure of oxygen in the storage environment, ingredients etc. (Anonymous 2003).

Water Activity (Moisture Content)

One of the most important factors affecting the growth of microorganisms is amount of available water in a food. For optimal growth, microorganisms generally require aw values that close to aw level of many fresh foods (0.97–0.99). The minimum

water demand for each microorganism is different. A large number of microorganisms can protect themselves in a hidden state in foods with low water levels, which can regain their growth ability after rehydration (Mossel et al. 1995; Anonymous 2019). Bacteria are more sensitive to low aw than molds and yeasts. Gram positive bacteria are generally more resistant to low aw than Gram negative bacteria (Wareing et al. 2011). Most bacteria require 0.9 aw for active metabolism, especially pathogenic and spoilage bacteria cannot grow in water activity less than 0.85 (Tortora et al. 2010a, b).

Nutrient Content

Organic compounds (amino acids, vitamins etc.) are growth factors for microorganisms and they could not be synthesized by microorganisms. These compounds are essential cellular components or precursors of these components and the growth rate of microorganism is limited by the availability of these components (Tortora et al. 2010a, b). The nutrient content and amount will determine what kind of microorganisms can grow (Lasztity 2004). In general, the nutrient requirement of the molds is minimal; it follows the yeasts and then the bacteria. The abundance of nutrients promotes the growth of foodborne pathogens. These nutrients include water, a source of energy, nitrogen, vitamins (e.g., thiamine, biotin, cobalamin, pyridoxine), and minerals (phosphorus, iron, magnesium, sulfur, manganese, calcium, and potassium) (Jay 2000; Moral et al. 2017).

Other Factors

Biological structure: Biological structures, especially in raw foods, can prevent the entry of microorganisms into food and thus make it difficult for a deep infection to occur (Anonymous 2019). Egg cuticle, testa of seeds, skin of fruits and vegetables, shell of nuts and membranes are some of these physical barriers (Jay 2000; Moral et al. 2017).

Natural substances: Antimicrobial substances have an inhibitory effect on the growth of microorganisms and are found naturally in certain foods. Some foods such as spices, mineral oils, garlic, mustard, honey contain natural substance capable of inhibiting the growth of some deteriorating bacteria. At the same time some vegetables (cabbage, broccoli, cauliflower, and radish) have antibacterial and antifungal effect due to their isothiocyanates content (Wareing et al. 2011).

Extrinsic Factors

The characteristics of the environment, where the food is kept, are effective on the properties of food and growth potential of the microorganisms.

Table 2 Microorganisms and temperature range for growth

Microorganisms	Minimum (°C)	Optimum (°C)	Maximum (°C)
Psychrophiles	(−15)–5	15–20	20–30
Psychrotrophs (facultative psychrophiles)	(−5)–7	25–30	30–40
Mesophiles	5–25	25–40	40–45
Thermophiles	40–45	55–65	70–90
Facultative thermophiles	35–40	45–55	60–80

Temperature

All microorganisms require a certain temperature to grow at maximum speed. Since temperature affects the enzymatic reactions, it is effective in promoting or preventing microbial growth. Five groups of microorganisms have been reported based on their temperature requirement for growth (Table 2) (Wareing et al. 2011). Additionally, hyperthermophiles, usually Archaea, grow at ≥80 °C (Tortora et al. 2010a, b).

Microbial growth occurs slowly if the temperature, that the microorganisms are exposed, is lower or higher than the optimum growth temperatures. However, microbial growth inactivates when temperatures are below the minimum value or above the maximum (Anonymous 2019). Heat-sensitive enzymes became inactive and structural cell components are denatured at high temperatures. Reaction rates of the enzymes in the microorganism become slower and the fluidity of the cytoplasmic membrane decreases at low temperatures (Mossel et al. 1995; Anonymous 2003). The growth of thermophiles and mesophiles could be controlled by cooling, thus preventing spoilage. Freezing keeps microorganisms in an inactive state without destroying them. Most pathogens could not be controlled through cooling because they can multiply at cooling temperatures (Wareing et al. 2011).

Atmosphere

Oxygen is mandatory for many microorganisms to survive, while some microorganisms cannot survive in the presence of oxygen. The organisms are classified as follows according to the usage of oxygen:

Aerobic; O_2 is essential for growth.
Facultative; can grow under either aerobic or anaerobic conditions.
Anaerobic; grow only in the absence of O_2 and brief exposure able to kill these organisms.
Aerotolerant; tolerate O_2 and grow with its presence even though they could not use O_2.
Microaerophilic; grow only at reduced O_2 concentrations (2–10%) (Tortora et al. 2010a, b).

The antimicrobial activity of gases on microorganisms is important in foods. Gases may have a direct toxic effect which may inhibit the growth and reproduction of microorganisms. The atmosphere has negative effects on the growth of particular microorganisms, while sometimes has positive effects. Since all microorganisms have special requirements for oxygen and carbon dioxide, the growth of microorganisms could be controlled by changing the atmosphere in a food package. These technologies include modified atmosphere packing (MAP), controlled atmosphere packaging (CAP), vacuum package, direct addition of carbon dioxide (DAC), and hypobaric storage (Loss and Hotchkiss 2002). The shelf life of foods could be extended by using these technologies.

Relative Humidity

The water activity of a food and thus the growth of microorganisms is related to the relative humidity in which the food is stored (Wareing et al. 2011). Especially high relative humidity supports microbial growth of surface-living creatures (aerobes). Foods should be kept in relatively low humidity conditions; otherwise, amount of water in foods will increase due to high humidity environment. Therefore, the risk of microbial proliferation is going to increase. The transition of moisture into the product can be limited by packaging (Anonymous 2019).

Interactions of Factors (Hurdle Technology)

Traditional food preservation techniques have used combinations of some growth factors, many preservatives and other inhibitory factors (Anonymous 2003). Each preservative factor is called as "hurdle". For instance, controlling temperature, redox potential, water activity and utilization of preservatives are frequently used hurdles for food preservation. Nowadays, the demand for the minimum processing of food is increasing and this leads to more use of hurdle technology. With this technology, cells are damaged by the conditions of a hurdle and become more susceptible to other hurdles. Consequently, microorganisms could be eliminated more easily (Wareing et al. 2011).

Microorganisms in Foods and Beverages

Natural Microflora of Meat and Fish

Meat could be simply described as edible tissue of mammals. Generally, skeletal muscles with associated fat is considered as meat, however it also includes organs, livers, brains, bone marrow, kidneys, lungs as well as skin (Thanigaivel and Anandhan 2015). Meat could be derived from various animals worldwide such as

goats, lambs, sheep, poultry, fish, pigs, shellfish, beef, cow, buffaloes, calf, frogs and alligators (Zakpaa et al. 2009). Pork is largely eaten meat species in the world representing up over 36% of the world meat consumption followed by poultry and beef with around 35% and 22% respectively (FAO 2014).

The fact that, especially to being the main source of animal protein makes meat indispensable part of human diet as well as it supplies vitamins, minerals, lipids and savory sensation. Meat is generally eaten as cooked but sometimes partially cooked or raw and many different foods are prepared using meat as a raw material (Thanigaivel and Anandhan 2015). Some common or traditional meat products in the world are salami (Europe), pastırma (Turkey), alheria sausage (Portugal), androlla sausage (Spain), kargyong (Tibet), suka ko masu, satchu, arjia, jamma, chilu, kheuri (India, Nepal, Bhutan, China) and naem sausage (Thailand) (Rai et al. 2010; Tamang et al. 2015).

Today there are very new array of tools such as Next Generation Sequencing (NGS) and omics-based technologies including genomics, transcriptomics, proteomics, metagenomics, and metabolomics along with the bioinformatics methods are providing a powerful way to analyze microbiology of meat based and all other types of foods. Microbiota of raw, processed or fermented meats are generally dominated by species of lactic acid bacteria even though some meat spoilage bacteria mostly *Staphylococcus* spp. and *Enterococcus* (*E.*) spp. Raw meats are rich in some lactic acid bacteria species as *Carnobacterium divergens*, *Lactobacillus (Lb.) sakei*, *Lb. curvatus*, *Lb. fuchuensis*, *Lactococcus (Lc.) piscium*, *Lc. plantarum*, *Lb. plantarum*, *Leuconostoc* (*Leu.*) *gelidum*, *Leu. mesenteroides*, *Weissella* (*W.*) spp., *Pediococcus* (*Ped.*) spp., while some yeasts as *Trichosporan* spp., *Rhodotorula* (*R.*) spp. and *Candida* spp. (Kröckel 2013; Thanigaivel and Anandhan 2015; Geeraerts et al. 2019). In addition, some bacterial and mold pathogens as *Esherichia coli*, *Staphylococcus aureus*, *Clostridium* (*C.*) *perfringes*, *Listeria monocytogenes*, *Pseudomonas* (*P.*) spp., *Hafnia alvei*, *Salmonella* spp., *A. niger*, *Mucor* spp., *Penicillium* spp., *Alternaria* spp. are isolated from different types of raw meats (Thanigaivel and Anandhan 2015; Geeraerts et al. 2019).

Fermented meats have broad range of different microorganisms. Some of them; *Lb. curvatus*, *Lb. paraplantarum*, *Lb. plantarum*, *Lb. sakei*, *Lb. brevis*, *Lb. carnis*, *Lb. casei*, *Lb. curvatus*, *Lb. divergens*, *Lb. sanfransiscensis*, *Leuconostoc carnosum*, *Leu. gelidium*, *Leu. pseudomesenteroides*, *Leu. citreum*, *Leu. mesenteroides*, *Ped. acidilactici*, *Ped. pentosaceus*, *W. cibaria*, *W. viridescens*, *B. lentus*, *B. licheniformis*, *B. mycoides*, *B. subtilis*, *B. thuringiensis*, *E. cecorum*, *E. durans*, *E. faecalis*, *E. faecium*, *E. hirae*, *D. hansenii*, *Deborymyces polymorphus*, *P. burtonii*, *P. anomala*, *C. famata* (Rai et al. 2010; Cocolin et al. 2011; Nguyen et al. 2013; Tamang et al. 2015).

Pastırma, a traditional dry-cured meat product, contain high number of *Lb. sakei*, *W. cibaria* and *W. confusa* with lesser extent *Ped. pentosaceus*, *Ped. acidilactici*, *Leu. carnosum*, *W. hellenica*, *Lb. plantarum*, *Lb. paraplantarum*, *Lb. curvatus*, *W. halotolerans*, *Lb. graminis*, *Lb. carnosus*, *Leu. citreum*, *Leu. mesenteroides* (Öz et al. 2017). A Portugal fermented sausage Alheira microflora dominated by lactic acid bacteria as *Lb. plantarum*, *E. faecalis*, *Lb. paraplantarum*, *Lb. brevis*, *Lb. rham-*

nosus, *Lb. sakei*, *Lb. zeae*, *Lb. paracasei*, *Leu. mesenteroides*, *Ped. pentosaceus*, *Ped. acidilactici*, *W. cibaria*, *W. viridescens* (Albano et al. 2009). *Lb. sakei*, *Lb. curvatus*, *Lb. plantarum* and *Pediococcus* spp. strains were reported in dry fermented Spanish Chorizo while *Lb. sakei*, *Lb. curvatus*, *Lb. plantarum*, *Lb. farciminis*, *Weissella* spp. and *E. faecium* were found in naturally fermented Greek salami (Kröckel 2013). *Leu. carnosum* and *W. viridesncens* frequently isolated from ham (Comi and Iacumin 2012).

Surface of marine fish includes *Acinetobacter calcoaceticus*, *Alcaligenes faecalis*, *B. cereus*, *B. firmus*, *Esherichia coli*, *Hyphomicrobium vulgare*, *Vibrio harveyi*, *Photobacterium angustum*, *Photobacterium logei*, *Prosthecomicrobium*, *P. fluorescens*, *P. marina*, and *Vibrio* spp. while gill microflora of marine fish is hosting *Achromobacter*, *Alcaligenes*, *Bacillus*, *Flavobacterium* and *Micrococcus* species (Austin 2002). Some bacterial genera determined in different types of fish skin as follows: salmon (*Pseudomonas* spp., *Vibrio* spp., *Flavobacterium* spp., *Micrococcus*, *Bacillus*, *Corynebacterium*, *Enterobacteria*), turbot (*Alcaligenes* spp., *Bacillus* spp., *Flexibacter* spp., *Lucibacterium harveyi*, *Acinetobacter calcoaceticus*, *Flavobacterium* spp.), sea mullet (*Micrococcus* spp., *Pseudomonas* spp., *Moraxella*, *Flavobacterium*, *Staphylococcus*, *Aeromonas* spp., *Vibrio* spp., *Bacillus* spp.) (Cahill 1990).

Natural Microflora of Fruits and Vegetables

Fruits and vegetables are significant part of a healthy diet of human and consumed daily by most civilizations at all times. Because, they are generally rich in terms of fiber, minerals, vitamins and antioxidants. In addition, fruits and vegetables do not contain cholesterol and they generally have low values of fat and calorie. Fruits and vegetables could be found with several types as fresh, cooked, hot or cold, canned, pickled, frozen and dried (Vicente et al. 2009; Rathi and Dhiman 2016).

Conventionally, fruits and vegetables have been considered as safe foods with regard to microbiological aspect unlike other processed foods such as meat, milk, eggs, seafood and poultry. Broad range of microbial communities belonging to diverse genera of bacteria, yeasts and fungi constitute the natural microflora of fruits and vegetables (Table 3). Type of fruits and vegetables, environmental factors, seasonality and harvesting circumstances substantially affect the microflora (Sajur et al. 2007). Epiphytic microflora of fruits and vegetables are generally composed of lactic acid bacteria and yeasts although sometimes pathogenic species are present. Some of these harmful microorganisms able to produce fungal metabolites which are dangerous for human. For instance, patulin, a secondary toxic metabolite, could be produced by *P. expansum* in apples (Cao et al. 2013) and *A. corbonarius* is account for accumulation of ochratoxin A (OTA), a immunotoxic—carcinogenic fungal metabolite, on grapes (Paola and Marco 2015).

Ajayi (2013), isolated numerous bacterial and fungal species as *Leuconostoc* spp., *Pediococcus* spp., *Bacillus* spp., *Streptococcus* (*St.*) spp., *Aspergillus* spp.,

Table 3 Microorganisms in some fresh fruits and vegetables

Product	Bacteria	Yeast-yeastlike-fungi	References
Tomato	*Leuconostoc* spp., *Pediococcus* spp., *Bacillus* spp., *Streptococcus* spp., *Micrococcus* spp., *Klebsiella/Raoultella* spp., *Pectobacterium* spp., *Leu. mesenteroides* ssp. *mesenteroides*	*Saccharomyces* spp., *Aspergillus* spp., *Neurospora* spp., *Penicillium* spp.	Sajur et al. (2007), Ajayi (2013), Leff and Fierer (2013), Harding et al. (2016)
Grape	*Lactobacillus* spp., *Pediococcus* spp., *Gluconobacter* spp., *Acetobacter* spp., *Bacillus* spp., *Ralstonia* spp., *Pseudomonas* spp., *Curtobacterium* spp., *Cellulomonas* spp.	*Deboryomyces hansenii*, *Metschnikowia pulcherrima*, *Hanseniaspora uvarum*, *Kloeckera apis*, *C. stellata*, *Issatchenkia* (*I.*) *terricola*, *Pichia* spp., *Saccharomyces* spp., *Rhodotorula* spp., *Botrytis cinerea*, *Penicillium spp.*, *Aspergillus spp.*	Delfini et al. (2002), Zahavi et al. (2002), König et al. (2009), Maulani et al. (2012), Martins et al. (2013), Kantor et al. (2015), Kasfi et al. (2018a, b)
Apple	*Acetobacter* spp., *Gluconobacter* spp., *Lactobacillus* spp., *Leuconostoc* spp., *Pediococcus* spp., *Photobacterium* spp., *Pantoea aglomerans*, *P. syringae*, *B. cereus*, *Erwinia amylovora*, *Clavibacter michiganense*, *Curtobacterium flaccumfaciens*	*P. expansum*, *C. pulcherrima*, *C. krusei*, *Saccharomyces* spp., *Deboryomyces* spp., *Hansenula* spp., *Aspergillus* spp., *Monilinia* spp., *Mucor* spp., *Aureobasidium pullulans*, *Cryptococcus magnus*, *R. minuta*, *Cryptococcus victoriae*, *Starmerella bombicola*	Doores (1983), Pusey et al. (2009), Cao et al. (2013), Leff and Fierer (2013)
Pear	*Escherichia coli*, *L. monocytogenes*, *Salmonella enteritidis*	*Aureobasidium pullulans*, *Cryptococcus albidus*, *R. glutinis*, *D. hansenii*, *Cryptococcus laurentii*, *Sporobolomyces roseus*, *Penicillium* spp.	Chand-Goyal and Spotts (1996), Raybaudi-Massilia et al. (2009)
Melon	*Acinetobacter* spp., *S. enterica*, *Escherichia coli*, *Salmonella* spp., *Listeria* spp.	*Alternaria* spp., *Cladosporium* spp., *Fusarium* (*F.*) spp., *Geotrichum* spp., *Penicillium* spp., *Rhizopus* spp., *Mucor* spp.	Richards and Beuchat (2005), Harding et al. (2016), Svoboda et al. (2016)
Cabbage	*Pseudomonas* spp., *Proteus vulgoris*	*P. olsonii*	Shobha (2014), Harding et al. (2016)
Onion	*Enterobacter* spp., *Pantoea agglomerans*, *Rahnella aquatilis*	*F. proliferatum*, *P. polonicum*, *P. glabrum*, *P. simplicissimum*, *F. proliferatum*, *A. niger*	Harding et al. (2016)

(continued)

Table 3 (continued)

Product	Bacteria	Yeast-yeastlike-fungi	References
Carrot	*Lactobacillus* spp., *Agrobacterium tumefaciens*, *Pantoea* spp., *P. putida*, *Serratia plymuthica*, *B. cereus*	*F. reticulatum*, *Saccharomyces* spp., *Pichia* spp., *Rhodotorula* spp., *A. flavus*, *P. islandicum*, *Geotrichum* spp., *A. terreus*	Aneja et al. (2014), Harding et al. (2016), Pylypenko et al. (2016)
Bean	*Rhodococcus* spp., *Pantoea* spp., *Klebsiella/Raoultella* spp.	*Alternaria alternata*, *Cladosporium oxysporum*, *Sclerotinia sclerotiorum*	Tournas (2005a), Leff and Fierer (2013), Harding et al. (2016)
Pea	*Pantoea agglomerans*	*A. niger*, *F. oxysporum*, *Mucor* spp., *A. ochraceus*, *F. verticilloides*, *A. oryzae*, *A. flavus*	Harding et al. (2016), Adjou et al. (2017)
Broccoli	*Serratia grimesii*	*F. equiseti*, *F. solani*	Harding et al. (2016)
Pepper	*Pantoea agglomerans*, *Stenotrophomonas* spp., *Escherichia coli*, *Streptococcus* spp., *Serratia* spp., *Bacillus* spp., *Staphylococcus* spp., *Klebsiella* spp.	*A. flavus*, *A. ochraceus*, *A. restrictus*	Christensen et al. (1967), Leff and Fierer (2013), Harding et al. (2016)
Celery	*Serratia plymuthica*, *Stenotrophomonas maltophilia*, *Pseudomonas* spp., *Aeromonas* spp., *Salmonella* spp., *Shigella* spp.	*Sclerotinia* spp., *Cephalosporium apii*	Buck et al. (2003), Tournas (2005a), Barth et al. (2009), Harding et al. (2016)
Squash	*Enterobacter cloacea*, *Acinetobacter calcoaceticus*, *Pantoea agglomerans*, *Escherichia coli*, *Salmonella* spp.	*Phoma* spp., *Stagonosporopsis* spp.	Castro-Rosas et al. (2010), Harding et al. (2016)
Potato	*P. fluorescens*, *Lactobacillus* spp., *B. cereus*, *Streptococcus* spp., *Staphylococcus aureus*, *Erwinia carotovora*, *P. marginalis*	*Aspergillus* spp., *Alternaria solani*, *Penicillium* spp., *Phytophthora infestans*, *Rhizopus stolonifer*, *F. sambucinum*, *F. solani* var. *coeruleum*	Tournas (2005a)
Strawberry	*Buchnera aphidicola*, *Bacillus* spp., *Pantoea* spp.	*Botrytis cinerea*, *Rhizopus spp.*, *Fusarium spp.*, *Alternaria spp.*, *Cladosporium spp.*, *Trichoderma spp.*, *Peniciilium spp.*	Tournas and Katsoudas (2005), Leff and Fierer (2013)
Cucumber	*Pseudomonas* spp., *Escherichia coli*, *L. monocytogenes*, *Salmonella* spp., *Erwinia carotovora*	*Cladosporium* spp., *Alternaria* spp., *Cladosporium cucumerinum*, *Colletotrichum coccode*	Tournas (2005b), Shobha (2014), Mritunjay and Kumar (2017)

(continued)

Table 3 (continued)

Product	Bacteria	Yeast-yeastlike-fungi	References
Eggplant	*Ralstonia solanacearum*, *Erwinia carotovora*	*Choanephora cucurbitarum*, *Diaporthe vexans*, *Alternaria tenuis*	Wang et al. (1998), Kwon and Jee (2005), Tournas (2005a)
Spinach	*Pantoea* spp., *Klebsiella/Raoultella* spp., *B. subtilis*, *Serratia marcescens*, *Lactobacillus* spp., *Proteus mirabilis*	*A. niger*, *A. flavus*, *Mucor mucedo*, *F. oxysporum*, *Penicillium* spp.	Oladele and Olakunle (2011), Leff and Fierer (2013)
Lettuce	*Xanthomonas* spp., *Pantoea* spp., *Pectobacterium* spp., *Leuconostoc* spp., *Janthinobacterium* spp., *Bacillus* spp., *Pseudomonas* spp., *Enterobacter* spp., *Erwinia carotovora* subsp. *atroseptica*	*Sclerotinia sclerotiorum*, *Sclerotinia minor*, *Botrytis cinerea*	Fucikovsky and Ortega (1997), Hou et al. (2013), Leff and Fierer (2013)
Mushroom	*Pseudomonas* spp., *Pedobacter* spp., *Enterobacter* spp., *Sphingomonas* spp., *Staphylococcus* spp., *Moraxella* spp., *Bacillus* spp.	*Trichoderma koningiopsis, Phomopsis* spp., *Mucor circinelloides*, *Cladosporium bruhnei*	Lim et al. (2008), Leff and Fierer (2013), Kim et al. (2013)
Orange	*Klebsiella pneumonia*, *Bacillus cereus*	*Haneniaspora uvarum*, *Hanseniaspora occidentalis*, *P. kluyveri*, *C. tropicalis*, *C. intermedia*, *I. orientalis*, *Torulaspora delbrueckii*, *Alternaria* spp., *Cladosporium* spp., *Peniciilium* spp., *Fusarium* spp., *Trichoderma* spp.	Arias et al. (2002), Tournas and Katsoudas (2005), Rathi and Dhiman (2016)
Lemon	*Bacillus subtilis*, *Micrococcus* spp., *Staphylococcus epidermidis*, *Enterobacter cloacae*, *Acinetobacter baumannii*, *P. putida*	*C. guilliermondii*, *C. tropicalis*, *C. lusitaniae*, *Alternaria* spp., *Fusarium* spp., *Trichoderma* spp., *Geotrichum* spp., *P. digitatum*, *P. italicum*	Tournas and Katsoudas (2005), Loving and Perz (2007)

Neurospora spp., *Clostridium* spp. and *Micrococcus* spp. from raw tomatoes. Microbial population of grape much diverse and commonly include *Pseudomonas* spp., *Sphingomonas* spp., *Micrococcus* spp., *Massilia* spp., *Enterobacter* spp., *Bacillus* spp., *Meyerozyma guilliermondii*, *C. membranafaciens*, *P. anomala*, *Hansenula anomala*, *Hanseniaspora uvarum*, *K. marxianus* and *R. mucilaginosa* (Martins et al. 2013; Kantor et al. 2015; Kasfi et al. 2018a, b). Some types of bacteria isolated from surface of apples are *Bacillus cereus*, *Pantoea agglomerans*, *P. syringae*, *Microbacterium laevaniformans*, *Arthrobacter globiformis*, *Rhizobium radiobacter*, *Clavibacter michiganense* and *Erwinia amylovora* (Pusey et al. 2009).

Natural Microflora of Cereal and Cereal Products

Cereals and cereal-based products are significant food sources for human worldwide. Cereal grains and legumes are used either raw materials or additives for preparation of many foods in most cultures. Since, cereals include rich source of nutrients as vitamins, minerals, carbohydrates, fats, oils and protein (Bullerman and Bianchini 2009). Most abundant cereals in the world are wheat, barley, rice, maize, millet, sorghum, rye, oats and triticale. Some common and traditional cereal products as follows: flour, bread, pasta, beer, noodles, sourdough, pastries, tarhana (Turkey), idli (India and Sri Lanka), kisra (Sudan), ogi (Nigeria), rabadi (India and Pakistan), selroti (India and Nepal) and Pitha (Bangladesh) (FAO 1999; Tamang et al. 2015).

Microflora of cereals and cereal fermentations composed of complex ecosystem. Although cereals microflora is characterized by various yeasts, molds and bacteria (psychrotrophic, mesophilic, and thermophilic, pathogens), fermented cereals are dominated mainly by lactic acid bacteria and yeasts (Bullerman and Bianchini 2009; Tamang et al. 2015). *B. cereus*, *C. botulinum*, *C. perfringens*, *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus* are reported as sources of bacterial contamination in cereal grains and cereal products. Grains with high moisture content could be host for some fungi such as *Alternaria*, *Cladosporium*, *Fusarium*, *Eurotium*, *Aspergillus*, *Penicillium* and *Helminthosporium*. These fungi cause some disease in cereal grains such as "blue eye" with related to presence of *Penicillium* and *Aspergillus* species (Bullerman and Bianchini 2009). Cereal fermentations is generally comprising of species as *Lactococcus*, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Streptococcus* and *Weissella*. *S. cerevisiae* is the main yeast for most bread fermentation. Non-*Saccharomyces* yeasts belonging to *Candida*, *Debaryomyces*, *Hansenula*, *Pichia*, *Trichosporon*, *Kazachstania* and *Yarrowia* genus are also important for many cereal based food fermentations (Tamang et al. 2016a, b).

Sourdough bread has been produced since ancient times. Sourdough is produced by lactic acid fermentation of mixture consist of flour (i.e. wheat, rye, maize) and water. Extensive distribution of lactic acid bacteria and yeasts species isolated from sourdough were reported in literature. Some of them as follows: *S. cerevisiae*, *S. exiguus*, *I. orientalis*, *C. humuli*s, *Lb. sanfranciscensis*, *Lb. brevis*, *Lb. buchneri*, *Lb. fermentum*, *Lb. reuteri*, *Lb. pontis*, *Lb. acidophilus*, *Lb. delbrueckii*, *Lb. plantarum*, *Lb. alimentarius*, *Lb. paralimentarius*, *Lb. mindensis*, *Lb. farciminis*, *Leu. mesenteroides* subsp. *dextranicum*, *Leu. mesenteroides* subsp. *mesenteroides*, *Ped. pentosaceus*, *Lb. curvatus*, *W. confusa*, *W. viridescens*, *Lb. reuteri* and *Lb. johnsonii* (De Vuyst and Neysens 2005). Tarhana is a traditional Anatolian fermented cereal product and wheat flour is one of the main components of its recipe. Tarhana fermentation is driven by lactic acid bacteria and yeasts such as *Lb. plantarum*, *Lb. brevis*, *Leu. mesenteroides*, *Lb. paralimentariu*s, *Lb. namurensis*, *Lb. alimentariu*s, *Lb. mindensis*, *Lb. casei*, *Ped. acidilactici*, *Lc. lactis*, *Lb. farciminis*, *Lb. vini*, *Lb. fabifermentans*, *W. cibaria*, *S. cerevisiae*, *C. humilis*, *P. kudriavzevii* and *C. glabrata* (Şimşek et al. 2017; Özdemir et al. 2018). *Ped. pentosaceus*, *Lb. plantarum*, *Lb.*

brevis, *Lc. lactis*, *Lb. fermentum* were isolated from fermentation of batter (Khade and Phirke 2015).

Microorganisms present in some fermented cereal foods in the world are reported as follows: boza (*Lactobacillus* spp., *Lactococcus* spp., *Pediococcus* spp., *Leuconostoc* spp., *S. cerevisiae*), idli (*Leu. mesenteroides*, *Lb. delbrueckii*, *Lb. fermenti*, *Lb. coryniformis*, *Ped. acidilactici*, *Ped. cerevisiae*, *Streptococcus* spp., *E. faecalis*, *Lc. lactis*, *B. amyloliquefaciens*, *C. cacaoi*, *C. fragicola*, *C. glabrata*, *C. kefyr*, *C. pseudotropicalis*, *C. sake*, *C. tropicalis*, *D. hansenii*, *D. tamarii*, *I. terricola*, *Rhizopus graminis*, *S. cerevisiae*, *Torulopsis candida*, *Torulopsis holmii*), Kunu-zaki (*Lb. plantarum*, *Lb. pantheris*, *Lb. vaccinostercus*, *Corynebacterium* spp., *Aerobacter* spp., *C. mycoderma*, *S. cerevisiae*, *Rhodotorula* spp., *Cephalosporium* spp., *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp.), kisra (*Ped. pentosaceus*, *W. confusa*, *Lb. brevis*, *Erwinia ananas*, *Klebsiella pneumoniae*, *E. cloacae*, *C. intermedia*, *D. hansenii*, *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Rhizopus* spp.), ogi (*Lb. plantarum*, *Lb. pantheris*, *Lb. vaccinostercus*, *Corynebacterium* spp., *Aerobacter* spp., *C. krusei*, *Clavispora lusitaniae*, *S. cerevisiae*, *Rhodotorula* spp., *Cephalosporium* spp., *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp.), rabadi (*Ped. acidilactici*, *Bacillus* spp., *Micrococcus* spp.), selroti (*Leu. mesenteroides*, *Enterococccus faecium*, *Ped. pentosaceus*, *Lb. curvatus*, *S. cerevisiae*, *S. kluyveri*, *D. hansenii*, *P. burtonii*, *Z. rouxii*), Dégué (*Lb. gasseri*, *Lb. fermentum*, *Lb. brevis*, *Lb. casei*, *Enterococcus* spp.), jalebi (*S. bayanus*, *Lb. fermentum*, *Lb. buchneri*, *Lc. lactis*, *E. faecalis*, *S. cerevisiae*), hulumur *(Leu. mesenteroides*, *Lb. plantarum*, *Lactobacillus* spp.) and lao-chao (*Rhizopus oryzae*, *Rhizopus chinensis*, *Chlamydomucor oryzae*, *Saccharomycopsis* spp.) (Tamang et al. 2015; Tamang et al. 2016a, b).

Natural Microflora of Other Foods

There are many different types of foods except the foods mentioned above. Tea, coffee, milk, cocoa, chocolate, coffee, eggs, frozen foods and canned foods have quite high consumption level throughout the world. People consume daily at least one of these foods and various microorganisms are taken into body through them. Wide range of bacteria, yeasts, fungi, yeast-like fungi, molds and pathogens belonging to different taxa are present intrinsically in these foods. Also, fermentation, different technological processing techniques and contamination (from soil, air, water, materials, storage etc.) led to existence of different microorganism apart from natural microflora of these foods.

Coffee and tea are the two most consumed beverages in the world. Coffee aroma is mainly formed during roasting and then brewing and post-harvest coffee processing (dry and wet). The role of fermentation step during coffee processing is to help removal of mucilage layer as well as contribute the aroma profile of coffee. Lactic acid bacteria such as *Leu. mesenteroides*, *Lb. brevis* and yeast species as *Kloeckera apiculata*, *C. guilliermondii*, *C. tropicalis*, *C. parapsilosis*, *Cryptococcus albidus*,

Cryptococcus laurentii, *P. kluyveri*, *P. anomala*, *Hanseniaspora uvarum*, *S. cerevisiae*, *D. hansenii*, *Torulaspora delbrueckii*, *K. marxianus*, *C. pseudointermedia*, *I. orientalis*, *P. ohmeri* and *R. mucilaginosa* were isolated from coffee fermentations. Additionally, some aerobic bacteria such as *Klebsiella ozaenae*, *Klebsiella oxytoca*, *Erwinia herbicola*, *Erwinia dissolvens*, *Hafnia* spp. and *Enterobacter aerogenes* were reported (Masoud et al. 2004; Lee et al. 2015). There are many types of tea according to processing method although common types raw (green tea) and fermented (black tea). Bacteria and mold species found in both green and black tea are *Bacillus* (*B.*) *amyloliquefaciens*, *B. cereus*, *B. thuringensis*, *B. licheniformis*, *B. subtilis*, *C. perfringens*, *A. niger*, *A. tubingensis*, *Penicillium commune*. *B. aryabattai*, *B. drentensis*, *B. endophyticus*, *Paenibacillus lactis*, *Paenibacillus taichungens*, *Penicillium rubens*, *Penicillium brevicompactum*, *Cryptococcus neoformans* were reported just in green tea while *Bacillus circulans*, *Bacillus pseudomycoides*, *Paenibacillus cineris*, *Pantoea gaviniae*, *P. psychrotolerans*, *Staphylococcus warneri*, *Rhizopus oryzae*, *Cladosporium ramotenellum*, *R. mucillaginosa* and *Sporidiobolis ruineniae* were determined in black tea (Carraturo et al. 2018).

Milk is rich in microorganisms because of its highly nutritious content which includes proteins, fats, carbohydrates, minerals, vitamins and essential amino acids. Although a large portion of commercial milk production obtained from cows, milk is supplied by numerous animal sources such as sheep, goats, buffalo, camel, yaks, donkeys, mares. Microbial population of raw milk based on the sources varies and it effects the flavor, taste and texture of the milk. Some of the milk microorganisms exhibit beneficial impact on human health, while there can be an important risk due to pathogens present in raw milk. Some dominant and subdominant microorganisms found in different milk types are; *Lb. casei*, *Lb. curvatus*, Lb. mindensis, *Lb. animalis*, *Lb. coryneformis*, *Lb. curvatus*, *Lb. delbrueckii*, *Lb. johnsonii*, *Lb. paracasei*, *Lb. paraplantarum, Lb. plantarum*, *Lb. rhamnosus*, *Lb. amylovorus*, *Lc. lactis*, *Lc. garvieae*, *Leu. mesenteroides*, *Leu. citreum*, *Leu. lactis*, *Acinetobacter johnsonii*, *Acinetobacter junii*, *Acinetobacter haemolyticus*, *Acinetobacter lwoffii*, *Bifidobacterium pseudolongum*, *Pantoea agglomerans*, *Rhodococcus erythropolis*, *Microbacterium oxydans*, *P. putida*, *P. aeruginosa*, *P. fulgida*, *Micrococcus caseolyticus*, *Staphylococcus epidermidis*, *Staphylococcus simulans*, *Staphylococcus caprae*, *Staphylococcus equorum*, *E. faecalis*, *E. saccharominimus*, *Escherichia coli* and *Salmonella* spp. (Quigley et al. 2013; Baur et al. 2015).

Eggs are another important widely consumed food with having important biological nutrients especially proteins and saturated/unsaturated fatty acids. Microbial ecology of eggs changes between pasteurized, unpasteurized and dried eggs and also among egg white and yolk because of the composition and indigenous antimicrobial substances. *Pseudomonas*, *Carnobacterium* and *Clostridium* species were found high abundance in raw eggs while *Enterococcus*, *Streptococcus*, *Enterobacteriaceae*, *Acitenobacter*, *Lactococcus*, *Lactobacillus*, *Arthrobacter*, *Psychrobacter*, *Shewanella*, *Paenibacillus*, *Comamonas* and *Moraxellaceae* were isolated less frequently (Vieira et al. 2019). The aim of canning foods is to destroy harmful microorganisms. Canned food could comprise of many different foods such as milk, sardines, meat, tomatoes and various vegetables. Generally, the probability

of heat stable thermophilic spore forming microorganisms in canned foods is more possible than others. *B. cereus*, *B. subtilis*, *C. perfringens* were isolated from canned meat, *B. coagulans*, *Bacillus* spp. from canned milk, *B. subtilis*, *S. aureus*, *Klebsiella* spp., *B. circulans* from canned mixed vegetables, *S. aureus*, *C. sporogenes*, *Klebsiella* spp. from canned sardine and *B. coagulans*, *S. aureus*, *S. epidermidis* from canned tomatoes (Us et al. 2012).

Frozen storage is a widely used prominent food preservation method for a long time, since it halts to the growth and proliferation of microorganisms which induce food spoilage and foodborne diseases. Furthermore, activity of enzymes causing decay of foods is stopped by freezing. Various foods are subject to freezing such as meat, fish, fruits and vegetables (i.e. cherry, potato chips, corn, peas), chicken, pastries (i.e. pizza, burger, patty). Psychrotrophic microorganisms are generally expected to found in frozen foods although different types of microorganisms could be found in them. Because, microorganisms are going to be re-activated once thawing of frozen foods. *S. aureus*, *Escherichia coli*, *Bacillus* spp., *Klebsiella* spp., *Salmonella* spp., *Flavobacterium* spp., *Listeria* spp., *Pseudomonas* spp., *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp. and *Trichodina* spp. were isolated from different kind of meats and fishes (Oranusi et al. 2014). Manani et al. (2006) reported *Pseudomonas*, *Klebsiella*, *Corynebacterium*, *Lactobacillus* spp., *Flavobacterium*, *S. aureus*, *L. monocytogenes* and *B. cereus* in frozen chips, peas and corn.

Main raw material for chocolate production is cocoa beans (*Theobroma cacao*). Chocolate process is starting with the fermentation of cocoa. Typical aroma and flavor of the product are formed in this step. Different types of microorganisms manage the cocoa fermentation such as yeasts (*Hanseniaspora guilliermondii*, *P. kudriavzevii*, *K. marxianus*, *S. cerevisiae*), LAB (*Lb. plantarum*, *Lb. fermentum*, *Lb. casei*, *Leu. mesenteroides*, *W. paramesenteroides*, *W. cibaria*) and acetic acid bacteria (*Acetobacter pasteurianus*, *Gluconobacter frateurii*) (Thuy Ho et al. 2014; Ouattara et al. 2017).

Recently, probiotic chocolates are used as functional products. It was indicated that chocolate matrix and production process is appropriate for probiotic microorganisms as well as prebiotic substances (Erdem et al. 2014). Utilization of chocolate as carrier agent for some probiotic strains such as *Lb. rhamnosus*, *L. acidophilus*, *Bifidobacterium* (*B.*) *lactis*, *L. brevis* subsp. *coagulans*, *L. paracasei* subsp. *paracasei*, *L. helveticus*, *B. longum*, *B. indicus* is reported (Konar et al. 2016).

Microbial Community of Fermented Foods

Fermentation technique has been used for the preservation of foods from ancient times. This process produces precious food products with high nutritional value, unique sensorial properties and long shelf life by bio-conversions of different raw materials used as a substrate such as fruits, vegetables, meat, milk, cocoa, flour and different beverages. There are many fermented regional and common food products all over the world. For instance; table olives, sauerkraut, vinegar, cheese, sausage,

wine, beer, pickles, yoghurt and bread are some of the common fermented foods while tarhana, kimchi, kefir, şalgam, dua muoi, tempoyak, pastırma and brovada are lesser known fermentation crops. Fermentation refers to decomposition of food-stuffs (particularly carbohydrates) achieved by divergent species of microorganisms in the presence or absence of oxygen. Major types of fermentation occurred in foods could be listed as; lactic acid, acetic acid, ethyl alcohol, propionic acid and butyric acid.

An extended variety of microflora are participating in diverse food fermentations (Table 4).

Table 4 Fermentation microflora of some common and regional foods

Product	Dominant microorganism in fermentation	Origin	References
Bread	*S. cerevisiae*, *S. exiguus*, *C. humilis*, *C. milleri*, *Issatchenkia orientalis*, *Ped. pentosaceus*, *Lb. confusus*, *Lb. brevis*, *Lb. acidophilus*, *Lb. delbrueckii*, *Lb. fermentum*, *Lb. reuteri*, *Debaryomyces* spp., *Hansenula* spp., *Pichia* spp., *Trichosporon* spp., *Yarrowia* spp.	Worldwide	Tamang et al. (2015)
Yogurt	*St. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus*, *Lb. casei*, *Lb. rhamnosus*, *Lb. gasseri*, *Lb. johnsonii*, *Bifidobacterium* spp.	Europe, Turkey	Tamime and Robinson (2007)
Cheese	*Lc. lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*, *Lb. delbrueckii* subsp. *delbrueckii*, *Lb. delbrueckii* subsp. *lactis*, *Lb. helveticus*, *Lb. casei*, *Lb. plantarum*, *Lb. salivarius*, *Leuconostoc* spp., *St. thermophilus*, *Ent. durans*, *Ent. faecium*, *Brevibacterium linens*, *Propionibacterium freudenreichii*, *D. hansenii*, *Geotrichum candidum*, *P. camemberti*, *P. roqueforti*	Worldwide	Quigley et al. (2011)
Kefir	*Lb. kefiranofaciens*, *Dekkera anomala*, *St. thermophilus*, *Lc. lactis*, *Enterococcus* spp., *Bacillus* spp., *Acetobacter fabarum*, *Acetobacter lovaniensis*, *Acetobacter orientalis*, *Kazachstania turicensis*	Caucasic, Turkey	Garofalo et al. (2015)
Vinegar	*Acetobacter aceti* subsp. *aceti*, *A. oryzae*, *A. pasteurianus*, *A. polyxygenes*, *C. lactis-condensi*, *C. stellata*, *Hanseniaspora valbyensis*, *S. cerevisiae*, *Z. bailii*, *Z. rouxii*	Worldwide	Solieri and Giudici (2008), Sengun and Karabiyikli (2011)
Table Olives	*Lb. plantarum*, *Lb. pentosus*, *Leuconostoc* spp., *Pediococcus* spp., *Lactococcus* spp., *S. cerevisiae*, *Wickerhamomyces anomalus*, *P. membranifaciens*, *C. boidinii*, *D. hansenii*, *R. mucilaginosa*	Worldwide	Erten et al. (2016)

(continued)

Table 4 (continued)

Product	Dominant microorganism in fermentation	Origin	References
Wine	*S. cerevisiae*, *Schizosaccharomyces pombe*, *Kloeckera* spp., *Candida* spp., *Metschnikowia* spp., *Dekkera* spp., *Pichia* spp., *Kluyveromyces* spp., *Issatchenkia* spp., *Saccharomycodes* spp., *Zygosaccharomyces* spp., *Torulaspora* spp., *Debaryomyces* spp., *Schizosaccharomyce* spp., *Kloeckera apiculata*, *Oenococcus oeni*, *Torulaspora delbrueckii*, *Metschnikowia pulcherrima*, *Lb. plantarum*, *Leu. mesenteroides*, *Ped. acidilactici*, *Acetobacter pasteurianus*, *Gluconobacter oxydans subsp. suboxydans*, *Brettanomyces* spp.	Worldwide	Díez et al. (2012), Erten et al. (2014), Sun et al. (2014)
Şalgam	*Lb. plantarum*, *Lb. paracasei* spp. *paracasei*, *Lb. buchneri*, *Lb. fermentum*, *Lb. brevis*, *Lc. lactis*, *Lb. delbrueckii* spp. *delbrueckii*, *Leu. mesenteroides* spp. *mesenteroides*, *Ped. pentosaceus*, *S. cerevisiae*	Turkey	Erten and Tanguler (2016)
Kimchii	*Lb. kimchii*, *Lb. brevis*, *Lb. casei*, *Lb. plantarum*, *Leu. mesenteroides*, *W. confusa*, *Ped. pentosaceus*	Korea	Patra et al. (2017)
Sauerkraut	*Leu. mesenteroides*, *Lb. plantarum*, *Lb. brevis*, *Ped. pentosaceus*, *Lb. sakei*	Worldwide	Wiander (2017)
Sausage	*Lb. pentosus*, *Lb. plantarum*, *Lb. brevis*, *Lb. paracasei*, *Lb. fermentum*, *Lb. rossiae*, *Lb. fuchuensis*, *Lb. namurensis*, *Lc. lactis*, *Leuc. citreum*, *Ped. acidilactici*, *Ped. pentosaceus*, *Ped. stilesii*, *W. cibaria*, *W. paramesenteroides*, *C. curvata*, *C. parapsilosis*, *C. zeylanoides*, *Trichosporon ovoides*, *Yarrowia lipolytica*, *Ent. faecalis*, *Ent. faecium*, *Ent. hirae*, *Leuc. mesenteroides*	Worldwide	Nguyen et al. (2011), Tamang et al. (2015)
Tempoyak	*Lb. brevis*, *Lb. mali*, *Lb. fermentum*, *Lb. durianis*, *Leu. mesenteroides*	Malaysia	Swain and Ananadharaj (2017)
Pickles	*Lb. brevis*, *Lb. plantarum*, *Lb. curvatus*, *Ped. pentosaceus*, *Leu. mesenteroides*, *Leu. fallax*, *Leu. lactis*, *Leu. citreum*, *Ent. durans*, *Lc. lactis*, *Ped. cerevisiae*, *Ped. acidilactici*, *S. cerevisiae*, *S. rosei*, *P. membranifaciens*, *Candida krusei*, *Zygosaccharomyces spp.*	Worldwide	Tamang et al. (2015), Franco et al. (2017)
Dua muoi	*Lb. fermentum*, *Lb. pentosus*, *Lb. plantarum*, *Lb. paracasei*, *Lb. pantheris*, *Ped. acidilactici*, *Ped. pentosaceus*	Vietnam	Swain and Ananadharaj (2017)
Brovada	*Lb. hilgardii*, *Ped. parvulus*, *Lb. plantarum*, *Lb. coryniformis*, *Hansenula* spp., *Candida* spp., *Issatchenkia* spp.	Italy	Erten et al. (2017)
Beer	*S. cerevisiae*, *S. pastorianus* (syn. *S. carlsbergensis*), *S. eubayanus*, *Meyerozyma guilliermondii*, *Debaryomyces* spp., *Pichia* spp., *Wickerhamomyces anomalus*, *Brettanomyces anomalus*, *Brettanomyces custersii*, *Brettanomyces bruxellensis*, *C. krusei*, *Cryptococcus keutzingii*, *Naumovia castellii*, *Kazachstania servazzii*, *R. mucilaginosa*, *Williopsis saturnus*, *Ped. claussenii*, *Lb. backii*, *L. Brevis*, *L. lindneri*	Worldwide	Geissler et al. (2016), Osburn et al. (2018), Varela and Varela (2019)

Functional Properties of Microorganisms in Different Types of Foods

Food sources include common microorganisms as the natural flora. Bacteria, mainly lactic acid bacteria, yeasts and moulds are responsible for food fermentations. During food fermentations, microorganisms use and convert organic compounds in the plant and animal sources to some metabolites. As a result of these conversions, some biochemical changes occur in foods. For example, sensorial properties of the final food products in terms of flavour and texture are enhanced, bioavailability of the nutrients are enriched, food safety are controlled due to the preservative effects, bioactive compounds are produced, anti-nutritive compounds are degraded and probiotic potential is increased (Tamang et al. 2009; Farhad et al. 2010; Bourdichon et al. 2012; Thapa and Tamang 2015; Tamang et al. 2016a, b).

Production of Bioactive Compounds

Bioactive compounds in foods are extra nutritional constituents showing physiological effects on the body to promote a better health (Kris-Etherton et al. 2002; Galanakis 2017). Many microorganisms have the ability to produce bioactive compounds during the fermentation process when microorganisms degrade substrates to various small molecules. During food fermentations, metabolism of different microorganisms leads to many end products depending on the pathway taken. The most common groups of microorganisms involved in food fermentations are bacteria, yeasts and moulds. The benefits of these microorganisms during food fermentations can be exerted through the interactions of ingested live microorganisms with the host as the probiotic effect or by ingestion of the microbial metabolites synthesized during fermentation as bioactive effect (Stanton et al. 2005; Gobbetti et al. 2010). Besides beneficial health effects, technological aspects of the produced foods are improved as a result of the fermentation with those microorganisms.

During fermentation, microorganisms follow different pathways and synthesize a large number of metabolites with assessed beneficial or detrimental properties for human health such as organic acids, enzymes, vitamins, bacteriocins, exopolysaccharides, amino acids and amino acid derivatives, bioactive peptides, biogen amines, phenolic compounds, ethanol, CO_2, bacteriocins and aroma compounds (Ray and Joshi 2015; Pessione and Cirrincione 2016).

Lactic acid bacteria are predominant bacteria in many food fermentations. Other groups of bacteria involved in food fermentations are acetic acid bacteria, *Bifidobacterium*, *Propionibacterium* and *Bacillus* species. Lactic acid bacteria produce lactic acid from the fermentation of carbohydrates as the major end product (Mayo et al. 2010). From a biochemical perspective, lactic acid bacteria can be grouped according to their ability to ferment glucose as homofermentative (homo-

lactic) or heterofermentative (heterolactic). Homofermentative lactic acid bacteria produce lactic acid as the sole fermentation end product (von Wright and Axelsson 2012), whereas heterofermentatives produce a variety of fermentation end products such as ethanol, CO_2, acetic acid, formic acid, hydrogen peroxide, acetaldehyde, diacetyl, acetoin, various bacteriocins, exopolysaccharides and bactericidal proteins in addition to main end product lactic acid (Kleerebezem and Hugenholtz 2003). Lactic acid, main compound produced by lactic acid bacteria, is an important organic acid used in various industries. In the food industry, it is used as an acidifier and flavour-enhancing agent (Papagianni 2012). Lactic acid production during fermentation improves the sensorial and preservation properties of the final product.

Increase in free amino acid content was reported by the activity of some lactic acid bacteria strains during cheese ripening (Mangia et al. 2008; Milesi et al. 2008), kefir (Simova et al. 2006) and sourdough fermentations (Gobbetti et al. 2005). Milesi et al. (2008) reported enhanced secondary proteolysis by *Lb. plantarum* strain resulting in noticeable increase in levels of total free amino acid during cheese fermentation. Proteolytic system of lactic acid bacteria enables degradation of proteins resulting in small peptides and amino acids. Proteolytic enzymes, proteinases and peptidases, have been isolated from different lactic acid bacteria strains belong to *Lc. lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. helveticus*, *Lb. delbrueckii* subsp. *lactis* and *St. thermophiles* species in cheese fermentations (Khalid and Marth 1990; Tjwan Tan et al. 1993; Wohlrab and Bockelmann 1992). Also proteinase and peptidase activities during sourdough fermentations were reported for different strains of the following species: *Lb. sanfranciscensis*, *Lb. brevis* subsp. *lindneri*, *Lb. plantarum*, *Lb. farciminis*, *Lb. casei* and *Ped. pentosaceus* (Gobbetti et al. 1996; Rollan et al. 2005; Gerez et al. 2006; Alvarez-Sieiro et al. 2016). The flour endogenous proteinases are considered to be important for proteolysis in sourdough fermentations. Besides primary proteolysis by cereal proteases, also proteolytic activity of bacteria strains contributes to proteolysis during sourdough fermentation. A limited degree of proteolysis of wheat and rye proteins during sourdough fermentation is very important for bread flavour, volume and texture (Gänzle et al. 2008). Gluten proteins in cereal flours are responsible for coeliac disease (gluten-sensitive enteropathy). It is a chronic gastrointestinal tract disorder and gluten proteins consumption must be prevented since the ingestion of gluten from wheat, rye and barley and their crossbred varieties leads to damage of the small intestinal mucosa by an autoimmune mechanism (Green and Cellier 2007; Tye-Din and Anderson 2008). Therefore, controlled proteolysis in wheat and rye sourdoughs may be used as a tool to reduce gluten levels to such an extent that the products can be tolerated by coeliac patients (Rizzello et al. 2007; Katina and Poutanen 2013).

Some lactic acid bacteria and *Bifidobacterium* strains produce B vitamins (thiamine, riboflavin, pyridoxine, cobalamin, folic acid, and biotin) during dairy fermentation (Taranto et al. 2003; LeBlanc et al. 2005; Burgess et al. 2006; Capozzi et al. 2012; Laiño et al. 2012; Patel et al. 2013; Linares et al. 2017). *Bifidobacterium* metabolizes glucose via the Bifidus pathway (Scardovi 1986) to yield lactic and acetic acids and this is a special pathway, unique to the genus, which clearly sepa-

rates them from lactic acid bacteria (Hammes and Hertel 2009; Endo and Dicks 2014).

Some *Propionibacterium* species can produce riboflavin, cobalamin, folic acid and biotin (Hugenholtz et al. 2002; Burgess et al. 2006). Using *Propionibacterium freudenreichii* strain in yogurt preparation increased its riboflavin content, thus increasing the commercial and nutritional value (LeBlanc et al. 2006). Conjugated linoleic acid production is important due to the numerous health benefits and several strains belong to lactic acid bacteria, *Bifidobacterium* and *Propionibacterium* species are capable of producing conjugated linoleic acid by converting linoleic acid (Maggiora et al. 2004; Ewaschuk et al. 2006; Gobbetti et al. 2010).

Bacillus spp. are spore forming bacteria and except for a few species the large majority have no pathogenic potential. *Bacillus* spp. produce various compounds such as vitamins, amino acids and enzymes (amylase, phospholipase, chitinase, protease) (Lövgren et al. 1990; Gurung et al. 2013). Also they produce polypeptide bacteriocins and bacteriocin like inhibitory substances showing antibiotic potential (Abriouel et al. 2011).

Acetic acid bacteria produce acetic acid as the main end product from ethanol. They have ability to produce organic acids, besides acetic acid such as tartaric, lactic, malic, formic, citric and succinic acids and some exopolysaccharides such as dextrans, levans, cellulose and acetan as a result of glucose metabolism (Ojinnaka et al. 1996; Gullo and Giudici 2008; Mercanoglu Taban and Saichana 2017).

Yeasts are eukaryotic microorganisms widespread in natural environments. In the food industry, alcoholic fermentation by yeast cells is a key process in the baking, brewing and winemaking industries (Hardin et al. 2012). Yeast cells break down glucose into ethanol and CO_2. In bread fermentation, CO_2 is trapped in the dough and causes the bread dough to rise. The CO_2 produced is responsible for the bubbles in beer and in sparkling wines and ethanol made by yeasts is the alcohol in alcoholic beverages (Tortora et al. 2010a, b; Hardin et al. 2012). Also some other secondary metabolites such as esters, acids, higher alcohols, aldehydes, ketones, polyols and volatile sulphur compounds are produced and enhance the sensorial properties of the final product depending on their amounts and threshold values (Lambrechts and Pretorius 2000; Fleet 2003). Besides alcoholic beverages, yeasts play an important role in the fermentation of cereal, dairy and plant based sources since synthesized aroma compounds contribute significantly to the sensorial properties of the final product.

Also yeasts and moulds are associated with biosynthesis of enzymes, antioxidants and melatonin (Mas et al. 2014). For example, lipases and proteases are produced by many species of fungi such as *Candida*, *Aspergillus*, *Penicillium*, *Rhizopus* (Nigam 2013). *A. niger* is known as a producer of extracellular enzymes and also organic acids (Lopes et al. 2011). Industrial organic acid production was reported through some yeast and mould species. For example, citric acid production by *A. niger*, *Yarrowia lipolytica*, some *Candida*, *Pichia* and *Rhodotorula* species is known (Yalcin et al. 2010). *Rhizopus oryzae* produce lactic acid through their amylolytic enzymatic activity during conversion of starch (Komesu et al. 2017).

Besides functional bioactive compounds, biogenic amines (BAs) can be produced by some microorganisms in foods. BAs, for example histamine, tyramine,

putrescine, cadaverine, tryptamine, 2-phenylethylamine, spermine, spermidine and agmatine, can be produced in foods and can cause adverse effects on the human health and food quality (Gardini et al. 2016). BAs are produced in fermented foods by microbial decarboxylation of the corresponding amino acids (Sahu et al. 2015). Foods likely to contain high levels of BA include fish, fish products, fermented foods, such as meat, dairy products and vegetables and alcoholic beverages, such as wine, cider and beer. Some species of bacteria and fungi are responsible for BAs accumulation in foods (Spano et al. 2010). The strains that have been described as BA producers are belong to some species of *Pediococcus*, *Lactobacillus*, *Leuconostoc*, *Oenococcus*, *Enterococcus* and *Streptococcus* in some fermented foods (Landete et al. 2007; Sahu et al. 2015).

Sensorial Properties

Compounds produced by different microorganisms through different metabolisms, mainly carbohydrate catabolism and also proteolytic and lipolytic degradation, affect the sensorial properties of the fermented product. Proteins are degraded to free amino acids and the liberated amino acids act as flavour precursors during fermentation (Thiele et al. 2002). Both yeasts and bacteria facilitate the flavour formation, either directly via metabolizing amino acids to flavour compounds or indirectly by transforming them into secondary compounds that can serve as new precursors for further conversions (Loponen 2006). Free amino acids can be converted into various volatile compounds such as alcohols, aldehydes, carboxylic acids and esters. Hydrolysis of lipids to free fatty acids is also important for aroma formation. Especially short chain free fatty acids have impact on aroma and also act as precursors of other aroma compounds such as methyl ketones, secondary alcohols, esters, lactones and small peptides and also free amino acids (Forde and Fitzgerald 2000; Van Hoorde and Van Landschoot 2014).

During lactic acid fermentation, lactic acid is the main end product and as a result of heterofermentative metabolism, some flavour compounds such as diacetyl, acetoin, acetaldehyde, acetic acid, formic acid and ethanol are produced (Pastink et al. 2008). Certain flavouring compounds including acetic acid, diacetyl, acetoin, CO_2 and 2,3 butanediol are also produced during citrate metabolism of mainly *Lactococcus* and *Leuconosoc* species. Especially diacetyl is an important flavour compound of dairy products such as butter, buttermilk and sour cream. During citrate metabolism, produced CO_2 leads to eye formation in certain cheese types (Tamine et al. 2006). Propionic acid bacteria contribute to the characteristic flavour and appearance of Swiss-type cheeses by propionic acid, acetic acid and CO_2. These end-products are key-components of this type of cheeses with propionic and acetic acids as flavour compounds and CO_2 responsible for the characteristic holes of these cheeses (Schwenninger et al. 2011).

Ratio of the certain metabolites contributes to the final sensorial quality of the fermented foods. In sourdough fermentation, the content of lactic and acetic acids is

very important for the taste and flavour of sourdough bread (Hansen and Hansen 1996). The molar ratio between lactic and acetic acid is defined as the fermentation quotient and should be around 4 in sourdoughs to obtain a balanced bread taste (Hansen 2012); with the optimum considered to be in the range of 2.0–2.7 (Hammes and Ganzle 1998).

Yeasts make positive contributions to wine flavour by the synthesis of secondary metabolites which are important for flavour and other sensorial properties. Esters, acids, alcohols, aldehydes, ketones, polyols, volatile sulphur compounds are produced and directly impact the wine flavour (Lambrechts and Pretorius 2000; Fleet 2003). In olive fermentations, in addition to lactic acid bacteria yeasts also improve the aromatic profile of fermented olives (Arroyo-Lopez et al. 2008).

Sensorial quality in terms of texture can be affected by microorganisms. Microbial exopolysaccharides (EPSs), long chain sugar polymers, are metabolites produced by bacteria, microalgae and to a lesser extent, yeasts and fungi (Sutherland 1972; De Vuyst and Degeest 1999). Many food-grade microorganisms produce EPS especially lactic acid bacteria, propionibacteria and bifidobacteria (Cerning 1990, 1995; Abbad Andaloussi et al. 1995). Some lactic acid bacteria species are a good source of EPS which are also recognized for their contribution to the texture, mouth feel, taste perception and stability of the final food product (Jolly et al. 2002; Gonzalez 2006). In some fermented products, lactic acid bacteria contribute to the textural characteristics of the final product by the production of EPS. EPS producer strains belong to the different species including *Lb. casei*, *Lb. acidophilus*, *Lb. brevis*, *Lb. curvatus*, *Lb. delbrueckii*, *Lb. helveticus*, *Lb. rhamnosus*, *Lb. plantarum*, *Lb. johnsonii* etc. (Jaiswal et al. 2014). In kefir grains, the main polysaccharide is kefiran, a heteropolysaccharide, and is mainly produced by *Lb. kefiranofaciens* (Zajsek et al. 2011). Most of the EPS-producing lactic acid bacteria strains were isolated from dairy products but it is known that some lactic acid bacteria species produce EPS and links between specific metabolic activities of sourdough cultures and product quality (Galle et al. 2010). Strains belonging to *Lb. sanfranciscensis*, *Lb. frumenti*, *Lb. pontis*, *Lb. reuteri*, *Lb. panis*, *W. confusa*, *W. cibaria*, *Lb. plantarum* and *Ped. pentosaceus* species were isolated from sourdough fermentations and reported as EPS producers (Korakli et al. 2001; Tieking et al. 2003; Di Cagno et al. 2006; Katina et al. 2009; Amari et al. 2013).

In some foods, EPS formation can negatively affect the final quality. Dextrans, levans and cellulose are the main EPSs produced by acetic acid bacteria, but, occurrence of polysaccharides in vinegar industry is a disadvantage since it negatively affects the filterability of the product (Gullo and Giudici 2008).

Food Safety and Preservation

Shelf life of the fermented foods increases as a result of the produced compounds. Organic acid production (especially lactic and acetic acids) reduces the pH and enables the food preservation by inhibiting spoilage microorganisms due to the low

pH levels and also the antimicrobial activity of the undissociated acid molecules (De Vuyst and Vandamme 1994; Salovaara and Gänzle 2012). A wide variety of inhibitory compounds are synthesised during the growth of fermentative microorganisms and those compounds can effectively inhibit pathogenic bacteria and fungi (Gänzle and Gobbetti 2013). Another mechanism of inhibition can be the microbial competition for space and essential nutrients, as well as the action of bacteriophages (Holzapfel et al. 1995; Chen and Hoover 2003).

In fermented foods, many lactic acid bacteria produce antimicrobial metabolites including lactic acid, acetic acid, hydrogen peroxide, diacetyl, reuterin, fatty acids and various inhibitory proteinaceous molecules commonly called bacteriocins which are ribosomally synthesised inhibitory peptides or proteins secreted by various bacteria against closely related microorganisms (Klaenhammer 1988; Holzapfel et al. 1995; Szkaradkiewicz and Karpiński 2013). Also some *Bacillus* species produce bacteriocins and bacteriocin like inhibitory substances showing antibiotic potential, for example bacitracin, polymyxin, gramicidin, subtilin, subtilosin, coagulin, megacin etc. (Abriouel et al. 2011).

Other Functions of Microorganisms in Fermented Foods

Microorganisms can improve the nutritional quality of the foods. Some microorganisms show important health benefits and accepted as probiotic. Probiotic is defined as the live microorganisms those confer a health benefit on the host when consumed in adequate amounts (FAO/WHO 2002) The best known probiotic strains are belonging to the *Lactobacillus* and *Bifidobacterium* genera including *Bifidobacteria bifidum*, *Bifidobacteria infantis*, *Lb. rhamnosus*, *Lb. casei*, *Lb. acidophilus* and *Lb. plantarum.* Also, some other bacteria strains belonging to *Enterococcus* species, *Streptococcus* species, *Escherichia coli*, *Bacillus* species and yeast *S. cerevisiae* subsp. *boulardii* have also been accepted as probiotics (Rauch and Lynch 2012; Harzallah and Belhadj 2013; Sivieri et al. 2013).

Moreover, microorganisms can degrade various anti-nutritive compounds. Phytic acid is found in most cereal grains, legumes and nuts and generates insoluble complexes with dietary cations resulting in prevention of mineral absorption in humans (Lopez et al. 2002; Rizzello et al. 2017). Phytase activity has been detected in some yeasts and lactic acid bacteria (Chaoui et al. 2003; De Angelis et al. 2003; Reale et al. 2004). The low pH values associated with fermentation lead to the solubilisation of the phytic acid complex as a result of the phytase activity of raw materials, lactic acid bacteria and yeasts; therefore, mineral bioavailability is increased (Chavan and Chavan 2011). Phytate degradation is also reported as a novel mechanism of probiotic functionality (Askelson et al. 2014).

Food Spoilage Microorganisms

Spoilage of Dairy Products

Aerobic psychrotrophic Gram-negative, heterofermentative *Lactobacilli* and spore forming bacteria, molds, and yeasts can be involved in spoilage of dairy products. Among them, psychrotrophic bacteria generally produce extracellular enzymes. Fungal spoilage of dairy products can result in off-flavour and odors, change in colors and texture due to production of a variety of by-products. In addition, other microorganisms such as yeasts, heterofermentative *Lactobacilli* and spore forming bacteria can cause high level of gases which defect particularly cheese products. These spoilages in dairy products can be prevented by reducing the pH via lactic acid fermentation or addition of acids and other conservatives, reducing water activity by adding salt or sugar, forming desired microflora to control the growth of undesired microorganisms, packaging techniques to restrict oxygen as well as keeping in low temperatures (freezing) (Ledenbach and Marshall 2009).

Psychotropic microorganisms that can be found in raw milk are 65–70% of *Pseudomonas* species (Griffiths et al. 1987; García et al. 1989) and others such as member of the genera *Bacillus*, *Aerococcus*, *Micrococcus* and *Lactococcus* and of the family *Enterobacteriaceae*. From them, *Pseudomonas* can grow lower temperatures (3–7 °C) and hydrolyse large molecules of proteins and fats for their growths. *Pseudomonas* and especially coliforms can convert diacetyl of buttermilk to acetaldehyde and cause yoghurt flavour due to unappreciated level of diacetyl to acetaldehyde ratio (Wang and Frank 1981). Moreover, cottage cheese is generally affected from psychotropic microorganisms due to insufficient salt content and moderate pH (5.0–5.3) level in which they have ability to grow. If cell density in raw milk is higher than 10^6 CFU/mL, they can reduce the yield and content of cheese curd (Nelson and Marshall 1977; Mohamed and Bassette 1979; Aylward et al. 1980; Fairbairn and Law 1986).

Besides psychotropic microorganisms, *Lactococci* cause high viscosity in sour cream and buttermilk and can reduce diacetyl via diacetyl reductase which causes yoghurt like flavour. In addition, heterofermentative lactic acid bacteria such as *Lactobacilli* and *Leuconostoc* can accumulate gas and off-flavour in cheeses during ripening. They generally accumulate lactate, acetate, ethanol and CO_2 from metabolism of lactose (Hutkins 2001). Facultative *Lactobacilli* can also produce CO_2 by metabolizing citrate and lactate. As a result of gas produced by this microorganism, cracks in cheese can occur that lower the quality (Zoon and Allersma 1996).

Yeasts have ability to grow on products with lower pH such as sour cream and buttermilk and usually produce off-flavour which is called yeasty or fermented. They can also decrease diacetyl content in these types of products. For instance, elsewhere was reported that *Geotrichum candidum* reduced 52–56% of diacetyl content in low fat cottage cheese during 15–19 days of storage at 4–7 °C (Antinone and Ledford 1993). In yoghurt, higher than 10^5–10^6 CFU/g of yeast growth can result in yeasty, fermented off-flavour and gassy look. Galactose-positive strains of

yeasts such as *Hansenula anomala* and *S. cerevisiae* were reported as yoghurt spoilage yeasts. In addition, cheeses with low pH and high nutritional content including peptides, amino acids and lactic acid are favourable medium for growth of spoilage yeasts. They often produce CO_2 and ethanol. Moreover, some yeast species produce sulphites and form an egg-odor. Contaminated cheeses usually include *K. marxianus*, *D. hansenii*, *Geotrichum candidum*, *Candida* spp. and *Pichia* spp. (Johnson 2001).

Molds usually show growth on the surface of cheeses with the presence of oxygen and low pH condition. The genus *Penicillium* is the most commonly founded mold on cheese surface. In order to protect cheese from microbiological spoilage, sorbate is commonly used but molds can degrade sorbic acid and potassium sorbate to trans-1,3-pentadiene which leads to an off-odor called as "kerosene". For example, *P. roqueforti* exhibits synthesis of this compound from sorbates containing cheeses. Furthermore, heat resistant mold *Byssochlamys nivea* which is responsible from spoilage of cream cheese, reported as microorganism capable to grow in limited oxygen atmosphere conditions (Taniwaki et al. 2010).

In case of spore forming bacteria, *B. licheniformis*, *B. cereus*, *B. subtilis*, *B. mycoides*, and *B. megaterium* can be counted as the most common spore-forming bacteria found in dairy products. They usually coagulate casein of milk by proteases produced by them at the high pH mediums (Choudhery and Mikolajcik 1970). In addition, as a result of excessive proteolysis of cheeses during ripening, quantity of amino acids and high pH favour the growth of *C. thyrobutyricum*. This microorganism generally produces gas and butyric acid that cause defect in cheeses called late blow defects. Cheeses such as Swiss, Gouda, Edam and Emmental which have low salt content and high pH and moisture content, are most commonly affected by *C. tyrobutyricum* (Klijn et al. 1995).

In addition, as another dairy spoilage microorganism, facultative anaerobe *Eubacterium* spp. can lead gassy effect in Cheddar type cheeses. Swiss cheese was also affected by *E. faecalis* subsp. *liquefaciens* which cause white-spot defect. Besides that, they had inhibitory effect on *Lb. fermentum* and *Propionibacteria*, causing poor eye development as well as absence of flavour in cheese (Nath and Kostak 1986).

Spoilage of Meat Products

Spoilage bacteria in red meats include Gram-negative *Acinetobacter*, *Aeromonas*, *Enterobacteriaceae*, *Pseudomonas*, *Psychrobacter*, and *Shewanella putrefaciens* and Gram-positive lactic acid bacteria (LAB) and *Brochothrix thermosphacta* (Borch et al. 1996; Barakat et al. 2000; Ercolini et al. 2006, 2009, 2011). Storage conditions of meat generally manage growth of these microorganisms. While *Moraxella*, *Acinetobacter* and *Psychrobacter* grow good in aerobic conditions, LAB and *B. thermosphacta* have the ability to grow under anaerobic or modified atmosphere packaging conditions. Moreover, *Pseudomonas* spp. are the most com-

mon spoilage microorganisms in meat products stored in refrigeration conditions (Molin and Ternstrom 1982, 1986; Shaw and Latty 1982; Labadie 1999; Rattanasomboon et al. 1999; Barakat et al. 2000; Gill 2003). Most isolated *Pseudomonas* spp. from spoiled meat products were reported as *P. lundensis*, *P. fragi*, *P. putida* and *P. fluorescens* (Nychas et al. 2008). Among them, *P. fragi* is the most frequently found species in chilled meat under aerobic storage conditions. It often causes surface spoilage, forming slime and undesired odor. Moreover, it can produce a fruity sour smell in meat (Liao 2006). Generally, this genus has considerable importance to establish shelf-life prediction due to their predominant effect on meat spoilage (Zhang et al. 2011).

LAB are also considered as spoilage microorganisms under anaerobic conditions. They usually present in meat stored at low temperatures and vacuum or modified atmosphere packaging. LAB can convert carbohydrates to secondary products which lead cheesy, sour and liver-like smells, accompanying sometimes with CO_2 production. This gas production causes blown pack in vacuum packaged products and negatively affects the appearance of meat. From LAB, most common meat spoilers were reported as *Leuconostoc*, *Lactobacillus*, *Lactococcus*, *Weissella*, *Carnobacterium*, *Enterococcus* and *Pediococcus* (Pothakos et al. 2015). Meat and meat products include most frequently *Lactobacillus* spp., *Leuconostoc* spp. and *Carnobacterium* spp. and their cell number can reach up to 10^7 CFU/cm^2 (Borch and Molin 1988). Moreover, vacuum packaged meat products generally contain *Lb. sakei*, as a dominant spoilage bacterium (Makela et al. 1992) which produces hydrogen sulphite and subsequently lead the greening of meat products (Egan et al. 1989). Gas production by *Leuconostocs* and *Lactobacilli* via heterofermentation can be also observed in meat products (von Holy et al. 1991). In addition, *Lb. fuchuensis* and *Lb. algidus* are linked with spoilage of meat products. Ham, stored in vacuum package can contain predominantly *Leu. carnosum* (Bjorkroth et al. 1998). *Leuconostoc* spp. can generate acetic acid, causing slime formation and cheesy like flavour in meat, accompanying by greening and CO_2 production (Nieminen et al. 2011). In addition, red meat can be spoiled by *Carnobacterium* spp. at the presence of limited oxygen conditions (Rieder et al. 2012). Vakuum packaged minced meat and other related products can be also deteriorated by *Weissella* spp. (Nieminen et al. 2011). Moreover, other LAB such as some *Enterococcus* and *Lactococcus* are responsible from meat spoilage (Pothakos et al. 2015). Recently, *L. gelidum* has been considered as spoilage bacteria in meat (Chaillou et al. 2014).

From *Enteroabacteriaceae*, *Escherichia coli*, *Serratia liquefaciens* and *Pantoea agglomerans* are known as minced beef spoilers. They can also spoil dry beef packaged in dark and firm vacuum package (Gribble et al. 2014). Aerobic spoilage by these microorganisms can cause discoloration, sulphite and ammonia odor, and slime formation whereas anaerobic spoilage by them can result in greening of surface and sulphite odor (Gill and Badoni 2004).

In case of genus *Brochothrix*, two species, *Brochothrix campestris* and *B. thermosphacta* were reported as meat spoilage microorganisms. *B. thermosphacta* is most commonly found in mutton, pork, beef and cured meat (Gill 1996). It is most often responsible from spoilage of vacuum packaged mutton even at lower tempera-

tures less than −1.5 °C (Gribble and Brightwell 2013). Meat spoilage by this microorganism is associated with undesired cheesy like odor, forming gas and discoloration with accompanying occurrence of green slime and greening effect (Gill and Badoni 2004; Gribble and Brightwell 2013; Gribble et al. 2014).

Other bacteria which cause spoilage in particularly bacon, vacuum packaged pork and other vacuum packaged meats at lower temperatures, were reported as *Achromobacter* spp., *Moraxella* spp. and *Clostridium* spp. (resulting in blown pack defect) respectively (Zhang and Zhang 2017).

In addition, spoilage of meat by yeast and molds occurs very rarely. However, some species such as *Yarrowia lipolytica*, *C. lipolytica* and *C. zeylanoides* were isolated from spoiled meats (Perez Chabela et al. 1999). It was also reported that yeasts were the main spoiler agents in cured meat such as fresh British sausages (Dalton et al. 1984).

Spoilage of Fruits and Vegetables

Vegetables with content of approximately carbohydrate of 5% and proteins of 1–2%, and except tomatoes, have high pH, are favourable products for the growth of many microorganisms. Most of the microorganisms can grow rapidly in fresh cut vegetables. High temperature, moisture and existence of air at storage conditions stimulate the spoilage of vegetables. The main spoilage microorganisms in vegetable products are molds such as genus *Aspergillus*, *Alternaria*, *Botrytis*, *Penicillium* and *Phytophthora.* Moreover, Bacteria: *Pseudomonas* spp., *Erwinia* spp., *Bacillus* spp., and *Clostridium* spp. are common species in vegetable spoilage (Rawat 2015). Microbial spoilage of vegetables is commonly described as term “rot” with the changes in appearance of them such as gray, black, pink, soft and stem-end rot (Hozbor et al. 2006). Among Bacteria, *Erwinia carotovora* is the most frequently spoilage bacterium found in many vegetables. It can found even in vegetables stored at the very low temperatures (Tournas 2005). In addition to *E. carotovora*, other bacteria such as *Pseudomonas* spp. and LAB are common spoilage bacteria in vegetables. During spoilage of vegetables, unpleasant odors and flavours, lactic acid and ethanol are produced by spoilage microorganisms through carbohydrate (sugars and starches) metabolism. Molds such as genus *Rhizopus*, *Alternaria* and *Botrytis* cause spoilage of vegetables by changing their texture, colour and pH. Their high moisture content stimulates fungi to proliferate (Rawat 2015).

Healthy fruits can inhibit the growth of many microorganisms until harvesting time. Ripening of them results in weaken of cell walls and decreasing the concentration of antifungal chemicals and physical damages during harvesting lead breaks on surface skin of fruits which allows activity of spoilage microorganisms. Molds have high tolerance to low pH and water activity, and known as main spoilage microorganisms in apples, pears, citrus and other related fruits. Main molds species found in spoilage fruits are *Botrytis*, *Rhizopus* and *Penicillium* (Calvo et al. 2007).

Moreover, fresh cut fruits are generally affected and spoiled by yeasts and some bacteria species such as *Xanthomonas* and *Erwinia* (Thomas and Davenport 1985).

In addition, fruit juices with their high level of sugar content and acidic property are suitable for the growth of molds, yeasts and some acid resistant bacteria. Molds can cause cloudiness and off-flavours by formation of surface pellicles or fibrous mats. This can be prevented by limiting oxygen in canned and bottled fruit juice drinks. Moreover, yeasts species such as *Saccharomyces* and *Zygosaccharomyces* can be found in several spoiled fruit juices due to their high resistance to thermal processing (Fitzgerald et al. 2004). A thermophilic and an acidophilic spore forming bacteria, *Alicyclobacillus,* can lead off-flavours and smoky spot in pasteurized fruit juices (Cocolin et al. 2004). Other high acid tolerant bacteria, *Propionibacterium cyclohexanicum* have ability to grow in many pasteurised fruit juices (Walker and Phillips 2007). In addition, LAB are involved in spoilage of tomato and orange juices. *Pseudomonads* and *Enterobacteriaceae* also play roles on spoilage of fruit juices (Rawat 2015).

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Emerging Technologies in Cereal Processing: Present Status and Future Potential

Khalid Bashir, Kulsum Jan, Sweta Joshi, Shumaila Jan, and Z. R. Azaz Ahmad Azad

Abstract Grain processing (cereal and pulse) occupies a large and important portion in food production chain in India, and is one of the oldest and most important of all food technologies. Cereal grains and pulses are grown widely throughout the world and their dietary and economic importance is globally appreciated and recognized. In Indian economy, cereals and pulses occupy a prominent place and remain the most consumed staple food. For ten consecutive years from 2006 to 2016 a rising trend in the production of food grains has been registered. Grains like wheat, paddy, maize, barley and pulses touched an increased level of 25, 20, 12, 2 and 1 lac tons, respectively. The production of millets, however, declined to a recorded level of 66,900 tons from 2010 to 2019. Cereal grains grown all over the country face potential losses during processing, hence posing a serious threat to the countries food reserves. Moreover, food security has always been the overriding goal of agricultural policy in India. The rapid growth in production followed by losses in cereals demands the utilization of the novel processing technologies. The introduction of novel technologies has improved the processing and utilization of cereal grains in different countries. Emerging technologies *viz.* radio frequency, microwave, irradiation and high-pressure processing have found potential application for storage and processing of cereals. The present chapter envisages the present and future scenario of the novel processing technologies to be used to reduce the losses in cereals.

Keywords Cereals · Radiofrequency · Microwave

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Introduction

The cereals sector occupies a very important place in the Indian economy and the value output among the production of all crops. Steady growth of food grains has been introduced due to the rise of high yielding rice and wheat varieties in late 1960s and early 1970s. India has been producing 20.6 Mt of rice during 1950–1951 and 89.48 Mt in 1999–2000. From 2000–2001 to 2009–2010 the production transformed with growth in area turning negative and in production and yield standing at 1.59% and 1.61%, respectively. Wheat also being the major crop of India, the country produced 6.5 Mt in 1950–1951, 76 Mt in 2000–2001. During 2000–2001 to 2009–2010 the growth in area in wheat has been 1.21% and in production and yield was 1.89% and 0.68% respectively. Similarly, production of coarse cereals has augmented from 15.4 to 32.0 Mt between 1950–1951 and 2000–2001. Because of little or no technological revolution in zone of total coarse cereals during 1980–1981 to 1989–1990 and 2000–2001 to 2009–2010 reproducing swing to other crops or fairly dry area continued to be unused. The production of wheat and pulses was estimated to be 81.47 million tons and 16.51 million tons, respectively in 2011. For the year 2012, the production of crops like rice, wheat, maize, pulses and millets was 1.58, 15.7, 9.5, 2.2, 0.96 and 1.07 Million tons, respectively. The production of the crops in the year 2013, 2014, 2015, 2016 and 2017 is given in Table 1.

Food security has always been an issue in India, despite the high amount of cereal grain production. It is found that 1.3 billion tons, roughly 1/3rd of the food is lost or wasted every year. The post-harvest losses comprise essentially on-farm losses and those in transportation and storage in different marketing channels. Storage losses annually is estimated to be 14 million ton of food grains worth $ 16,000 million per year (FAO). While in low income countries, losses primarily occur during the early and middle stages of the supply chain (FAO, 2011). Post-harvest losses have led to economic losses, Table 2 lists the individual estimation of economic post-harvest losses. Higher losses during post-harvest processing have lead scientists to develop new techniques for the safe storage of foods in an economical manner.

The accurate post-harvest practices and proper storage of crops can show a major role in extending shelf life and reducing spoilage. Important technologies (radio frequency, microwave, irradiation and high-pressure processing) have also been found to have potential application for storage and processing of cereals. Existing food processing techniques create a few limits which has led to the development of innovative technologies (Toepfl et al. 2006). These innovative techniques are environment friendly and reduce the water use. High hydrostatic pressure (HHP), pulsed electric fields (PEFs), ultrasound (US), and cold plasma (CP) are used to exemplify scalable and flexible food manufacturing techniques. Significant, science-based achievements have been made to better understand the basic principles underlying HHP and PEF processing (Hendrickx and Knorr 2002, Raso and Heinz 2006). Understanding the impact of such technologies on foods will enable to design suitable foods and to create process-structure-function relationships. The needs of consumer in terms of preference, acceptance and quality can be fulfilled by the use

Table 1 Production of different crops

Crop	Year	Production (million tons)
Rice	2013	15.9
	2014	15.7
	2015	15.6
	2016	16.3
	2017	16.8
Wheat	2013	9.3
	2014	9.5
	2015	8.6
	2016	9.2
	2017	9.8
Maize	2013	2.4
	2014	2.4
	2015	2.2
	2016	2.5
	2017	2.8
Pulses	2013	0.1
	2014	0.1
	2015	0.09
	2016	0.09
	2017	0.09
Millets	2013	1.0
	2014	1.1
	2015	1.1
	2016	1.0
	2017	1.1

FAO 2019 (http://www.fao.org/faostat/en/#data/QC)

Table 2 Individual estimation of economic value of the losses in India

Crop/commodity	Estimate of economic value of the losses, $ million doll
Cereals	1949
Pulses	268
Oilseeds	790

of non-thermal processes. The current review will emphasize on the present and future scenario of the emerging non-thermal processes and their potential application for storage and processing of cereals.

Irradiation Technology

Food convenience and approachability can be amplified by increasing food production, improvements in supply chain and inhibition of post-harvest losses as being the critical section for certifying future food security. About 20–30% of normal food is lost during harvesting due to insect infestation alone during storage.

Most of the agricultural crops are usually contaminated with insects, pests and pathogenic non-spore forming bacteria, which are responsible for majority of the health problems. According to the UNICEF, WHO and UNDP report of 2018, each year approximately 3.5 million people are killed by the infectious and parasitic diseases, most of which occur in the developing countries. During the past two decades several technologies have emerged in the food sector to enhance the shelf life and do the value addition to the food. Although, there exist several methods for controlling the post-harvest losses like spraying insecticides and fumigants (including ethylene dibromide, malathion, pyrethrins, phosgene) but, the residues of these chemicals has led to a substantial decline in their usage because of their toxicity effects to humans. The most promising alternative to these materials appears to be gamma-irradiation which can be given after packing the food and there are least chances of re-contamination (Christensen 1982; Kleinkopf et al. 2003).

History and Present Status

In India, the first irradiation pilot plant was set up in 1967 at Food Irradiation and Processing Laboratory (FIPLY), Trombay for the irradiation of wheat (to inhibit insect growth) and potatoes (to inhibit sprouting). During 1967–1973 several experimental trials were carried out for the irradiation of many fruits, vegetables, pulses and sea foods for the enhancement of shelf life and the data generated was reviewed jointly by Indian Council of Medical Research (ICMR), National Institute of Nutrition (NIN), Food Corporation of India (FCI), Joint FAO/WHO Expert Committee on Food Irradiation (Loaharanu and Ahmad 1991). During 1991, the standards for food irradiation were drafted and in 1994, first consent for the use of irradiation technology for spices, onion and potato was received from the Ministry of Health and Family Welfare under the Prevention of Food Adulteration Act (PFA) Rules then (now Food Safety and Standard Authority of India). The first functional irradiation plant was set up in Navi Mumbai and the plant is functional since 2000, with a capacity of 30 tons raw materials (spices and dry vegetables) per day. During 2003, another plant was built in Nasik Maharashtra with a capacity of 10 tons of raw materials (onions, cereals and cut flowers) per day (Kume and Todoriki 2013). During 2015, 2100 tons of spices and dehydrated vegetables such as coriander, turmeric and red pepper, and 108 tons of onion were irradiated. During 2016–17 more than 700 tons of mangoes were exported to the US after irradiation. A bilateral agreement with the USA for irradiated fresh fruits was established and the export of

irradiated mango has increased (FSSAI 2016- http://www.fssai.gov.in/). Mangoes are irradiated only in USDA approved units, these are Krushak, Lasalgaon, and Maharashtra State Agricultural Marketing Board, Mumbai. Maharashtra-based Kay-Bee Exports became the first Indian company to export pomegranates to the North American market. By the end of 2017, India had 15 commercial irradiation plants. During, 2016, a bilateral agreement was signed between the Indian Agricultural Association, Hindustan Agro Cooperation Ltd. and United Innovation Corporation (a subsidiary of ROSATOM State Atomic Energy Corporation of Russia) for the development of 25 food irradiation centers (Ihsanullah and Rashid 2017).

Plasma Technology

Plasma is referred to as the fourth state of matter as continual addition of energy between the states of matter, gas molecules will become ionized and so carry a net positive charge. If enough molecules are ionized to effect the overall electrical characteristics of the gas the result is called a plasma. A plasma contains electrons, positive ions, neutral gas atoms or molecules, UV light and also excited gas atoms and molecules, which can convey a huge amount of internal energy. Plasma treatments are performed in an evacuated enclosure, or chamber. The air is pumped out and a gas is allowed to flow in at low pressure before energy in the form of electrical power is applied. Plasma is a source of green processing technology satisfying the requirements of food industry. The technology modifies food components to be used for different attributes with its overall quality criterions like nutritional and microbial. Plasma technique is one of the modern technologies applied worldwide for several applications (Garofulic et al. 2015; Misra et al. 2015).

Plasma, a quasi-neutral gas, comprises a number of active elements, like radicals, ions, electrons, metastable excited species, and vacuum ultraviolet radiation that have enough energy to breakdown covalent bonds and start new reactions to form volatile compounds (Şen et al. 2012). Plasma technology is safe for its usage as the active components (phytocomponents) vanish instantly once it is turned off (Misra et al. 2011). Nutritional, functional, and sensory properties are retained by plasma technology, hence, confirming its quality standards. Plasma as a non-thermal process is majorly used for the cereals based industry, which covers more than 50% of consumption (Poutanen et al. 2014). In addition to the decontamination, this technology promises several requirements for cereal industry like maintaining the nutrition content of food. This process is used for the processing of brown rice to maintain its nutritional content, the process basically modifies the cooking quality which makes it more suitable for consumption (Mir et al. 2017).

It has been sated in different studies that plasma pretreatment of seeds encourage their germination and reduce bacterial and fungal growth. It was observed that in case of plasma 13.56 MHz and 300 MHz −300 GHz or below that viz. 50–100 Hz region of electromagnetic fields can reduce the growth of pathogens. A patent by

Menashi (1968) stated the sterilizing effect of plasma, after which a lot published about the sterilizing effect of plasma technology (Lerouge et al. 2001; Moisan et al. 2001; Boudam et al. 2006; Violleau et al. 2008). These articles focused on the corn seeds treated with oxygen plasma technique, it was reported that the rate of germination of corn seed increased. Studies on the effect of plasma technology was also reported for legumes wherein it was reported that the sowing quality and production increased (Filatova et al. 2011). Brown rice (long grain) was modified using plasma technology by Chen et al. (2014). Low pressure plasma technique was used by Denis et al. (2015) for the decontamination of wheat grains. Thirumdas et al. (2016) stated that the hydrophilicity of brown rice increased after treating it with radio frequency low pressure plasma technology. Effect of corona discharge plasma was studied on sprouting of rape seeds by Puligundla et al. (2017).

High Pressure Processing (HPP)

Development of new high quality foods require non-conventional method for food processing like high pressure (HP). The range of pressure varies from some tens of MPa in homogenizers or supercritical fluid extractors to several hundreds of MPa in ultra-HP homogenizers or HP pasteurization units. Several other exciting applications of HPP, viz., food structure engineering is available, in addition to the inactivation of microorganisms (Diels and Michiels 2006, Knorr et al. 2008, Sharma and Yadav 2008). Other applications include improvement of the functional properties such as emulsifying, texture, dough-forming and whipping properties of foods and food ingredients (Hoover et al. 1989). It has been studied recently that HPP may reduce the anti-nutritional factors in grains while preserving food quality and constituents, in addition to serve as a promising non-thermal technology applied to food products.

It has been reported that allergenic proteins (7S globulins) of rice grains gets solubilized in other treatments, while no changes occur at modest pressure in treated grains in terms of superficial variation in character color. Tofu subjected to HHP has shown increasing protein digestibility when treated with HPP in addition to reduce the load of microorganisms.

Rice grains immersed in water exhibited solubilization and consequent release of rice allergenic proteins was studied by Kato et al. (2000). It was studied that 0.2–0.5 mg per gram of rice was released with maximum amounts obtained in the pressure range of 300–400 MPa. According to Kato et al. (2000) pressure treatment induces some modifications in the rice grains such as loss of the endosperm, a tightly packed structure within the bulk of starch granules and consequently, breaking it down to single starch granules.

Development of off flavors was prevented by treating with high pressure without affecting the nutritional content and limits the conditions of storage needed to extend shelf life of food products. The off flavors depend on the action of lipoxygenase (Baker and Mustakas 1972). Thermal inactivation of enzymes at atmospheric pressure occurs in the temperature range 60–70 °C.

In contrast, pressure–temperature inactivation occurs in the pressure range 50–650 MPa at temperatures between 10 and 64 °C. Multicycling is the multiple application of pressure alone or in combination with temperature for the same total treatment time but with various numbers of cycles. Ludikhuyze et al. (1998) reported the multicycling application of pressure to inactivate lipoxygenase. These authors found that in the pressure range of 350–525 MPa and thermal treatment at 10–40 °C, the use of multicycles exerted an additional inactivation effect on lipoxygenase, compared to single cycle treatments.

Recommendations

Food safety is a crucial issue in food industry, making decontamination an essential step in many food processing industries. It is gaining importance due to more restrictive food laws and higher quality expectations. Cereal grains are naturally contaminated by a variety of microorganisms which find their ways from air and dust, water and soil, and also from animal feces. The emerging technologies like plasma technology, high pressure process technology and gamma irradiation seem to be the promising techniques of future as well as to meet the ever growing consumer demands for safe food, food security and enhanced food shelf life so as to feed the huge population and to approach the distant markets while maintaining high quality of the food. At present more than 500,000 metric tons of food is getting processed annually worldwide. Considering the bans being imposed on using chemical fumigants in foods, these technologies are expected to gain wide popularity to reduce the post-harvest losses, increase shelf life of products and inhibit the enzymatic and microbial growth in foods. Moreover, these days focus is being given on developing mobile irradiation facilities, which can be taken to the site and used as soon as the food is harvested or produced. As on today food irradiation has now been approved by the various international regulatory bodies such as CODEX, IAEA, FAO and WHO and the regulatory authorities of over 60 countries globally for radiation processing of foods. These novel techniques lead to environmental friendly and sustainable food manufacturing processes with least energy requirements to provide shelf life extension and safety.

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Emerging Technologies in Dairy Processing: Present Status and Future Potential

Sweta Joshi, Ahmed Mobeen, Kulsum Jan, Khalid Bashir, and Z. R. Azaz Ahmad Azad

Abstract Milk and Milk products are consumed by people across all ages and countries. Being highly nutritious, dairy products are known to be susceptible to microbial and enzymatic spoilage and thus mandate improved processing methods. In recent years, the development of various non-thermal technologies like high pressure processing (HPP), pulsed electric field, ultra-sonication, membrane filtration and cold plasma, have demonstrated the potential to produce shelf stable dairy products with retained nutritional parameters. On one hand where growing awareness about the effect of nutrition and bioactive compounds on human health has paved the way for emergence of state-of-art methods of food fortification, on the other, the liability of sustaining the ever-increasing and dispersing population resulted in innovations in food processing technologies; together which supported motto of 'healthy food for all'. Specifically, focusing on impacts on safety, quality and nutritional value, the chapter discusses the principle, scope, merits and limitations of emerging technologies with respect to dairy products.

Keywords Milk products · Spoilage · Thermal processing · Cold processing · Pulse electric field · High pressure processing

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Introduction

A part of dairy processing is art, while other part is science. All dairy products start with milk, but each product entails a precise amount of proteins, fats, and nutrients. A report published by European Milk Forum in 2017, describes milk products to compose lactose, casein, fatty acids, vitamins, minerals, and various essential trace elements. The demand for dairy products is growing rapidly since they are nutrient rich and supposed to provide nutrient security. Moreover, the health benefits of dairy products have been established in prevention of various metabolic disorders osteoporosis, cardiovascular disease, and cognitive disorders (Hess et al. 2016). In agreement, Food and Agriculture Organization (FAO) reported that by 2025, there would be around 12.5% rise in world per capita dairy consumption (IDFA 2016). Further, dairy product processing provides small-scale dairy producers an opportunity to reach urban markets and earn higher cash incomes. Also, the processing of raw milk into processed milk and products, aid in generating employment in the form of milk collection, transportation, processing and marketing, and, help in dealing with seasonal dairy product shortage. Therefore, there is cumulative necessity to evolve efficient dairy processing techniques to meet product safety standards and shelf-life.

Thermal processing which involves and sterilization and pasteurization is predominantly used in dairy industry for microbiological safety (Misra et al. 2017). However, it causes extensive protein denaturation, deterioration of nutritional value with vitamins and flavour loss, reduction in physiological and sensory properties, non-enzymatic browning and freezing point depression (Mosqueda-Melgar et al. 2008; Barba et al. 2012). Therefore, to meet the consumer demand for nutritious, minimally processed, appetizing, safe and healthy dairy product with increased shelf-life, many non-thermal processes have recently been developed. The non-thermal techniques should meet microbial food safety standards with improved aroma, flavour, nutritional and physiochemical characteristics (Amaral et al. 2017; Barba et al. 2017).

To identify the emerging dairy processing techniques, the related abstracts from PubMed database was acquired and through text-mining a word cloud was obtained (Fig. 1). The lightly highlighted word (with small frequency and thus size) is representative of potential new techniques on the verge of exploration. These methods include cold plasma, high hydrostatic pressure, pulsed electric field, ultrasound, ultra sonication and membrane filtration.

Plasma

Quite popularly, plasma is defined as the fourth state of matter. The plasma state is achieved when gas (noble) is subjected to magnetic, thermal or electric energy at radio or microwave frequencies resulting in the ionization of gas. The plasma is electrically neutral in nature, i.e., it has negative charges and positive charges in

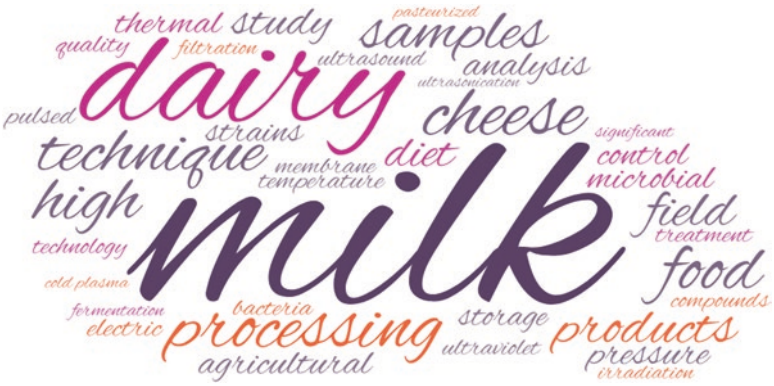

Fig. 1 Word cloud of the abstracts obtained for "Dairy processing technique" from PubMed search. A preprocessing of word-list data is done for developing an informative cloud

equal concentration (Ekezie et al. 2017).The nature of plasma will depend upon the process parameters and gas used (Phan et al. 2017).

Types of Plasma

Depending on the method of generation, plasmas can be divided into two groups as thermal plasma (TP) and non-thermal plasma (NTP) or cold plasma or non-equilibrium plasma. Cold plasma is generated under atmospheric pressure or vacuum at 30–60 °C, requiring low energy. The electrons are at high temperatures, whereas protons and neutrons have lower temperatures and are at non- equilibrium state (Thirumdas et al. 2015). Generation of thermal plasma takes place at higher pressures (>10^5 Pa) and high power (up to 50 MW) (Liao et al. 2017). Thermal plasmas are in thermal equilibrium state and are suitable for treating heat-sensitive foods because the uncharged molecules and ions are at low temperatures (Phan et al. 2017; Pankaj et al. 2018). Based on pressure differences, plasmas can be categorized as high-pressure, atmospheric and low-pressure plasma. The cold plasma based processing has recently gained much popularity due to its excellent performance with respect to -maintaining nutritional and sensory properties of dairy products, and -increasing shelf life through microbial inactivation (Coutinho et al. 2018).

Generation of Cold Plasma

The common sources for the cold plasma generation at atmospheric pressure are dielectric barrier discharges (DBD),corona discharges, microwave discharges and plasma jets.

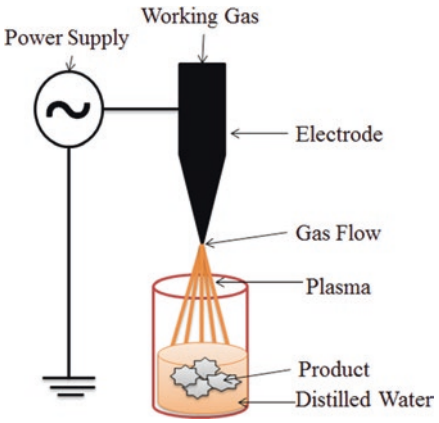

Fig. 2 Simple schematic flow of cold plasma processing

Dielectric barrier discharge (DBD) devices comprises of two metal electrodes, where one of the electrode is concealed with a dielectric barrier which stops electric current to flow and prevents the formation of sparks (Moreau et al. 2008). Plasma jet consists of two concentric electrodes between which noble gases such as helium or argon flows at a high rates (>10 slm). The inner electrode is connected to a radio frequency power at 13.56 MHz resulting in ionization of the gas, producing excited atoms, molecules and free radicals which leave the nozzle at high velocity giving a 'jet-like' appearance (Misra et al. 2016).Microwave discharges are produced by magnetron, directed to the process chamber using a waveguide, where they extent to the electrons present in the working gas (Tolouie et al. 2017). These microwaves are absorbed by the electrons causing an increase in their kinetic energy and thus resulting in gas ionization (Schlüter and Fröhling 2014).Corona discharge is the luminous plasma veil which is produced when the air surrounding a conductor or electrode gets ionized (Phan et al. 2017). Irrespective of the source, cold plasma processing can be simply represented as been depicted in Fig. 2.

Mechanism of Action

The composition of cold plasma is so complex that different reactive agents produced during plasma formation contribute to microbial inactivation. The microbial cell death by plasma is attributed to the reactive species mainly reactive oxygen species (ROS) such as atomic oxygen O, singlet oxygen $^1O_2$, superoxide anion $O^-_2$and ozone O_3; and reactive nitrogen species (RNS) such as atomic nitrogen N, excited nitrogen N_2(A), nitric oxide NO which causes damage to microbial cells possibly by oxidation of cytoplasmic membrane, protein and DNA strands (Bourke et al. 2017)

Application

Song et al. 2009 reported the effect of cold plasma on sliced cheese against *Listeria monocytogenes*. The study showed reduction in viable cell count after 125 s exposure to plasma treatment at atmospheric pressure.

Gurol et al. 2012 studied the effect of plasma against *Escherichia coli* in whole, semi-skimmed and skimmed milk at different time intervals in the range of 0–20 min. The noteworthy reduction in *E. coli* population was detected after 3 min irrespective of the fat content.

Kim et al. 2015 inoculated raw milk with *S. typhimurium, E coli and L. monocytogenes*, and demonstrated that 10 min plasma treatment reduced the bacterial count by around 2.4 log cfu/ml.

Ultrasonication

Ultrasound is one of the emerging techniques in food processing sector deployed to enhance the quality and safety of food products. The sound waves greater than the frequency of human hearing(>16 KHz) are known as ultrasound waves.

Types of Ultrasound

The ultrasound technology can be categorized into two, based on difference in frequency ranges i.e., low and high energy ultrasound. The low intensity ultrasound has frequency higher than 100 kHz at intensities below 1 Wcm^{-2} whereas, high-energy (high-intensity) ultrasound works at frequencies between 20 and 500 kHz at intensities higher than 1 Wcm^{-2} (Mason et al. 2011). The frequency range commonly employed in ultrasonic technology lies between 20 and 500 MHz (Yusaf and Al-Juboori 2014).

Mechanism of Action

The propagation of sound waves through the liquid medium involves alternating expansion and compression cycles. As ultrasound waves travels through the medium, the liquid bubbles oscillate and expand in size to the point when they can no longer absorb enough energy, causing bubbles to collapse known as cavitation which results in the mechanical, chemical and thermal effects. Mechanical effects comprise of collapse pressure, turbulences, and shear stresses (Yusaf and Al-Juboori 2014), while the chemical effects consist of free radicals (H^+ and OH^-) generation (Lateef et al. 2007). The cavitation causes formation of localized hot spots with

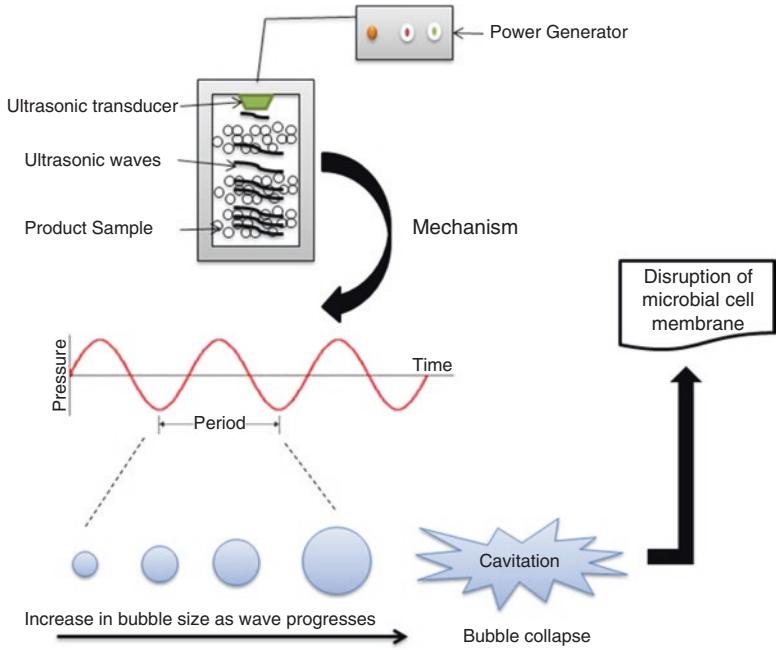

Fig. 3 Schematic diagram for ultrasound mediated product processing

enormously high temperatures of (5000 K) and pressures of 1000 atmosphere (Soria and Villamiel 2010). This pressure change produces shock waves that cause the disruption of bacterial cell membranes resulting in cell lysis(Cameron et al. 2008). Figure 3 illustrates the ultrasound mediated product processing.

Ultrasound can be applied by following methods

- Applying directly to the product.
- Coupling with the device.
- Submerging in an ultrasonic bath

Application

Adulkar and Rathod (2014) illustrated the application of ultrasound treatment in the removal of fat from dairy wastewater by lipase enzyme as a catalyst. Ultrasonic imaging has been used to study the rheological properties of cheese (Lee et al. 1992), changes in the structure of cheese due to heating (Mulet et al. 1999) and cheese maturity (Benedito et al. 2000). The studies conducted has shown the more potential effect of ultrasound combined with other techniques such as heating (thermosonication) and pressure(manothermosonication) against the spoilage microbes and

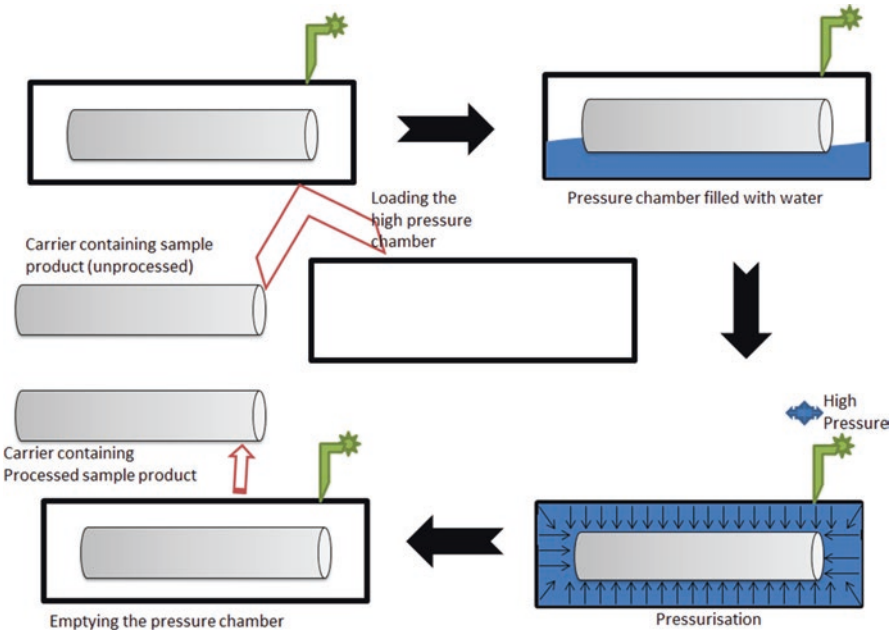

Fig. 4 Schematic flow diagram for high pressure processing

enzymes. Bermúdez-Aguirre et al. 2009, reported the enhanced inactivation effect of thermosonication for *Listeria innocua* and mesophilic bacteria in raw whole milk. Khanal et al. 2014, depicted the positive effect of high-intensity(80–100% amplitude, 1–10 min) ultrasonication for the inactivation of endospores of *Bacillus licheniformis, Bacillus coagulans* and *Geobacillus stearothermophilus* in nonfat milk.

High Pressure Processing

High Pressure Processing (HPP) inactivates harmful pathogens and vegetative spoilage microorganism by using intense pressure instead of heat. The technique involves working at pressure range of 100–1000 MPa. The HPP can be used to treat liquid as well as solid (water-containing) foods (Fig. 4).

Mechanism of Action

In HPP the packed food product is loaded in the pressure vessel, the process chamber is filled with the pressure transmitting medium commonly water. The chamber is pressurized up to the desired pressure-time combination. The food product attains

the pressure of the surrounding medium and the pressure is distributed uniformly throughout the product. After the desired hold time, the chamber is depressurized and the processed food product is removed. The high pressure technique involves the following two principles:

Le Chatelier's principle: whenever stress (high pressure) is applied to a system in equilibrium, the system will counteract to the applied stress, promoting reactions that result in reduced volume causing the inactivation of microorganisms and enzymes.

Isostatic principle: It states that compression of food products occurs due to uniform pressure from every direction and regains their original shape on release of pressure. The compression of the products is independent of the size and shape of the product, because transmission of pressure to the center is mass/time independent (Carlez et al. 1994).

Though HPP is a non-thermal technique, it results in adiabatic rise in temperature (Ohlsson and Bengtsson 2002). The rendering of high pressure causes increase in the temperature of the liquid component of the food by approximately 3 °C/100 MPa. In case of food with significant amount of fat (butter/cream), the temperature increases by 8–9 °C/100 MPa (Rasanayagam et al. 2003).

Application

Jankowska et al. (2005) revealed that use of high pressure treated milk for manufacturing of yoghurt produced curd with improved firmness and reduced its syneresis. The studies showed HP-treated cheese have higher retention for total free amino acids, salt and moisture content as compared to raw or pasteurized milk cheeses (Trujillo et al. 2002). Vachon et al. 2002 reported that dynamic high pressure treatment of milk causes the in inactivation of *Listeria monocytogenes*, *Escherichia coli* and *Salmonella enteritidis* present in it. The pressure processing combined with mild heat treatment activates spores to germinate after which they lose their resistance against pressure and heat and get destroyed (Gould and Sale 1970; Knorr 1995; Gould 2000). The high pressure resistant gram-positive microorganisms requires pressure of 500–600 MPa at 25 °C for 10 min to inactivate whereas gram-negative microbes are inactivated at 300–400 MPa pressure, 25 °C temperature for 10 min (Alpas and Bozoglu 2002). Rodriguez et al. 2005 claimed the HPP treated milk causes inactivation of *E. coli* O157:H7 at 300 MPa pressure 10 °C for 10 min. Huppertz et al, 2006 has reviewed the available literature regarding the effect of HPP on bovine milk and provided a detailed account of reported changes in protein composition due to casein micelles formation; however, the efficacy of HPP on dairy products remain inconclusive due to limited equipment and instrumentation facility.

Pulsed Electric Field

The pulsed electric field (PEF) technology is considered to be one of the efficient techniques for microbial and enzymatic inactivation at mild temperatures. PEF in combination with sub-pasteurization temperatures can attain the potential levels of microbial inactivation in milk at par to conventional thermal pasteurization.

Mechanism of Action

Pulsed electric field (PEF) technology involves small bursts of high intensity electric fields (10–80 kV/cm) applied for microseconds to liquid food positioned between two electrodes. The electrical current is transmitted to each point in the liquid due to the presence of the charged molecules present it (Zhang et al. 1995 and Jose et al. 2010). There are two widely accepted theories of microbial inactivation through PEF: electrical breakdown and loss of cell membrane functionality. The former hypothesizes the microbial cell to be a membrane bound dielectric cytoplasm matrix as capacitor and the surrounding food system as medium with differing electrostatic setting: thus creating a difference in electrical conductivity on either side of microbial cell membrane thereby generating transmembrane potential. In the instance of an external electric field, the ions in the two medium moves, where ions with opposing charge are attracted towards each other causing reduction in membrane thickness and eventually pore formation and membrane breakdown (Jeyamkondan et al. 1999). The latter hypothesis suggests that due to application of PEF, the integrity and arrangement of membrane biomolecules, like proteins and phospholipid, is disordered, causing the prolonged opening of ion/protein channels thereby forming irreversible pores and disruption of microbial cell (Buckow et al. 2014). Nevertheless, a general scheme of PEF based processing is represented through Fig. 5.

Application

Craven et al. 2008 showed the effect of PEF application on ultra-high temperature treated skim milk inoculated with *Pseudomonas* spp., the major spoilage microorganism in milk. The treatment was significantly effective in the inactivation of the microbial population at 31 $kVcm^{-1}$, 19.2 μs, 55 °C thereby extending the shelf life of the treated milk up to 8 days at 4 °C.

Bermudez-Aguirre et al. 2012 studied the effect of PEF in skim milk (0.3% fat) against *B. cereus*, known to cause food-borne illness like diarrhoea and abdominal cramps, nausea and vomiting. The study showed that treatment at 40 $kVcm^{-1}$, 50 μs, 60 °C resulted in 1.5 $\log_{10}$ reduction whereas at 35 $kVcm^{-1}$, 25 μs, 78°Conditions, 3

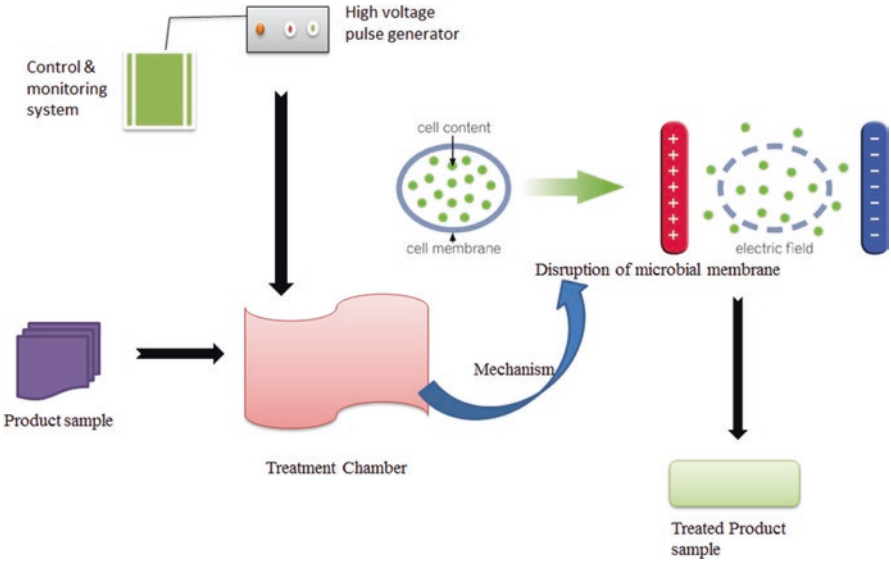

Fig. 5 Schematic flow diagram for Pulse Electric field based product processing

$\log_{10}$ reduction in the microbial population was observed. Jaeger et al. (2010) demonstrated the effect of PEF in raw milk against Alkaline Phosphatase enzyme. The treatment at 38 $kVcm^{-1}$, 12.3 μs, 44 °C resulted in 10% inactivation of enzyme while treatment at 38 $kVcm^{-1}$, 21.2 μs, 60 °C showed 27% enzyme inactivation. In a similar experiment, the continuous PEF treatment with 2 ms monopolar pulses and monopolar square wave at 35 $kVcm^{-1}$, 19.6 μs, 60 °C inactivated the activity of alkaline phosphatase by 67% (Shamsi et al. 2008).

Membrane Filtration

The membrane filtration refers to the separation processes by using different types of semi-permeable membranes. Specific types of membranes are used in the food industry for various purposes such as concentration, fractionation and purification of food products, extending the shelf life of many food products. The usage of membrane ensures that any undesired components like, sediment or microorganism, which may deteriorate the quality of product, be removed; thus increasing the shelf life of the final product.

Mechanism of Action

Membrane filtration employs a specific semi-permeable membrane which allows the passage of selective compounds, known as "permeates", through it to fractionate a liquid into various constituents. The effectiveness of membranes is dependent on the concentration gradient of the liquids and thus formed resultant transmembrane potential across the membrane (Winston Ho and Sirkar 1992).

Types of Membrane Filtration

The types of membrane based separation technologies in the descending order of pore size are: nano-filtration, micro-filtration, reverse osmosis (RO)and ultra-filtration. Microfiltration (MF) process is analogous to ultrafiltration (UF) but has larger membrane pore size allowing passage of 0.1–10 μm size particles and uses less pressure than that of UF process. The UF membranes having1–100 nm membrane pore size are occasionally used for the retaining proteins and other macromolecules (van Reis and Zydney 2007). The procedure involves the usage of 40 psi pressure, temperatures of 50–60 °C with a cutoff value of 10,000 MW using polysulfone membranes. A type of reverse osmosis technique, nanofiltration (NF) allows the passage of monovalent ions. Reverse Osmosis (RO) is a membrane filtration process driven by usage of high pressure thus giving entrance to solutes with very low molecular weight. The process requires700 psi, with a cutoff value of 100 MW, and temperature of 40 °C and 70–80 °C for cellulose acetate and composite membranes respectively (Kumar et al. 2013).Electro Dialysis (ED) involves the movement of ions under the applied electric potential through the membrane which is cation- or anion-selective, allowing either positive ions or negative ions to pass through. Cation-selective membranes are polyelectrolytes having negatively charged matter, passing positively charged ions whereas opposing the entry of negatively charged ions and vice-versa.

Application

The MF casein concentrated milk is more suitable for cheese production due to enhanced firmness of curd. The cheese has improved shelf-life quality by removal of bacteria and spores, accelerated ripening and reduced additive concentration (Pierre et al. 1992; Caron et al. 1997; Maubois 2002; Schafroth et al. 2005).The utility of microfiltration has been well demonstrated for the separation of whey protein from skimmed milk (Govindasamy-Lucey et al. 2007; Lawrence et al. 2008).Nanofiltration has been used for desalting whey and production of lactose free milk. Greiter et al. (2002) conducted the demineralization of salt and acid rich

Table 1 Advantages and Limitations of Dairy processing techniques

Technique	Advantages	Limitations	Reference
Cold Plasma	– Low running cost	– Suitable gas should be used (basically noble gases)	
Ultrasonication	– Non-toxic – Environment friendly – Cheap – No need of sophisticated machinery	– Formation of free radicals leading to undesirable changes in food	Majid et al. 2015
High Pressure Processing	– Increases the microbiological safety – Modifies functional properties of foods	–Works for food with water as entire process is based on compression –Increases free fatty acid levels –Denatures whey	Kim et al. 2008 Chawla et al. 2011
Pulsed electric field	–Requires less processing time –Low processing temperature		Jose et al. 2010
Membrane Filtration	–Simple –Cost effective method	– Fouling of membranes which requires timely membrane cleaning	Kumar et al. 2013

cheese whey using electrodialysis and ion exchange process to prevent the environment menace on being used.

Advantages and Limitations

Although the modern processing techniques have several merits over the conventional method of thermal processing; these techniques also have some limitations. The advantages and shortcomings of the recent processing techniques are mentioned in Table 1.

Conclusions

Dairy processing is growing promptly around the globe to meet the growing appetite for milk-products from an ever-growing population. Milk is a valuable nutritious food having short shelf-life. It requires careful handling, since it is an excellent medium for the growth of microorganisms and thus highly perishable. The appropriate processing technique allows the preservation for extended period of time and thus reducing the microbial load, and thereby reduces chances of spoilage and that of food-borne illnesses. The thermal processing techniques has demonstrated its merits but with many caveats, such as nutritional and physiochemical quality loss.

Consequently, with growing concern, many other methods have emerged. These techniques have high perspective in food industry and better alternative to thermal processing. Nevertheless, each method has some advantages and limitations. Therefore, the selection of technique is based on the context of requirement: duration of shelf-life desired, safe and healthy, or better sensory appeal.

The aforementioned emerging technologies have promising role in obtaining dairy products with enhanced sensorial, nutritional and microbiological aspects; however, a comparative study across all the techniques is warranted for inferring the most suitable method for particular product type. In the era of precision medicine, further research and analysis is required to be properly channelized towards meeting the consumer health requirement and efficacy. There is always the scope for improvement of existing one and exploration of new method for realizing the goal of 'white revolution'.

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Insect Pests Infestation During Field and Storage of Fruits and Vegetables

M. Shafiq Ansari, Rabiya Basri, and Surendra Singh Shekhawat

Abstract This is an overview of insect pests associated with tropical, subtropical and temperate fruits and vegetables grown in different agro-climatic zones of India. India is known as a fruit basket of world as well as second highest in vegetable production. Fruits and vegetables are attacked by insect pests of Heteroptera, Homoptera, Diptera, Coleoptera and Lepidoptera. Homopterans are sucking pests that result in decreasing plant vigour and also transmitting more than 260 plant viruses. Bunchy top of banana is transmitted by *Pentalonia nigronervosa*, potato leaf curl virus by *Myzus persicae*, papaya ring spot and chilli leaf curl by *Bemisia tabaci*. Bacterial diseases of plants; *Erwinia amylovora* is transmitted by wasps and bees, *E. carotovora* by onion maggot fly and *E. tracheiphila* by spotted and stripped cucumber beetles. Fruit flies, *Bactrocera* spp. are the major threats to mango, guava, papaya, peach, pear and cucurbits in India. Major insect pests of fruits are mango hoppers; *Idioscopus* sp., *Amritodus atkinsonii*, banana rhizome weevil; *Cosmopolites sordidus*, papaya mealy bug; *Paracoccus marginatus*, citrus butterfly; *Papilio demoleus*, citrus psylla; *Diaphorina citri*, mealy bug; *Ferrisia virgata*, stem girdler; *Sthenias grisator*, San Jose scale; *Quadraspidiotus perniciosus*, codling moth; *Cydia pomonella*, and woolly aphid; *Eriosoma lanigerum*. Other than these, *Helicoverpa armigera*, *Spodoptera litura*, *Plutella xylostella*, *Scirtothrips dorsalis, Phthorimaea operculella*, *Tuta absoluta*, *B. tabaci*, *Thrips tabaci* and *Tetranychus urticae* are major threat in vegetables production causing enormous losses. Fruitflies, stone weevil, codling moth, potato tuber moth, sweet potato weevil, almond moth, redflour beetle and khapra beetle are mainly affecting produce in storage.

Keywords Fruits · Vegetables · Infestation · Insect pests · Losses · Management

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Introduction

India is the second largest producer of the fruits and vegetables in the world, contributing 10.23% and 14.45% of the total world's production, respectively (Anonymous 2017b). India is the major producer of mango, banana, papaya, cashew-nut, areca-nut, peas, potato, cauliflower, okra, ginger, onion, cabbage, and brinjal (Raj et al. 2017). India produces 92.84 mt of fruits cultivated in 6.3 mha and 178.17 mt vegetables in 10.23 mha in 2016–2017 (Anonymous 2017c). India exported fruits worth of 655.90 million USD and vegetables 804.03 million USD that share 1% of global market. Highest exported fruits are mango, walnut, grape, banana, and pomegranate while among the vegetables; onion, okra, bitter gourd, green chilli, mushroom and potato (Anonymous 2017a) but the acceptance of horticulture produce from India is increasing day by day.

Despite high production, productivity is very low in India compared to China, USA, Brazil, Italy and the Philippines (Anonymous 2017c). Biotic and abiotic factors are responsible for limiting productivity such as seed quality, soil and climatic conditions, weeds, insect pests and diseases.

Insect pests are causing both qualitative and quantitative damage: by feeding (defoliators and borers) and by sucking sap (Peterson and Higley 1993; Ram and Byrne 2009). They are responsible for causing 25–30% yield loss in vegetables (Rahman 2006). Arthropods supposed to be destroying an estimated 18–20% of the annual crop production worldwide estimated at a value of more than US$470 billion (Oerke 2006; Sharma et al. 2017). Overall crop losses have declined from 23.3% to 17.5% in post-green revolution era (Dhaliwal et al. 2010). In India, expected annual production losses due to pests are as high as US$ 42.66 million (Sushil 2016).

India produces tropical, subtropical and temperate fruits:

1. Tropical fruits: mango, guava, banana and papaya
2. Subtropical fruits: ber, citrus, grape, pomegranate and litchi
3. Temperate fruits: pear, apple, peach, cherry, walnut and almond

Tropical Fruits

Mango

India is the largest producer of mango contributing about 40% of world's production. India has exported about 1.6 mt (167.04 USD) in 2017–2018 (Anonymous 2019a, b, c, d). Uttar Pradesh is a leading producer of mango with 0.045 mt followed by Andhra Pradesh 0.0404 mt which contribute to the total production of 0.22 mt in India in 2017–18. Diseases and insect pests cause severe damage. Tandon et al. (1978) reported as many as 492 insect species infesting mango crop. More than 300 insect pests are reported from all over the world to infest mango crop (Patel et al. 2004) and 45% alone are reported from India. They are responsible for the heavy losses in terms of quality and quantity.

Insect Pests of National Significance

Mango hoppers: *Idioscopus clypealis* (Letheirry), *I. nitidus* Walker, *I. niveoparsus*, *Amritodus atkinsonii* (Letheirry) (Homoptera: Cicadellidae)

Mango mealybug: *Drosicha mangiferae* Green (Homoptera: Margarodidae)

Fruit flies: *Bactrocera dorsalis* Hendel, *B. correcta* Bezzi, *B. zonata* Saunders (Diptera: Tephritidae)

Inflorescence midges: *Erosomyia indica* Grover, *Procystiphovra mangiferae* Bitancourt and Jenkin, *Procontarinia mettiana* Kieffer & Cecconi (Diptera: Cecidomyiidae)

Stem borer: *Batocera rufomaculata* De Geer (Coleoptera:Cerambycidae)

Bark eating caterpillar: *Indarbela quadrinotata* Walker (Lepidoptera: Metarbelidae)

Stone weevil: *Sternochaetus mangiferae* (Fab.) (Coleoptera: Curculionidae)

Leaf Webber: *Orthaga exvinascea* Walker (Lepidoptera: Pyralidae)

Red ant: *Oecophylla smaragdina* (Fab.) (Hymenoptera: Formicidae)

Eriophyid mite: *Aceria mangiferae* Sayed (Prostigmata: Eriophyidae)

Termites: *Odontotermes obesus*, (Rambur) *Microtermes obesi* (Holmgren) (Isoptera: Termitidae)

Regional Significance

Red spider mite: *Oligonychus mangiferus* Rahman and Spara (Trombidiformes: Tetranychidae)—Bihar

Scale insects: *Aspidiotus destructor* Signoret (Homoptera: Diaspididae)—Uttar Pradesh, Karnataka

Shoot borer: *Chlumetia transversa* Walker (Lepidoptera: Noctuidae)—Karnataka, Rajasthan, Maharashtra, Himachal Pradesh, Bihar, U.P., Gujarat

Shoot gall psylla: *Apsylla cistellata* Beckton (Homoptera: Psyllidae)—Bihar, U.P.

Thrips: *Scirtothrips dorsalis* Hood, *Caliothrips indicus*, *Rhipiphorothrips cruentatus* (Thysanoptera: Thripidae)—Gujarat, West Bengal

Mango hoppers, *Idioscopus clypealis*, *I. nitidus*, *I. niveoparsus*, *Amritodus atkinsonii*

Biology

Recently 12 species of hoppers are reported from India (Naraynashetty et al. 2017) belong to Cicadellidae with two subfamilies: Idiocerinae and Typhlocybinae. Adults are 3 mm, greyish, having three brown spots on head, median band and two black spots on pronotum. Triangular markings are present on scutellum. They are active

in flowering and fruiting season with respect to oviposition and their development. They lay the eggs singly and embedded in plant tissues about 100–200 eggs/female (Sharma and Tara 2014). Incubation period is 4–7 days. Nymphs are seen at the end of Feb or early March. First and fourth nymphal instars lasted for 2–3 days and the second, third and fifth instars lasted for 2–4 days in *A. atkinsoni* whereas, each of the five instars lasted for 2–4 days in *I. clypealis* (Baro et al. 1997). Life cycle is completed in 15–19 days. Adults of second brood emerged in September and hibernate in winter. Immatures are greenish with black or brown markings on scutellum and move very fast. Both nymphs and adults are wedge-shaped. Adults are golden brown to dark brown, looks like a small cicada and measure about 4–5 mm in length (Fig. 1). It jumps off the plant with a clicking sound while get disturb.

Damage

They cause injury by egg laying in the inflorescence and sucking the sap voraciously, which leads to the withering of the inflorescence. Heavy infestation results in fruit dropping and growth of sooty mould on honeydew excreted by hoppers.

Parasitoids: *Polynema* spp., *Gonatocerus* sp., *Tetrastichus* sp.

Predators: *Mallada boninensis*, *Plexippus paykullii*

Management

(a) Spraying of imidacloprid (0.005%, 0.3 ml/l) at an early stage of panicle formation in case of more than five hoppers/panicle.
(b) Thiamethoxam (0.005%, 0.2 g/l water) or acephate (1.5 g/l water) applied after fruit setting.

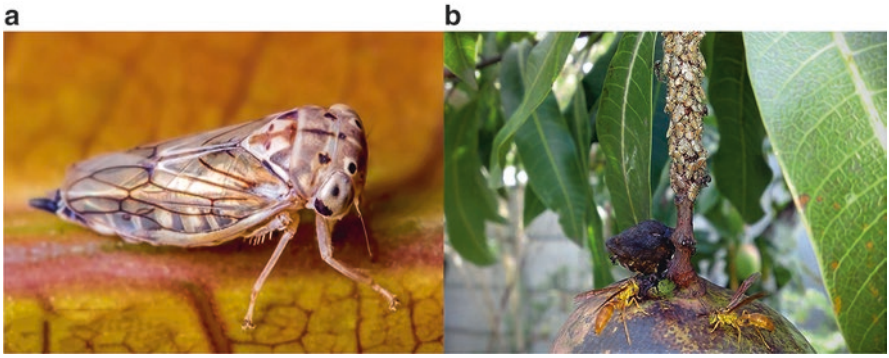

Fig. 1 Mango hopper (**a**) Adult (Source: https://cststudy.blogspot.com)(**b**) Infested shoot © Gauzz 2020 (2009)

(c) Farmers are advised not to apply any spray after the occurrence of 50% flowering because it will affect the insect pollinators, which leads to less fruit setting.
(d) Keep orchard clean by regular removal of weeds and pruning of overcrowded and overlapping branches in December (Anonymous 2011a, b).

Mango mealybug, *Drosicha mangiferae*

Biology

Females are wingless larviform, body covered over by waxy or mealy powder (Fig. 2c). They are sexually dimorphic. Adults males are winged and non-feeding stage, and short-lived. They only inseminate females and then die. After mating females are laying eggs in silken ovisac, then they descend in soil/cracks/crevices to find out shelter. Eggs present in ovisac are in diapaused condition for 9 months. Eggs hatch in the body of a female in December–January, and they feed upon the content of the mother's body and emerge out. The first instar nymphs were finding a suitable place and fixed its proboscis to suck the sap from the weeds and other plants of the orchard. The second instar nymphs start ascending on the mango tree to suck the sap from tender twigs, shoots, and fruits. The third instars were already present on the plant and change to adult. It passes to four instars to become adult female, while five instars required for the development of males. Temperature and relative humidity are major ecological factors which affect mealybug as well as their natural enemies (Chong and Oetting 2006; Gutierrez et al. 2008; Nakahira and Arakawa 2006; Walton and Pringle 2005).

Fig. 2 (**a**) Alkathene banding (https://blog.realenglishfruit.co.uk/2012/10/10/grease-banding-fruit-trees/) (**b**) Alkathene banding. (https://blog.realenglishfruit.co.uk/2012/10/10/grease-banding-fruit-trees/) (**c**) Mango mealy bug. (http://www.nbair.res.in/insectpests/Drosicha-mangiferae.php)

Damage

Nymphs attack on the inflorescence which leads to flower dropping or fruit setting. Excretion of honeydew facilitates the development of sooty mould, which affects the photosynthesis (Pruthi and Batra 1960).

Predators: *Menochilus sexmaculatus*, *Rodolia fumida*, *Cryptolaemus montrouzieri*, *Suminus renardi*

Management

(a) Flooding of the orchard in October and along with deep ploughing in November.
(b) Fastening of 25 cm alkathene band of 400-gauge wide sheet and later mud plastering of the trunk at 30 cm above the ground can be done in the middle of December (Fig. 2a, b)
(c) Application of chlorpyriphos dust (1.5%) @ 250 g/tree by loosens the soil around the tree trunk, which helps in reduction of mealybug population.

Mango nut weevil, *Sternochaetus mangiferae*

It is a monophagous pest and cosmo-tropical distribution in mango growing regions of South Asia, Central Africa, Australia and Pacific Island (Tandon and Verghese 1985). Warner (1956) has used *Sternochetus* as the genus.

Biology

Weevil has a compact body of about 8 mm long (Fig. 3c). Usually, they are active at dusk. They mimic like dead when they get disturbed. Females start egg laying in the marble size fruits after 3–4 days of mating. Adults feed on mango leaves, tender shoots or flower buds. They can survive for 2 years. Eggs are small, elliptical about 0.8 mm in length and 0.3 mm in width and laid singly in small cavities under the skin of young fruits and into inflorescence. Freshly laid eggs are creamy white (Pinese and Holmes 2005). Females used to cover their eggs with brown exudate

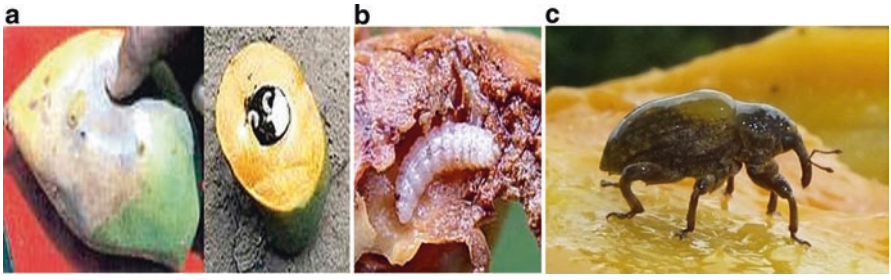

Fig. 3 (**a**) Symptom of egg laying and grub inside (**b**) Grub (**c**) Mango nut weevil (http://agritech.tnau.ac.in/crop_protection/mango)

and also cut very small semicircular area of about 0.3 mm in the fruit at the immediate back of the egg. This injury results in sap flow, which covers the egg by making a protective layer. Females may deposit several eggs in each fruit. Incubation period is 5–7 days. Grubs are white with a brown head. The body of newly hatched grub is curved in 'C' shape (Pinese and Holmes 2005), extremely slender, elongated and about 1 mm long without legs. Mature grub is about 17 mm long in size. Larvae enter into the flesh of the fruit and seed immediately after hatching. Generally, the development of the larva completes within the mature seed, but sometimes it also occurs in fruit pulp. The pupa is white but later turns pale red just before the adult emergence. Pupation occurs in the seed inside the fruit stone (Balock 1961). Life cycle is completed in 40–50 days. Emerged adults are inactive and rescue breeding in next season.

Damage

This pest has been a barrier in the export of fresh mangoes. Grubs feed on unripe tissue by making tunnels in pulp and then bore into cotyledons (Fig. 3a, b). Injuries caused by feeding as well as egg-laying results in fruit dropping. External sign of infestation is undetectable (Pertanian 2004) and carried with the fruit to storage, therefore, may cause damage in storage also.

Predators: *Camponotus* spp., *Oecophylla smaragdina*

Management

(a) Field sanitation required to collect and destroy all fallen fruits at weekly interval.
(b) Soil below the tree must be raked up in October or November and March to destroy weevils which are hiding under fallen leaves.
(c) Spraying of chlorpyriphos 0.05% should be done in November and December to manage the hiding beetles in the bark.

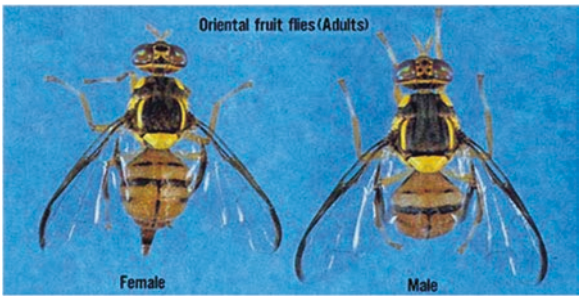

Fig. 4 *Bactrocera dorsalis* (Hendel) (http://entnemdept.ufl.edu/creatures/fruit/tropical/oriental_fruit_fly.htm)

Fruit fly, *Bactrocera dorsalis*

Biology

The adults are brown, having transparent wings, yellow legs. They have two horizontal black stripes and longitudinal median stripe on the abdomen from the third segment to the last abdominal segment and appear like "T" shaped pattern (Fig. 4). They are active in summer season, hibernate in November to March and emerged in April. After mating females start laying eggs on ripened fruits and vegetables of the season and later, they shift to mango. A female lays about 150–200 eggs in 1 month under field condition. Eggs are laid in a cluster of about 10–50 eggs under the skin of fruits. They are white or cream, elongated, elliptical and measure about 8–9 mm long. Generally, hatching occurs in 2–3 days in March to April, 1–1.5 days in summer but it may extend up to 10 days during the winter season. Maggots are elongated, cone-shaped and without a leg. The mouth is oriented at the pointed end of the body. There are three larval stages, usually complete in 11–15 days. Full grown larvae left the fruit and dropped in soil to pupate by forming yellowish brown puparium. Adult emergence takes place after 6–44 days.

Damage

Females choose half ripened/ripened fruits for egg laying by puncturing epidermal layer. Maggots may be carried with the fruit from field to storage. Maggots are feeding (Fig. 5) upon fruit pulp results into rotting and also provides an avenue to secondary infection in field as well as in storage of the fruits, which makes them unfit for human consumption.

Management

(a) Collection and destruction of dropped infested fruits and deep ploughing of orchards.

Fig. 5 Maggot of fruit fly infesting mango (https://www.cbp.gov/newsroom/local-media-release/cbp-la-intercepts-fruit-flies-mangos)

(b) Use of 100 ml water emulsion with methyl eugenol (0.1%) + malathion (0.1%) as a trap during April–June will help in reduction of the infestation of fruit flies.
(c) Bait spray of carbaryl (0.15%) + protein hydrolysate (0.1%) or molasses could be sprayed at 21 days of interval beginning from the first week of April is effective for management of adult flies.

Guava

Insect Pests of National significance

Fruit flies: *Bactrocera correcta* (Bezzi), *B. zonata*, *B. dorsalis* (Hendel) (Diptera: Tephritidae)

Castor capsule borer: *Conogathes punctiferalis* Guenee (Lepidoptera: Crambidae)

Pomegranate butterfly: *Deudorix isocrates* Fabricius (Lepidoptera: Lycaenidae)

Bark eating caterpillar: *Indarbella tetraonis* (Moore) (Lepidoptera: Cossidae)

Mealy bugs: *Ferrisia virgata* (Cockerell), *Maconellicoccus hirsutus* Green (Homoptera: Pseudococcidae)

Guava fruit fly, *Bactrocera correcta*

Biology

Adults are brownish or dark brown having hyaline wings and yellow legs. Flies of *B. correcta* (Fig. 6a) are similar to *B. orientalis*, but have darker or almost black thorax and also smaller in size. They are distinguished from *B. zonata* due to presence of facial spots which are almost united to form a black transverse band (Ansari et al. 2015; Weems 1987; Hardy 1973). A white color cross band on the second abdominal segment is less developed. The hind tibiae is remarkably tuberculate in

Fig. 6 (**a**) *Bactrocera correcta* (Bezzi) (https://entnemdept.ifas.ufl.edu/creatures/fruit/tropical/guava_fruit_fly.htm) (**b**) *Bactrocera invadens* (https://www.researchgate.net/publication/262185464_Monitoring_of_Tephritidae_of_Fruit_Trees_and_Their_Level_of_Infestation_in_South_Kordofan_State_Sudan). (**c**) Maggots of Fruit fly infesting Guava (http://eagri.org/eagri50/ENTO331/lecture19/guava)

males (Hardy 1973). A female can lay 150–200 eggs in 1 month under field conditions. The total development time is about 16 days in the summer season. Mature larva drops down to the ground just after emergence from the fruit for pupation in soil into tan to dark brown puparium. Adult fly attains sexual maturity in about nine days after emergence. Temperature, rainfall and relative humidity greatly influence their population (Siswanto et al. 2008). Maximum fly populations were noticed during the third week of June, whereas the lowest numbers were observed during the last week of August (Agarwal and Kumar 1999).

Damage

The adults attack semi-ripened fruits for depositing eggs by puncturing the fruits. Maggots feed on the fruit pulp which leads to turning of flesh into a semi-liquid mass having a bad odor (Fig. 6c). Maggots are carried inside the fruit as hidden infestation after harvesting.

Management

(a) Infested fallen fruits should be removed and dumped in 40–60 cm deep pit.
(b) Deep ploughing about 5–10 cm.
(c) Methyl eugenol trap @ 5/acre and bait traps used in mango.

Banana

India leads in the production of banana with 29 mt of annual production in the area of 0.8 mha (Anonymous 2017c) Nonetheless, Cavendish bananas are supreme among the banana export in the world. Although, traditional bananas like Nendran, Ney Poovan, and Red banana have built their value in hyper malls of West Asia and South East Asia markets on the demand of Indian populations. Nendran variety is commercially grown in Kerala, Tamil Nadu, and parts of Karnataka including 50% of the total area in these states. Adequate profit of the business is getting diminished due to the export of fruits through air cargo. Indian government has signed memorandum of understanding (MoU) for a consultancy project "Development of Sea Protocol for the Trial Sea Shipment of Traditional Nendran banana to Dubai" on the kind attempt of ICAR-NRCB, Trichy and APEDA, New Delhi to set a new voyage by sea to Dubai with its 'Made in India' Nendran Bananas.

Insect Pests of National Significance

Banana rhizome weevil: *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae)
Banana stem weevil: *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae)

Banana aphid: *Pentalonia nigronervosa* Coquerel (Homoptera: Aphididae)
Rust thrips: *Chaetanothrips signipennis* Bagnall (Thysanoptera: Thripidae)

Regional Importance

Hard scale: *Aspidiotus destructor* Signoret (Homoptera: Diaspididae)
Fruit fly: *Bactrocera dorsalis* Hendel (Diptera: Tephritidae)—Karnataka
Banana scab moth: *Nacoleia octasema* Meyer (Lepidoptera: Pyralidae)—Uttar Pradesh

Banana rhizome weevil, *Cosmopolites sordidus*

It may cause yield loss up to 100% (Sengooba 1986; Koppenhoeffer et al. 1994) because of sucker death, toppling and snapping and shortens plantation life spans if not controlled (Rukazambuga et al. 1998). They are able to migrate from one farm to the other (Gold and Bagabe 1997; Gold et al. 2002).

Biology

Newly emerged weevil is red-brown but later becomes dark and measure 10–15 mm in length. Adults are active at night. Females lay white eggs singly on the upper part of the rhizome by making hole using rostrum. Grubs are yellowish white with a red head (Fig. 7) and pupa is white. Pupation occurs in the tunnel formed by larva inside the corm. Longevity of adult is 1–4 years. Weevil can survive without food for several months. Oviposition rate is 1 egg/day or 1 egg/week. Incubation period is 7–10 days. Flowering plants and crop residues are preferred site for oviposition. Oviposition occurred throughout the banana cycle with egg density increasing with

Fig. 7 (**a**) *Cosmopolites sordidus* infestation (http://nrcb.res.in/album/Corm%20weevil%2C%20Cosmopolites%20sordidus/index.html) (**b**) *Cosmopolites sordidus* (Source: http://www.ces.csiro.au)

plant age. Larva passes through 5–8 instars and completed the development in 14–42 days. Life cycle is completed in 5–7 days (Abera et al. 2000).

Damage

Grubs bore into rhizome cause withering of leaves and ultimately results in the death of the plant (Fig. 7). Debris around banana plants provides a place to beetles for hiding during day time. Adults are attracted by volatiles emanating from host plants. Cut rhizomes are especially attractive (Gold and Massiaen 2000).

Management

(a) Destroy shelter and feeding places of weevils.
(b) Suckers should be treated with 0.1% quinalphos before planting.
(c) Spray phosphamidon @ 315 ml or dimethoate @ 100 ml or 625 ml in 625 l of water/ha.

Banana aphid, *Pentalonia nigronervosa*

Biology

Adults are small to medium sized and shiny red to dark brown or almost black (Fig. 8b). They have prominent dark veins. Adults start producing offspring after reaching sexual maturity. Nymphs are oval and slightly elongated. They are reddish brown. They can give birth to 4 nymphs /day with an average of 14 aphids per female. Life cycle is almost anholocyclic. Sexual morphs have been reported only in India and Nepal (Blackman and Eastop 1984). Apterous and pterous forms are found at the same time on the plants. Adults and nymphs are sucking the sap from the soft portion. There are three or four nymphal instars to become young. Pterous aphids breed sexually to lay the eggs.

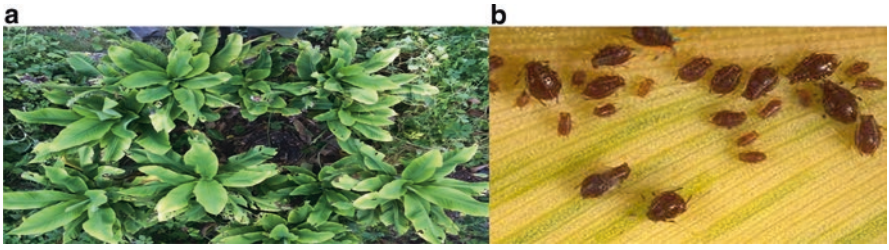

Fig. 8 (**a**) Bunchy top of banana (https://www.flickr.com/photos/scotnelson/15186492036) (**b**) *Pentalonia nigronervosa* (Source: nbair.res.in)

Damage

Infestation leads to upward curling and rosette formation with wavy leaf margins. The banana aphid is a significant pest of banana and acts as vector for virus causing banana bunchy top (Hu et al. 1996; Elayabalan et al. 2015).

Management

Spray of dimethoate (30EC) @ 1.7 l or oxydemeton methyl @ 1.25 l in 1250 l of water/ha.

Papaya

Insect Pests of National Significance

Mealybug: *Paracoccus marginatus* Williams and Granara de Willink (Homoptera: Pseudococcidae)

Ak grasshopper: *Poekilocerus pictus* (Fab.) (Orthoptera: Pyrgomorphidae)

Regional Significance

Papaya whitefly: *Bemisia tabaci* Gennadius (Homoptera: Aleurodidae)

Aphids: *Aphis gossypii* Glover, *Myzus persicae* Sulzer (Homoptera: Aphididae)

Fruit flies: *B. papayae* (Drew and Hancock), *B. diversus* Coquilett, *B. cucurbitae* (Coquilett) (Diptera: Tephritidae)

Grey weevil: *Myllocerus viridans* (Fab.) (Coleoptera: Curculionidae)

Red spider spotted mite: *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae)

Papaya mealybug, *Paracoccus marginatus*

Papaya mealy bug is a native to Mexico and Central America, where it never acquires the status of a serious pest, probably due to the presence of an endemic natural enemy complex (Tanwar et al. 2010). In India, it was recorded in July 2007 at Tamil Nadu Agricultural University, Coimbatore and subsequently spread to neighbouring districts (Muniappan 2009). It has a broad range of host plants of over 60 species including some economically important such as guava, papaya, maize, brinjal etc. (Chen et al. 2011; Seni and Chongtham 2013). It assumed the status of a major pest in 2009 when it caused severe damage to economically important crops in Coimbatore, Erode, Tirupur and Salem districts of Tamil Nadu (Tanwar et al. 2010).

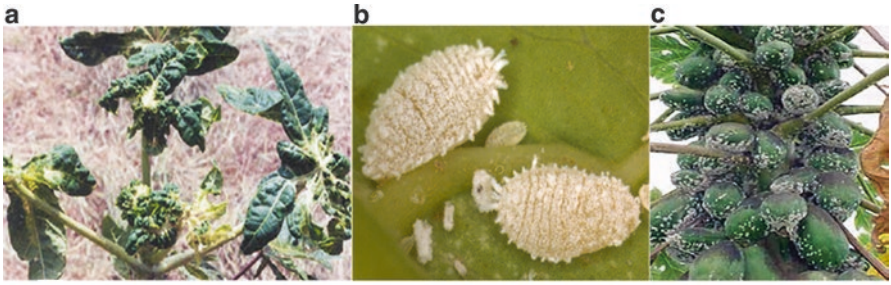

Fig. 9 (**a**) Leaf deformation caused by Papaya mealy bug (http://nemdept.ufl.edu/creatures/fruit/mealybugs/papaya_mealybug.htm) (**b**) *Paracoccus marginatus* (Source: nbair.res.in) (**c**) Mealybug infestation on papaya (moa.gov.in)

Biology

It is a polyphagous pest (Miller and Miller 2002). The adult female is greenish yellow (Miller et al. 1999) with a white waxy covering (Fig. 9b) whereas males are pink (Anonymous 2011a, b) but appear yellow in the first and second nymphal stage. Females measure about 3 mm long with 1.4 mm width while adult males are about 1 mm in size. Antennae are eight segmented (Walker et al. 2003). Genitalia of male is sclerotized, aedeagus apparents, wings approximately as long as the body with small basal vein (Anonymous 2008). Females generally lays 100–600 greenish yellow eggs in three to four times longer ovisac than the body length and entirely covered with white wax. Oviposition period lasts for 1–2 weeks. The incubation period is 10 days. Nymphs or crawlers start searching actively for feeding sites just after emergence from the eggs.

Damage

Affected plant turns brown due to loss of chlorophyll and dries away. They appear like a cluster of cotton masses on the underside of leaves, stem, and fruits. The growth of sooty mould also occurs on the honeydew secreted by mealybugs.

Parasitoids: *Acerophagus papayae*, *Phygadium* spp.

Predators: *Spalgis epius* (West wood), *C. montrouzieri*, *R. fumida*

Management

(a) Destruction of infested plant parts and avoid transportation of infested plant material.
(b) Spraying plants with a soap + kerosene oil + water mixture.
(c) Wrapping polythene/spongy tapes impregnated with insecticides around tree trunks to exclude ants from the canopy (Galanihe et al. 2010).
(d) Thiamethoxam 25%WG @ 1 g/l; mineral oil at the rate of 5 ml/l.

Whitefly, *Bemisia tabaci*

Biology

Female lays about 200–300 eggs near the veins on the underside of leaves. Eggs are stalked, pear-shaped, approximately 0.25 mm in size and vertically attached to the leaf surface through a pedicel. Freshly laid eggs are white but later turn into brown color. First instar larva moves on the leaf surface to locate a suitable feeding site immediately after hatching. Therefore, first instar larva commonly called as "crawler." Newly hatched nymph starts sucking the plant sap from the phloem and remain there till emergence. The emergence of adult takes place through T-shaped ecdysial cleavage line of puparium leaving behind empty pupal cases or exuviae. The body and wings are covered over by white powder with crimson red eyes (Fig. 10b). The body is light yellow. Males are slightly smaller in size than the females. Adult longevity is about 1–3 weeks.

Damage

Infestation reduces the plant vigor and results in yellowing and dropping of leaves. They secrete a considerable amount of honeydew, which favors the growth of sooty mould on the leaf surface, as a result, reduces the photosynthetic rate of plants. It is a vector of papaya leaf curl virus (Fig. 10a, b). Main symptoms of papaya leaf curl disease are inward/outward curling of plant leaves, vein thickening, and stunted plant growth with small distorted fruits or no fruits (Saxena and Varun 2017).

Parasitoids: *Encarsia formosa*, *Eretmocerus* spp., *Chrysocharis pentheus*

Predators: *Dicyphus hesperus*, Lacewing, ladybird beetle, mirid bugs (*Geocoris* sp.)

Management

(a) Installation of 4–5 yellow sticky trap/acre.
(b) Water sprays to dislodge the adult flies.

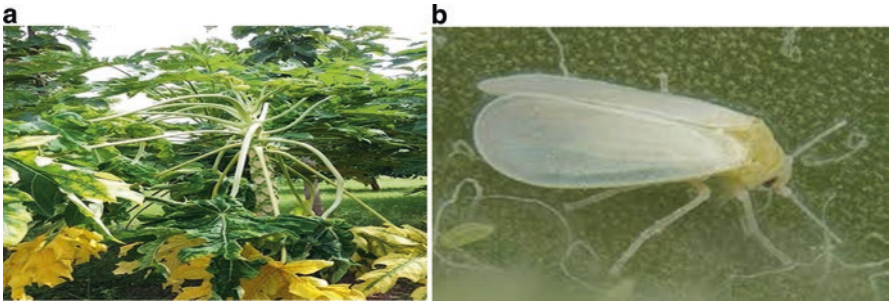

Fig. 10 (**a**) Papaya leaf curl transmitted by white fly (https://plantix.net/plant-disease/leaf-curl-virus) (**b**) *Bemisia tabaci* (Source: nbair.res.in)

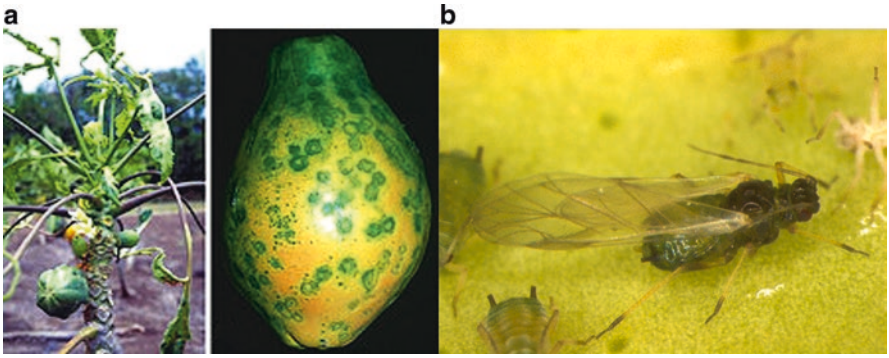

Fig. 11 (**a**) Papaya Ring Spot virus (https://en.wikipedia.org/wiki/Papaya_ringspot_virus) (**b**) *Aphis gossypii* (Source: nbair.res.in)

Aphid, *Aphis gossypii*

Biology

Adults are pterous and apterous and both of them are present on the same host plant. Immature completes the development in 7–10 days. Adults are soft-bodied measuring about 1–4 mm with two long antennae (Fig. 11b). It has two short cornicles present on fifth and sixth abdominal segments of the body.

Damage

Infestation cause curling and crinkling of leaves with a stunted growth of plants (Fig. 11a). Sooty mould develops on the honeydew exuded by aphids.

Parasitoids: *Aphidius colemani*, *Aphelinus* sp.

Predators: Fire ant, robber flies, praying mantis, lacewing, ladybird beetle, spiders, etc.

Management

Monoculturing of papaya should be avoided, especially when adjacent crops or weed plants support enormous populations of any one of the vector species (Kalleshwaraswamy and Kumar 2008).

Sub-tropical Fruits

Ber

India stands second in the ber production in world ranking after China. It occupied around 0.1 mha area and cultivated all over India mainly in Maharashtra, Madhya Pradesh, Gujarat, Punjab, Haryana, Rajasthan, Karnataka, Andhra Pradesh, Tamil Nadu, Bihar, West Bengal, and Assam (Bal 2014).

Insect Pests of National Significance

Fruit fly: *Carpomyia vesuviana* Costa (Diptera: Tephritidae)
Green slug caterpillar: *Thosea* sp. (Lepidoptera: Limacodidae)
Mite: *Laryacarus transitans* Ewing (Tetranychoidea: Tenuipalpidae)

Regional Significance

Stone Weevil: *Aubeus himalyanus* Voss (Coleoptera: Curculionidae)
Bark eating caterpillar: *Indarbela quadrinotata* Walker (Lepidoptera: Metarbedelidae)
Mite: *Eriophyes cernus* Massee (Acari: Eriophyidae)
Laef miner: *Cameraria* spp. (Lepidoptera: Gracillariidae)
Grey weevil: *Myllocerus undecimpustulatus* Marshall Faust (Coleoptera: Curculionidae)

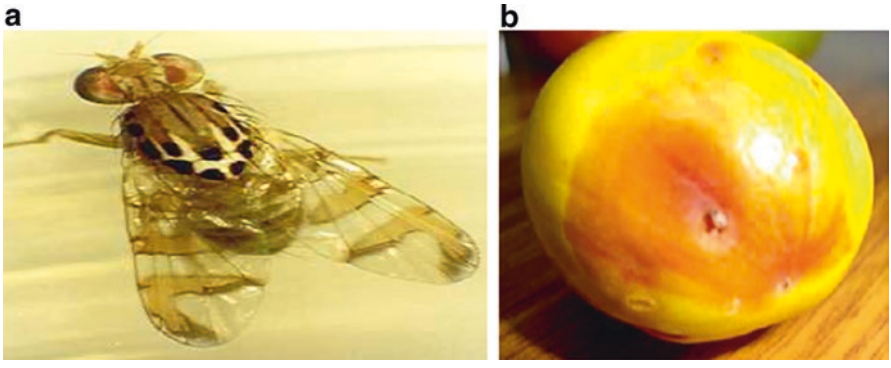

Fig. 12 (**a**) Fruit fly: *Carpomyia vesuviana* (Source: Vikaspedia) (**b**) Damage caused by maggots (http://vikaspedia.in/agriculture/crop-production/integrated-pest-managment/ipm-for-fruit-crops/ipm-strategies-for-ber/ber-insect-and-mite-pests)

Ber fruit fly, *Carpomyia vesuviana*

It is a monophagous pest of *Zizyphus* sp. growing in semi-arid and arid region in Oriental and temperate Asia, middle east, China, Europe including India (Kapoor 2005; Stonehouse et al. 2002; Farrar et al. 2004; Hu et al. 2010). The pest contributes to low yield and poor quality of fruits and causing loss up to 80% under severe infestation (Karuppaiah et al. 2010). Incidence of *C. vesuviana* reduce the yield from 13 to 20% per plant (Bagle 1992) but in severe condition it may damage up to 90–100% (Joshi and Shinde 1971).

Biology

Adult has two horizontal black stripes and longitudinal median stripe on the abdomen from the third segment to the last abdominal segment and appear like "T" shaped pattern (Fig. 12a). Females start egg laying after 8 days of pre-oviposition period and laying 10–50 eggs in cluster 1 mm below the skin of fruits. Eggs are white and spindle-shaped. The incubation period is about 1–1.5 days. Larva is cone shaped apodous known as maggot. The mouth is oriented at the pointed end of the body. There are three larval instars. Larval development completed in 11–15 days. Last instar drops to the ground for the pupation in the soil inside seed like yellowish brown puparium. Emergence takes place after 10 days of pupation.

Damage

Infestation starts with the onset of fruiting. The larvae make galleries inside the fruit. The fecal matter starts accumulating inside those galleries and leads to the rotting of fruits (Fig. 12b). Excessive infestation results in retarded growth and fruit dropping.

Parasitoids: *Fopius arisanus*, *Diachasmimorpha kraussi*

Management

(a) Collect and destroy the infested fallen fruit.
(b) Deep ploughing during summer to expose pupae to their natural enemies.
(c) Harvesting of fruits at green and firm stage.

Stone weevil, *Aubeus himalyanus*

Aubeus himalayanus is recently recorded as a pest of ber. It was first time reported as a pest of ber from Andhra Pradesh, Rahuri, Maharashtra, Jobner, Rajasthan in 1996 and Bikaner, Rajasthan in 2010.

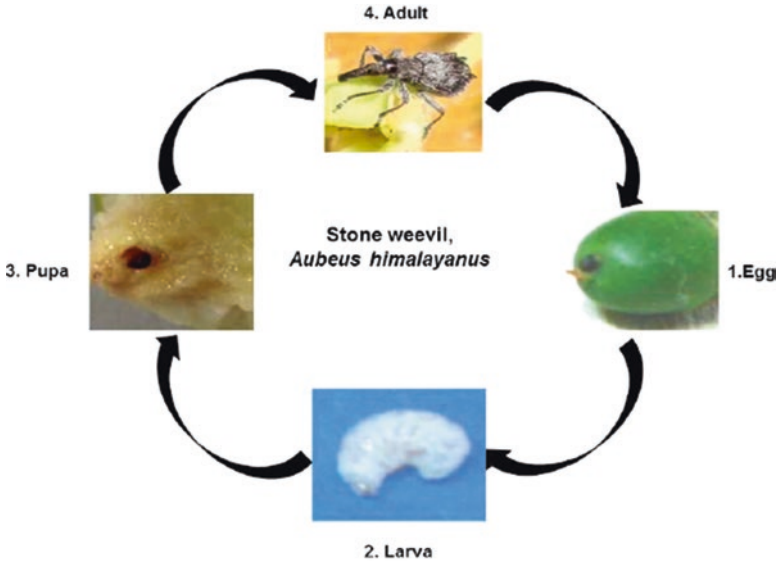

Fig. 13 Life cycle of stone weevil (http://vikaspedia.in/agriculture/crop-production/integrated-pest-managment)

Biology

Adults are small dark in color with snout (Fig. 13). Grubs are white with red marking on the body. Adults are generally active in the morning and evening hours. They used to present inside the seeds (Karuppaiah et al. 2010). The life cycle of stone weevil has not been studied so far.

Damage

Adult punctures fruit for oviposition and lays eggs on the stylar end of fruits. They consume the inner content of the seed and make hollow galleries for pupation. Grubs make tunnels into the seed of fruits. Infested fruits become abnormal in shape and appear reddish brown. Infested fruit doesn't reach to maturity.

Management

(a) Spraying of carbaryl 50 WDP 0.1% just before the fruit setting and repeat the sprays at 3 weeks interval (Anonymous 2004).
(b) Collect and destroy the adult weevils immediately after detection that can also reduce the population. Infested fallen fruits should be burried to break the generation cycle (Karuppaiah 2013).

Citrus

Citrus is the leading fruit crop in the world and it is native to South East Asia. In world, India ranks sixth in production of the citrus. Next to mango and banana, citrus fruit represents the third largest fruit industry of India (Aruna et al. 2017). More than 30% of citrus production in the country is lost every year as a result of damage caused by insects and mites.

Insect Pests of National Significance

Citrus aphid: *Toxoptera aurantii* (Boyer de Fonscolombe), *Aphis citricola*, *Myzus persicae* (Homoptera: Aphididae)

Citrus psylla: *Diaphorina citri* (Kuwayama) (Homoptera: Liviidae)

Citrus leaf miner: *Phyllocnistis citrella* (Stainton) (Lepidopter: Gracillariidae)

Mealy bug: *Planococcus citri* (Risso) (Homoptera: Pseudococcidae)

Fruit sucking moth: *Eudocima phalonia* (Linn.), *E. maternal* Linn. (Lepidoptera: Erebidae)

Citrus lemon butterfly: *Papilio demoleus* Linn., *P. polytes* (Linn.) (Lepidoptera: Papilionidae)

Regional Significance

Citrus blackfly; *Aleurocanthus woglumi* (Ashby) (Homoptera: Aleyrodidae)—Punjab, Maharashtra, Karnataka, West Bengal, and Madhya Pradesh

Armored scale; *Aonidiella aurantii* (Maskell) (Homoptera: Diaspididae)—Meghalaya, Punjab, Uttar Pradesh, Maharashtra, West Bengal, and Karnataka

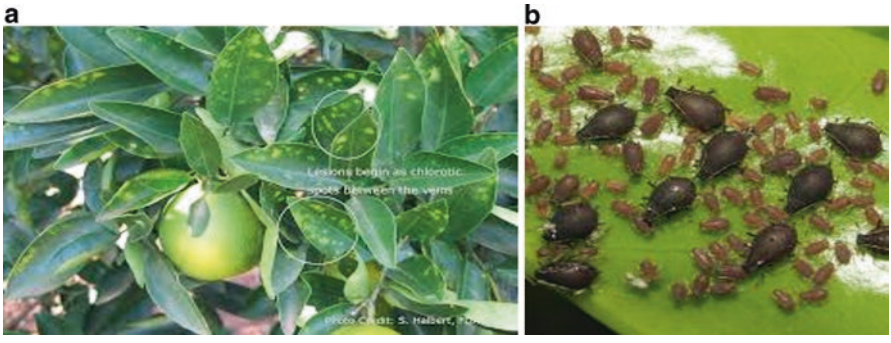

Fig. 14 (**a**) Symptoms of citrus tristeza virus on citrus plant (https://crec.ifas.ufl.edu/extension/plant_pathology/cvc_gallery) (**b**) Colony of *Toxoptera aurantii* (https://www.agrobaseapp.com/canada/pest/black-citrus-aphid-3)

Stem borer; *Chlorigolum alcmene* (Guenee) (Lepidoptera:Noctuidae)—Tamil Nadu

Mite; *Eutetranychus orientalis* (Klein) (Prostigmata:Tetranychidae)—Meghalaya, Punjab, Madhya Pradesh, Rajasthan, Haryana, Karnataka, Delhi, Jammu, and Kashmir

Citrus aphid, *Toxoptera aurantii*

Biology

They are shiny black, reddish brown or brownish black in color and oval (Fig. 14b). Wings may be present or absent. Body is 1/25 in. broad and 1/12-in.-long with short black and white antennae. Winged aphids are slightly thinner and have dark abdomen. Females reproduce parthenogenetically or viviparous exclusively on the undersurface of flush leaves (Firempong 1997). There are three nymphal instars which are brownish. Entire population consist of females. The development of winged individuals depends on the population density and the stage of leaf. Aphids produce a stridulating sound like crickets. Development of aphids completely depends on temperature. The optimum temperature for the development of aphid is 20–25°C. They are capable of completing 30 generations/year. Disastrous epidemics of citrus tristeza virus (CTV) have occurred in Argentina, Brazil, Colombia and Peru (Rocha-Pena et al. 1995).

Damage

It is a serious vector of citrus tristeza virus (CTV) (Fig. 14a). They cause wilting and dropping of a flowers with cupping and crinkling of leaves.

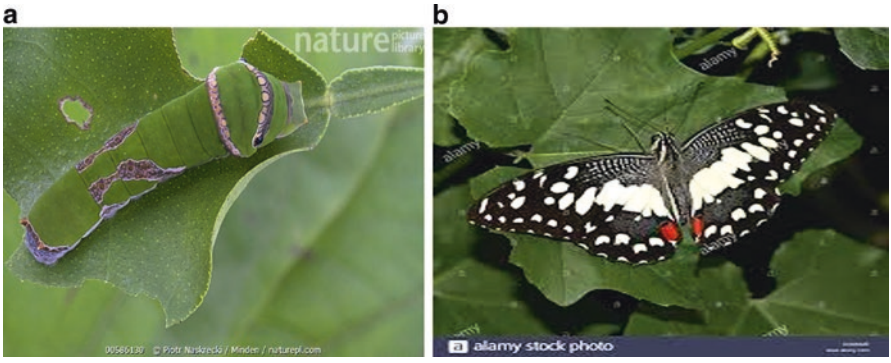

Fig. 15 (**a**) Larva of *Papillio demoleus* (**b**) Adult

Management

Spray of thiamethoxam (25WG) @ 400 g or imidacloprid (17.8 SL) @ 500 ml or dimethoate (30 EC) @ 3.25 l or oxydemeton methyl (25 EC) @ 250 ml in 1250 l water/ha.

Citrus/lemon butterfly, *Papilio demoleus*

Biology

Adults of *P. demoleus* are beautiful butterflies having white and black markings on both fore and hind wings. Wingspan is about 80–100 mm (Evans 1932). It has a brick red oval patch near the anal margin of rear wings (Fig. 15b). The tail-like extension is not present in this butterfly, but it is a common factor of family Papilionidae. Males of *P. polytes* are black while females vary in form. Adults of *P. helenus* has black wings with white distal spots. Females lay eggs on tender leaves and shoots. Eggs are smooth, round, yellowish white and about 1.5 mm (Lewis 2009; Kunte 2000). The incubation period is about 3–8 days. Newly hatched larvae are dark brown and later on irregular white markings develop on their body, which resembles like bird droppings. Typically, they undergo five instars (Lewis 2009), voracious feeders and defoliate entire seedlings or tree leaving behind only midribs. Caterpillar attaches themselves to the branches for pupation with silken structure. The pupa is rugose (wrinkled), stout, and 30 mm in length, has two projections to the front on its head and also one on its thorax, and resembles that of the common Mormon (*Papilio polytes*), the difference being that the common Mormon pupa has a deeper cut between the projections and its abdomen is more protruded on the sides, having a small point (Lewis 2009; Kunte 2000). Pupal period is 2–3 weeks. Population density is high from July to December. The lemon butterfly can survive at low temperatures even dropped down to 0°C.

Damage

Caterpillar (Fig. 15a) feeds voraciously on tender leaves leaving behind only midribs. The entire tree gets defoliated in case of severe infestation.

Management

(a) Hand picking of various stages of the pest in nurseries of new orchards.
(b) Spray *Bacillus thuringiensis* 1 g/l or neem seed kernel extract @ 3%.
(c) Quinalphos 25 EC or carbaryl 50 WP 2.0 l in 1500–2000 l water/ha during April and October.

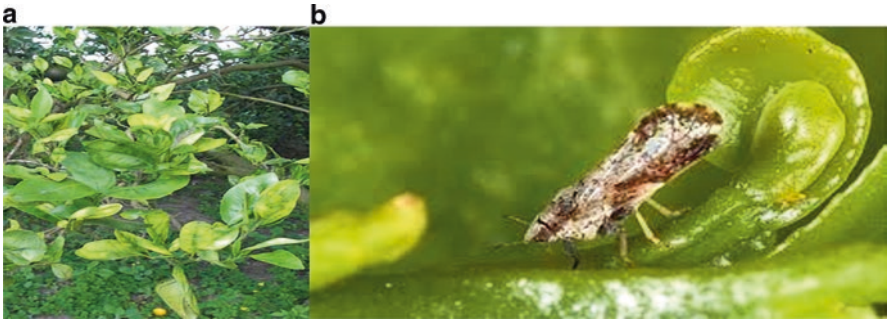

Fig. 16 (**a**) Citrus greening caused by citrus psylla (https://entnemdept.ifas.ufl.edu/creatures) (**b**) *Diaphorina citri* (https://www.ars.usda.gov/ARSUserFiles/35403/HallDiaphorinabiologyhistoryworldstatus2008.pdf)

Citrus psylla, *Diaphorina citri*

It is known to occur in China, India, Myanmar, Taiwan, Philippine Islands, Malaysia, Indonesia, Sri Lanka, Pakistan, Thailand, Nepal, Hong Kong, Ryukyu Islands, Afghanistan, Saudi Arabia, Réunion and Mauritius (Mead 1997; Halbert and Manjunath 2004).

Biology

Adults are tiny, mottled brown with wings (Fig. 16b). It damages the curry leaf plant by sucking sap from the young leaves. Adults measure 1/16–1/8 in. with red eyes and short antennae. Female lays 500–800 eggs pear-shaped, which are very small in size measure about 0.2–0.3 mm (Hoy and Nguyen 1996; Bhagat and Nehru 1999). Eggs are initially orange to yellow but later turn darker just before hatching. Nymphs are yellow, orange or brown with flattened bodies and have wing pads in the later instars. They are generally not visible due to its very small size of about 0.25 mm. Summer and rainy seasons are suitable for population growth. Life cycle is completed in 2–7 weeks depending upon the temperature of the environment (Husain and Nath 1927).

Damage

Adults and nymphs suck the sap from leaves and stems of the plant and leads to drying up (Tondon 1993). It is potent vector of greening disease of citrus (Fig. 16a) in Indian sub-continent (Boykin et al. 2012; Bindra and Chhabra 1967; Capoor et al. 1967) and other parts of the world. It's feeding leads to twisting and curling of leaves which gives witches' broom appearance. Heavy infestation results in the fruit dropping. The growth of sooty mold occurs on the secreted honeydew.

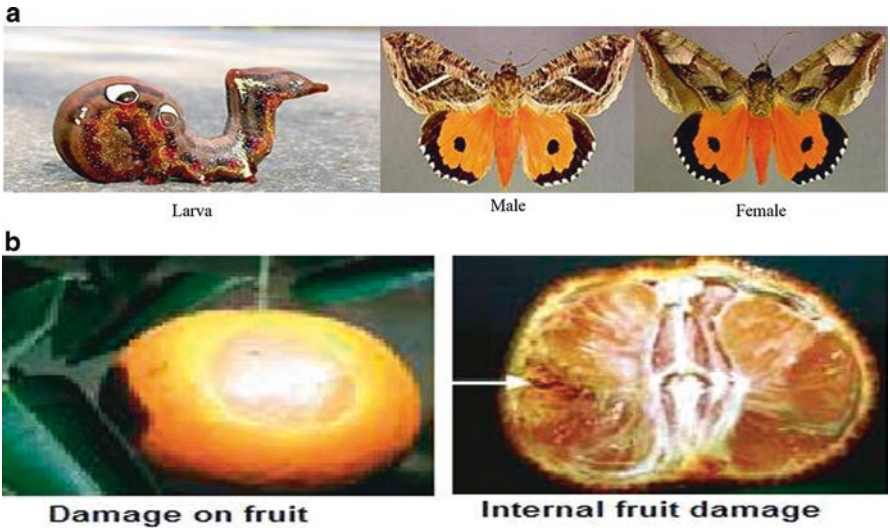

Fig. 17 (**a**) Larva and adults of fruit sucking moth, *Eudocima fullonia* (http://agritech.tnau.ac.in/crop_protection/amla/amla_3.html) (**b**) Damage caused by moth (http://vikaspedia.in/agriculture/crop-production/integrated-pest-managment/ipm-for-fruit-crops/ipm-strategies-for-citrus/insect-and-nematode-pests-management#section-6)

Management

Spray of thiamethoxam (25WG) @ 400 g or imidacloprid (17.8 SL) @ 500 ml or dimethoate (30 EC) @ 3.25 l or oxydemeton methyl (25 EC) @ 250 ml or monocrotophos 36 SL @ 1.5 l in 1250 l of water/ha.

Fruit sucking moth, *Eudocima phalonia*

Biology

Adult moths are large and robust with large eyes. Thorax is pale to purple-brown with pale brown abdomen ending with a bright yellowish orange base. Forewings are olive to purple-brown with white and green colored flecks (Fig. 17a). Sexual dimorphism is found in the edge of the forewings of moth. Outer edge of forewing in male moth is curved while female has the scalloped or toothed edge. A black border with white dots and black comma-shaped mark are present on the fringed, bright orange hind wings. Females lay eggs singly or in masses containing 50–750 eggs usually on the underside of leaves. Total development time is about 21 days with five instars. Larvae are generally feed from 5 pm to 10 am. Young larvae tend to drop down to the ground on the stimulus of danger whereas older larvae show the typical characteristic of aggressive posture and swaying motion. Pupal period completes in about 12.5–17.5 days inside a cocoon onto the tree or drops to the ground after drying.

Damage

It is a severe pest of mature mandarin fruits. Adults puncture the ripened fruits (Fig. 17b) which result in dropping and rotting, which leads to considerable fruit loss. Microorganisms introduced due to feeding by moths also cause rotting and premature fruit fall (Sands et al. 1993). Damaged fruits are unmarketable and, if undetected and packed, pose a threat to sound fruit through pathogenic breakdown (Fay and Halfpapp 2006).

Management

(a) Dispose the infested fallen fruits.
(b) Bait consisting molasses 200 g + malathion 50 EC @ 2 ml + water 2 liter in wide mouthed bottle can be used to manage moth.

Grapes

Insect Pests of National Significance

Mealy bugs: *Ferrisia virgata* Cockerell, *Maconellicoccus hirsutus* Green (Homoptera: Pseudococcidae)
Grape leaf folder: *Desmia funeralis* (Hubner) (Lepidoptera: Crambidae)
Girdler beetle/Grape cane girdler: *Sthenias grisator* (Coleoptera: Cerambycidae)
Thrips: *Rhipiphorothrips cruentatus* Hood (Thysanoptera: Thripidae)
Stem borer: *Celosterna scabrator* F. (Coleoptera: Cerambycidae)

Regional significance

Red spider mite: *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae)—Maharashtra
Grape leaf roller: *Sylepta lunalis* Guen. (Lepidoptera: Pyralidae)—Delhi
Scale insects: *Aspidiotus lantaniae* Signoret (Homoptera: Diaspididae)

Mealybug, *Ferrisia virgata*

Biology

Females are pinkish in case of *M. hirsutus* whereas yellowish white in *P. citri* and covered with a layer of white wax. The life cycle of female comprises three nymphal instars while four in males. The adult male has a pair of wings. Females are mainly responsible for more damage while males are found very rare in the field. They reproduce parthenogenetically throughout the year. Females lay orange colored

Fig. 18 Mealy bug infestation on grapes (http://vikaspedia.in/agriculture/crop-production/integrated-pest-managment)

(*M. hirsutus*) and yellowish white (*P. citri*) eggs. Incubation period is about 5 days. The first instar is mobile, orange (*M. hirsutus)* and yellowish white (*P. citri*). The nymphal development of male completes in 19 days whereas 21 days in females. Male nymph pupates inside a cottony cocoon in the winter season. Its life cycle takes 30 days to complete.

Damage

Both nymphs and adults suck the sap from buds, flower panicles, leaves, spurs, nodes, shoots, bunches, and aerial roots (Fig. 18). Infestation leads to malformation of leaves and shoots tips. Excretion of honeydew gives rise to the sooty mould. As a result, fruits get covered with cottony wax masses which makes it unfit for marketing. Heavy infestation often leads to the death of the plant and cause loss of up to 100%.

Predators: *C. montrouzieri*.

Management

(a) Collection and destruction of mealybugs along with infested branches at the time of harvesting in March–April. Removal of weeds and alternate host plants around the vineyard.
(b) Soil drenching with imidacloprid (200 SL) @1.50 ml/plant in the basins around the trunk or through drip irrigation @ 400 ml/acre in April and May.
(c) Releasing the *C. montrouzieri* @ 5000/ha in August and September.

Fig. 19 Symptoms of grape leaf folder infestation (http://vikaspedia.in/agriculture/crop-production/integrated-pest-managment/ipm-for-fruit-crops/ipm-strategies-for-grapes-insects-pests-management)

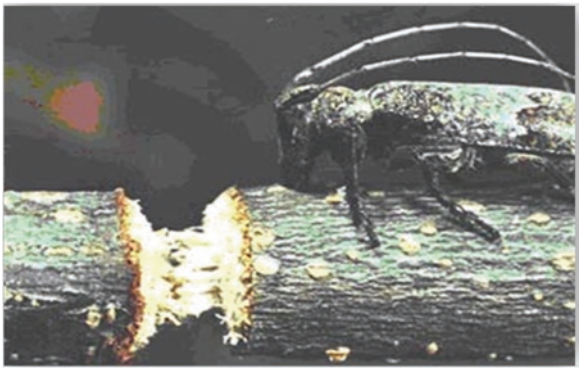

Fig. 20 Damage caused by girdler (Source: agropedia.iitk.ac.in)

Grape leaf folder, *Desmia funeralis*

Biology

Adults are dark brown to black with two white spots on forewings. Hindwings of female have two white spots whereas the male has one large white spot. Two white bands are present across the abdomen. The female has smooth antennae whereas male's antennae are thickened and distorted at the center. Females lay egg singly on the underside of leaves, often in the angles between a vein and the leaf surface. Eggs are flat and elliptical. Larvae are yellow-green, glossy and translucent with the presence of scattered fine yellow hairs on each segment. The head and prothoracic shield are light brown with two spots present on the sides of the first two thoracic segments. The larvae drop down to the ground with dynamic wiggling movement when get disturbed. Pupae are light brown immediately after pupation but later turn dark.

Damage

The larva folds the leaves by a silken thread. It feeds inside the fold and skeletonizes the leaf (Fig. 19). Heavy infestation causes the patchy appearance of leaves which becomes visible at a distance.

Larval Parasitoid: *Bracon cushmani*, *Cardiochiles* spp.
Predators: Lacewing, spiders etc.

Girdle beetle, *Sthenias grisator*

Biology

Adults are grey with a white spot at the center of each elytron and medium-size stout beetle. Grubs are dark brown having a pair of strong mandibles. Larvae make tunnels into the stem right after the emergence and complete their life cycle including pupation inside the stem.

Damage

Female beetles girdle the grapevine for oviposition (Fig. 20). It results in drying up of the region beneath the girdled portion. The grubs start making tunnel right after emergence into the dry wood form by the female during girdling.

Management

(a) Removal of loose barks at the time of pruning is to be done to prevent oviposition
(b) Wet piece of cloth in insecticide like chlorpyriphos can be used for wrapping around the stem.
(c) Spray application of phosalone 35 EC 0.07%, quinalphos 25 EC 0.05% or carbaryl 50 WP 0.1%

Pomegranate

Insect Pests of National Significance

Anar butterfly: *Deudorix isocrates* Fabricius (Lepidoptera: Lycaenidae)
Stem borer: *Coelosterna spinator* Fabricius (Coleoptera: Cerambycidae)
Ash whitefly: *Siphorinus phillyreae* Haliday (Homoptera: Aleyrodidae)
Thrips: *Scirtothrips dorsalis* Hood, *Rhipiphorothrips cruentatus* Hood (Thysanoptera: Thripidae)
Fruit borers: *Conogethes punctiferalis* (Guenee) (Lepidoptera: Crambidae)

Regional Importance

Pomegranate aphid: *Aphis punicae* Passerini (Homoptera: Aphididae)
Mealybug: *Ferrisia virgata* Cockerell (Homoptera: Pseudococcidae)

Fig. 21 Symptoms of pomegranate butterfly/fruit borer (Source: http://agropedia.iitk.ac.in)

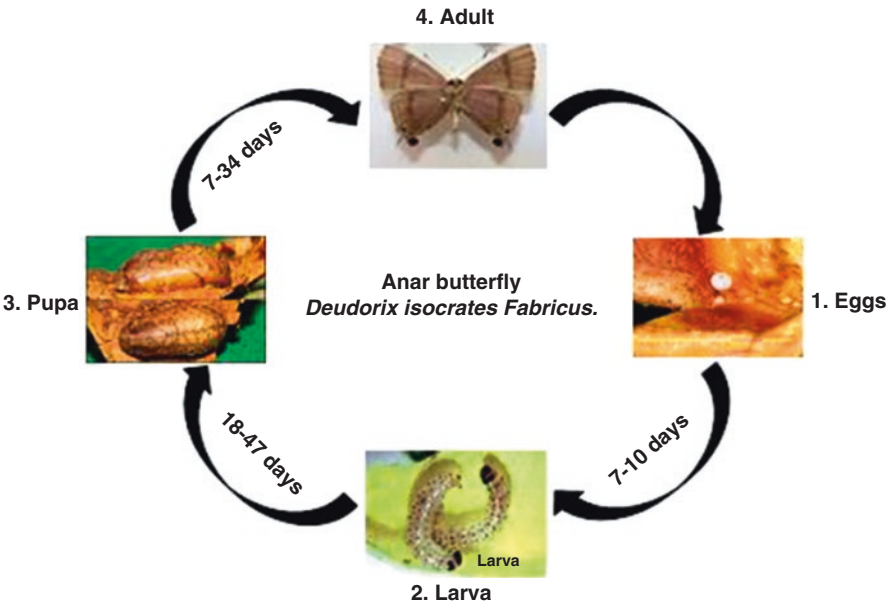

Fig. 22 Life cycle of anar butterfly (http://vikaspedia.in/agriculture/crop-)

Fruit borer: *Spodoptera litura* (Lepidoptera: Noctuidae)
Fruit sucking moth: *Eudocima fullonia* (Lepidoptera: Noctuidae)

Anar butterfly/pomegranate fruit borer, *Deudorix isocrates*

Pomegranate butterfly, *Deudorix isocrates* is one the most obnoxious pest on pomegranate crop incurring about 65–70% of yield loss worldwide (Kumar et al. 2017).

Biology

Adult butterflies are brown (Fig. 21). Females lay eggs on tender leaves, flower buds, and stalks. Larvae are dark brown in color, stout and covered with short hairs. The larval period completes in 18–47 days. Pupation occurs inside the damaged fruits or its stalk and ends in about 7–34 days. The total life cycle takes 1–2 month to complete.

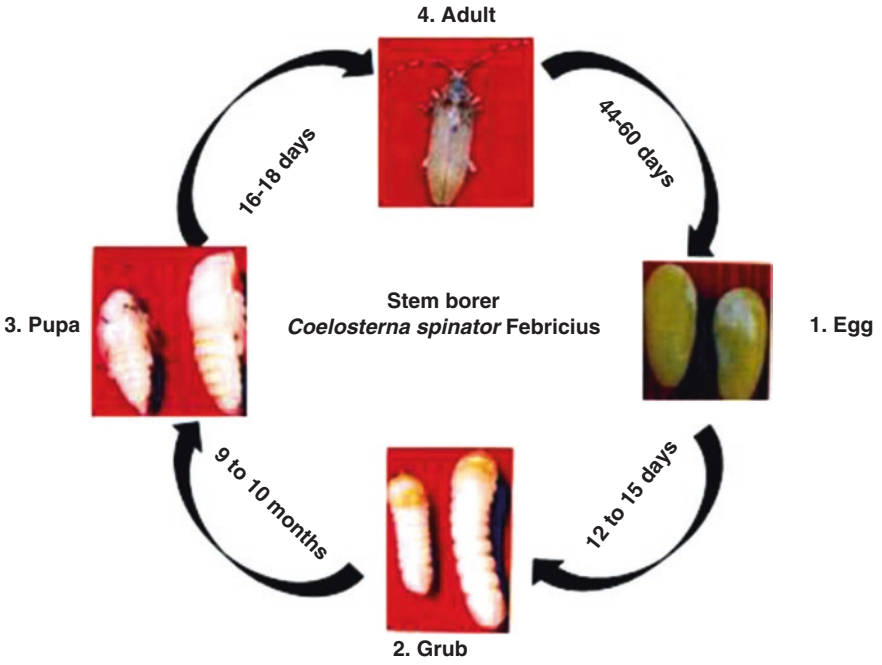

Fig. 23 Life cycle of stem borer (Source: Vikaspedia)

Damage

Larvae cause damage by boring into fruits and feed on pulp (Fig. 22). It results in fruit dropping due to excessive infestation. Active during February end to April beginning. Fruit damage caused by this insect between the age of 30 and 50 days.

Management

(a) Bagging of fruits before maturity.
(b) Collection and destruction of infested fallen fruits.
(c) Removal of weeds from the vicinity.
(d) Application of dimethoate 30EC @1.5 ml/*l*

Stem borer, *Coelosterna spinator*

Biology

Adults are pale yellowish brown (Fig. 23) and light grey elytra with a large number of black spots. It measures about 40–50 mm along wingspan. Females lay about 20–40 eggs under the bark of stems. Full grown larva measure about 17–20 mm. Larva feeds on the soft tissues after emergence and further bore into the stem and roots. Larval period completes in 9–10 month. The length of the pupal period is 16–18 days. The adult beetle emerges through the bark by cutting a hole.They complete only one generation per year with 45–60 days of adult longevity.

Damage

The larvae bore inside the trunk and continue feeding on sapwood. Adults are diurnal and feed on the green bark of shoots. Excreta and dry powder are visible outside the hole on the bark of stem, which is the typical sign of its presence inside the bark.

Management

Insert Aluminium phosphide tablet @ 1 g/live hole or dichlorvos (76 EC) @ 80 ml/live hole (Kumari and Vijaya 2015).

Litchi

Insect Pests of National Significance

Mealybug: *Planococcus litchi* Cox (Homoptera: Pseudococcidae)
Fruit borer: *Conopomorpha sinensis* Bradley (Lepidoptera: Gracillariidae)
Litchi mite: *Aceria litchi* (Trombidiformes: Eriophyidae)

Leaf roller: *Statherotis discana* (Felder and Rogenhofer) (Lepidoptera: Tortricidae)

Regional Significance

Bark eating caterpillar: *Indarbela quadrinotata* Walker (Lepidoptera: Metarbelidae)
Litchi nut borer: *Blastobasis* sp. (Lepidoptera: Blastobasidae)
Leaf-cutting weevil: *Myllocerus undatus* Marshall (Coleoptera: Curculionidae)
Litchi semilooper: *Anisodes illepidaria* Guenée (Lepidoptera: Geometridae)

Mealybug, *Planococcus litchi*

Biology

The female color varies from white to light brown and bears dull grey stripe along their dorsal side. They have brown legs and antennae, but wings are absent in females, and body covers with white wax. A pair of long waxy filaments are present at the end of their abdomen whereas, short waxy filaments are visible around the margins of their body. Females deposit their eggs in a group of 5–20 eggs in ovisacs as white cottony masses. Fecundity is about 300–600 eggs per female. Eggs are oval, light color, glossy and measure about 0.3 mm in length. Newly emerged nymphs settle down on the twigs and along the side of midribs and veins of leaves. Honeydew and wax secreted by crawlers are the indicators of infestation. The yellow colored nymphs are oval-shaped having red eyes and covered with a layer of white wax. Females have four instars and resemble with adult females while male nymphs are more elongated than the adult one. The measurement of adult size varies from 3 mm of female to 4.5 mm length in case of males.

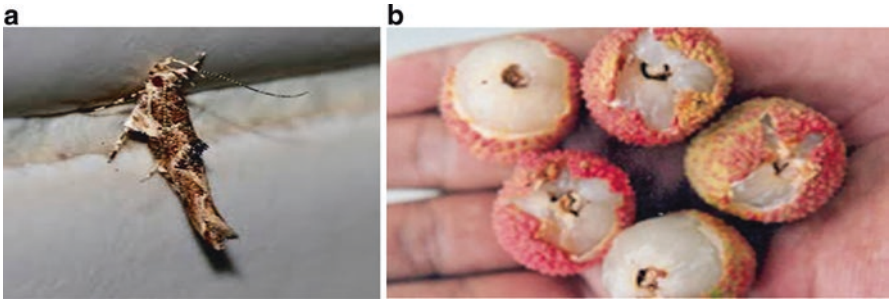

Fig. 24 (**a**) *Conopomorpha sinensis* Adult (Source: www.inaturalist.org) (**b**) Damage symptoms caused by *Conopomorpha sinensis* (Source: Vikaspedia)

Damage

The young plants are more susceptible to heavy infestation. Both nymphs and adults attack almost all parts of the plant including buds, leaves, berries, tender branches, roots, etc. Heavy infestation leads to chlorotic leaves, aborted flower buds and reduced size of berries. The honeydew secreted by mealybugs gives rise to the growth of sooty mould which affects photosynthesis.

Parasitoid: *Leptomastix dactylopii*

Predators: Coccinellid beetle, *C. montrouzieri*, spiders, reduviid bug, etc.

Litchi fruit borer, *Conopomorpha sinensis*

Biology

Females lay egg singly on the underside of a leaf or near the calyx of fruits. The larvae are milky white with a distinct light brown head and slender in shape. The newly emerged larvae start boring into the fruits to feed on its pulp (Fig. 24).

Damage

The young larvae initially mine in the lamina and later bore into the midrib of young leaves and fruits (Fig. 24). It results in the withering and dropping of branches.

Parasitoids: *Trichogramma chilonis*

Predators: Mirid bug, *Campyloneura* sp., Coccinellid beetles, *C. sexmaculata*, seven spotted beetle *C. septempunctata*, three spotted beetle *Brumoides suturalis*, Lacewing *C. carnea*, reduviid bug, praying mantis, big-eyed eyes (*Geocoris* sp.), Pentatomid bug, *Eocanthecona furcellata*

Management

(a) Spraying of neem oil 4 ml/l to avoid egg laying and hatching.
(b) Spray of cypermethrin (25 EC) @ 0.5 ml/l or emamectin benzoate (5 SG) @ 0.4 ml/l.

Temperate Fruits

Pear

Insect Pest of National Significance

San Jose scale: *Quadraspidiotus perniciosus* Comstock (Homoptera: Diaspididae)
Citrus psylla: *Diaphorina citri* Kuwayama (Homoptera: Liviidae)
Pear psylla: *Cacopsylla pyricola* (Forester) (Homoptera: Psyllidae)

Green peach aphid: *Myzus persicae* (Sulzer) (Homoptera: Aphididae)
Chaffer beetle: *Protactia neglecta* (Coleoptera: Melolonthidae)
Stem borer: *Aeolesthes sarta* Solsky (Coleoptera: Cerambycidae)
Root borer *Dorysthenes huegelii* Redt. (Coleoptera: Cerambycidae)

Regional Significance

Codling moth: *Cydia pomonella* L. (Lepidoptera: Tortricidae)
Mites: *Eutranychus orientalis* Klein, *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae)
Flat-headed borer: *Sphenoptera lafertei* Thompson (Coleoptera: Buprestidae)
Leaf roller: *Archips argyrospilus* Walker (Lepidoptera: Tortricidae)
Tent caterpillar: *Malacosoma indica*, *M. kashmiriensis* Walker (Lepidoptera: Lasiocampidae)
Bark eating caterpillar: *Indarbela quadrinotata* Walker (Lepidoptera: Metarbelidae)
Fruit fly: *Bactrocera dorsalis* Hendel (Diptera: Tephritidae)

San Jose scale, *Quadraspidiotus perniciosus*

Biology

Females lay about 200–400 eggs in 6 days. First instar hatch and come out from the scale covering and wander for some time to find a suitable place for the feeding and remain there for entire life. First instars are yellow and secrete white waxy material known as white cap stage. This waxy covering turns black with various shades of grey at the later stage and also known as the black cap stage. Identification of males

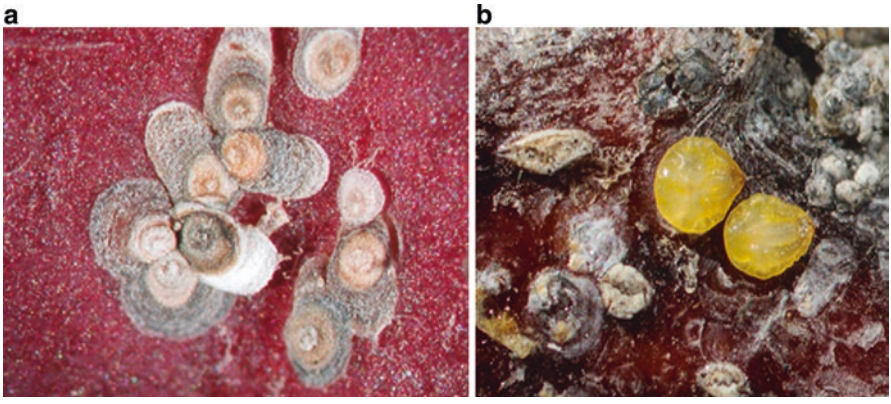

Fig. 25 (**a**) San Jose scale infestation (jennytfrec.wsu.edu) (**b**) Female San Jose scales (covering removed) (https://fff.hort.purdue.edu/article/san-jose-scale/)

and females is not distinguishable at the stage of the first instar. Later on, covering of male scale begin to elongate while females remain circular. Four molting occurs in male, and finally, a yellow insect emerges from the scale with smoky wings and mate with the females covering with grey scales. Females remain inside the grey covering. Females are a yellow lemon in color (Fig. 25b). Developmental time of male is approximately 25 days whereas 31 days of the female. This scale insect completes at least two overlapping generations at higher altitudes (Dinabandhoo and Bhalla 1980; Masoodi and Trali 1987) and four generations at lower altitudes (Sahai and Joshi 1965).

Damage

Infested parts of the plant turn purplish red. Heavy infestation ultimately results in the death of plant. Infested fruits have distinct "measles-like spots" on the surface (Fig. 25a).

Parasitoids: Augmentative and inoculative releases of two exotic parasitoids, *Encarsia perniciosi* (Tower) and *Aphytis proclia* (Walker)/*A. diaspidis* (Howard) @2000/tree has given promising (Rao et al. 1971).

Predators: Coccinellid beetles, *Chilocorus infernalis*, *Pharoscymnus flexibilis*

Management

(a) Spray emulsion of diesel oil + Bordeaux mixture (diesel oil 68 l + copper sulphate 15 kg + unsacked lime 3.75 kg) and diluted 5–6 times before spray.
(b) Spray of ESSO tree oil emulsion @ 7.5 l in 250 l water/ha during winter season.

Fig. 26 Green peach aphids (Source: Entomology Dept, UFl)

Green peach aphid, *Myzus persicae*

Biology

Aphids are polymorphic. Adults are wingless and winged. Wingless forms of females are breeding parthenogenetically, viviparous and paedogenetic. Nymphs are slender, pinkish in the first instar then become yellowish-green with three darker stripes on the dorsum of the abdomen (Fig. 26). The head and thorax of a winged adult are black, and a yellowish green abdomen has a dark brown patch on the top of it. Winged form produces two or three generations, then migrate to summer hosts and reproducing sexually and laying the eggs.

Damage

Adults and nymphs are sucking from tender parts of leaves. Plants become curled, and flowers or newly formed fruit will abort. High populations can also reduce tree vigor and retard shoot growth. Therefore, the average growth of fruits would be distorted, and fruits become unmarketable.

Parasitoid: *Aphelinus mali*

Predators: Syrphid flies, Lygaeid bug, Coccinellids, Lacewings

Apple

Insect Pests of National Significance

San Jose scale: *Quadraspidiotus perniciosus* Comstock (Homoptera: Diaspididae)

Codling moth: *Cydia pomonella* Linn. (Lepidoptera: Tortricidae)

Woolly apple aphid: *Eriosoma lanigerum* Hausman (Homoptera: Aphididae)

Apple leaf folder and fruit scrapper: *Archips termias* (Meyrick) (Lepidoptera: Tortricidae)

Root borer: *Dorysthenes hugelli* (Redtenbacher) (Coleoptera: Cerambycidae)

Apple stem borer: *Apriona cinerea* Cheverlot (Coleoptera: Cerambycidae)

Spider mites: *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae)

Regional Significance

Apple fruit moth: *Argyresthia conjugella* Zeller (Lepidoptera: Yponomeutidae)—Himachal Pradesh

Tent caterpillar: *Malacosoma indica*, *M. kashmiriensis* Walker (Lepidoptera: Lasiocampidae)—J&K

Indian gypsy moth: *Lymantria obfuscata* Walker (Lepidoptera: Lymantriidae)

Fig. 27 (**a**) Codling moth (growveg.com) (**b**) Frass from codling moth larva feeding in fruit (utahpests.usu.edu) (**c**) Larva (http://www.countryfarm-lifestyles.com/codling-moth)

Codling moth, *Cydia pomonella*

It is a major pest of apple, distributed widely in all the apple growing areas of Ladakh and Kashmir. It causes a tremendous loss in the yield due to direct fruit damage. If left neglected, the infestation may occur up to 80% (Mohi-ud-Din and Ahmad 2018). It was recorded first time in Ladakh in 1964 and believed to have entered in India from Northwestern border of Pakistan and Afghanistan. Henceforth, it was declared as a quarantine pest, decades back, hence the implementation of strict embargo on the transportation of apple outside Ladakh, as a safety measure for other apple growing areas of Jammu and Kashmir and also adjoining Himachal Pradesh (Mohi-ud-Din and Ahmad 2018).

Biology

It completes two-and-a-half generations a year. The overwintering larvae pupates in the April. The first-generation adults appear in May–June. Actively flying adults are mating at dusk. They spent the day resting on the tree trunk, branches, and leaves. Females lay 50–100 eggs, small oval and flat shaped. Newly hatched larvae enter into fruit through calyx, attack the core and flesh and ultimately infested fruits fell. They come out of the fruit for pupation under the bark or debris. The second generation adults appear during the last week of June to the first week of July. The brood of this generation attacks the fruit not through calyx but other side of the fruits. Codling moth (Fig. 27a) passes the winter as full-grown larvae in resting stage inside a thick silken cocoon, preferably under the bark of the tree.

Damage

Larvae bore into the fruit and leave fecal matter outside the tunnel (Fig. 27b, c). Hidden infestation is carried to the storage inside the fruit which also leads to the secondary infection.

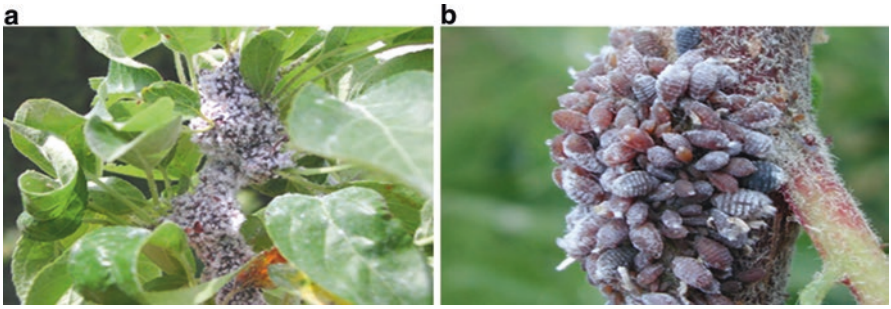

Fig. 28 Woolly aphid (Colonies) (**a**) with wax layer (**b**) without wax layer (http://apples.ahdb.org.uk/woolly-aphid.asp)

Management

(a) Removal of loose bark from tree trunks should be followed during August, to discourage larvae for shelter for overwintering.
(b) The banding of the trunk with gunny bags (in two to three layers from base to middle of a trunk of a tree) during Mid-August to September provide shelter for overwintering of larvae. They can be mechanically killed by unwrapping the bands or burnt down from November to April.
(c) Release of *Trichogramma cacoeciae* or *T. embryophagum* @ 2500–5000/tree twice in a year. The first release at the end of May to the first week of June, i.e., 10–15 days after the chemical spray and second release in the last week of June to the first week of July.
(d) Pheromone trap @ 4 /acre should be applied against first and second generations of the pest. Lure must be replaced after every 15 days.
(e) Spray dimethoate 30 EC @ 100 ml/100 l water after 2–3 weeks after the first spray. If infestation persists, then repeat the same spray in July (Mohi-ud-Din and Ahmad 2018)

Woolly apple aphid, *Eriosoma lanigerum*

Biology

Adults are apterous, viviparous reproduce asexually and parthenogenetically. It is a native of USA and first noticed in 1909 in Shimla on nursery stocks imported from England. There is a partial migration from aerial parts to the roots of the infested plant in December. Reverse migration takes place in February and March. Adults and nymphs are reddish brown and covered with waxy cottony white filaments (Fig. 28). The winged aphids developed in both the aerial and the root colonies during fall. They again migrate back to the elm, where they gave birth to wingless males and females. Females gave birth to 30–116 nymphs in its lifetime @ 1–4 nymphs/day. Eggs are usually laid in the cracks or crevices of bark. The females lay

a single, long, oval, cinnamon-colored egg almost as large as her body. Eggs hatched in the spring season. The wingless nymphs emerge as, parthenogenetic and viviparous stem mothers. They hibernate on the roots of the tree. Four nymphal instars completed the development in 35–42 days. They feed on buds and leaves during May and June for two generations. It leads to the curling of leaves into a rosette. They complete about 13 generations a year.

Damage

Both the nymphs and the adults suck the cell sap from the tender twigs and also attack the roots, which develop large knots. Feeding on underground parts produces large knots on roots. Heavily infested plants have a short fibrous root system and yellowish foliage which can be easily uprooted.

Parasitoid: *Aphelinus mali*

Management

Imidacloprid provides the most consistent suppression of nymphal movement and mid-summer establishment of aerial colonies.

Tent caterpillar, *Malacosoma indica*, *M. kashmiriensis*

Biology

Important pest of apple, in northwestern India, being more severe in Shimla hills. Adult moths are brown and yellowish with two diagonal markings on the front wings. Their wingspan is about 2.5 cm. They are attracted to lights and found very abundant. The moths are short lived. In late spring adults deposit an egg mass of 200–400 encircling small twigs or on the tree, frothy substance secreted by female called spumaline and also used as a hard-protective covering around the egg mass. This pest is active from March–May and passes in the egg stage for 9 months in a year. The hatching of the eggs occurs in early spring. Caterpillars feeds on young leaves. They form small webs in a few days and enlarging the networks as they grow further. Damage can be found for a few distances away around the web. The caterpillars feed gregariously and lead to defoliation. The large webs formed by caterpillar can be recognized from a distance. Adult is light reddish-brown with two whitish stripes running across each of the forewings. Last larval stage wanders in search of pupation sites in the late spring. Larval period completes in 39–68 days. They spin white or yellowish crystalline cocoons under bark, in dead plant refugee, or inside a rolled leaf. Pupal period lasts 8–22 days. Adults emerged in the third week of May or June. There is only one generation per year. Male is short-lived and female may survive for 2–5 days.

Damage

Caterpillars rest at their nest during the day time, and feed on leaves during night time. The entire plant may defoliate in case of severe infestation.

Management

(a) Destroy all egg bands at the bands at the time of pruning in December–January.
(b) Spray of carbaryl (50WP) 2.5 kg in 1250 l of water/ha.

Peach

Insect Pests of National Significance

Stem borer*: Aeolesthes sarta* Solsky (Coleoptera: Cerambycidae)
Flat-headed borers: *Chrysobothris mali* Eschscholtz, (Coleoptera: Buprestidae)
Peach tree borer: *Synanthedon exitiosa* Say (Lepidoptera: Sesiidae)
Defoliating beetle: *Protactia neglecta* (Coleoptera: Scarabaeidae)
San Jose scale: *Quadraspidiotus perniciosus* Comstock (Homoptera: Diaspididae)
Apricot brown scale: *Lecanium corni* (Bouché) (Homoptera: Coccidae)
Peach leaf curl aphid: *Brachycaudus helichrysi* Kaltenbach (Homoptera: Aphididae)

Regional Significance

Green peach aphid*: Myzus persicae* Sulzer (Homoptera: Aphididae)

Fig. 29 *Brachycaudus helichrysi* (Peach leaf curl aphid) (Source: National Bureau of Agricultural Insect)

Tent caterpillar*: Malacosoma indica* Walker (Lepidoptera: Lasiocampidae)

Peach twig borer/Apricot fruit borer: *Anarsia lineatella* Zeller (Lepidoptera: Gelechiidae)

Root borer: *Dorysthenes hugelli* Ridt (Coleoptera: Cerambycidae)

Peach fruit fly: *Bactrocera zonata* (Saunders), *B. dorsalis* Hendel (Diptera: Tephritidae)

Oriental fruit moth: *Grapholita molesta* Busck (Lepidoptera: Tortricidae)

Codling moth: *Cydia pomonella* Linn. (Lepidoptera: Tortricidae)

Peach leaf curl aphid, *Brachycaudus helichrysi*

Biology

Adults are small yellow, dark stripes on the head (Fig. 29). Paired cornicles are present on fifth and sixth abdominal segments. They are active from February–March on temperate fruits and June–October on golden rod trees. Overwintering in egg stage at the base of the bud. Eggs are hatched during spring and sucking the sap from primordial leaves. Development completes in 4 weeks. Female reproduce parthenogenetically and giving birth to 50 nymphs in 13 days with 8–22 nymphs/day. Three to four generations completed on fruit plants. On the onset of summer, females are produced and migrate to another plant. They breed asexually. Four to five generations are completed on the goldenrod from June to October. Early November, winged females are produced then migrate back to peach and plum, etc. At this time old leaves are shed, and females lay eggs at the base of bud upto mid-December. The life cycle takes 22–25 days to complete. It has 12–14 generations per year (Singh and Sharma 2012).

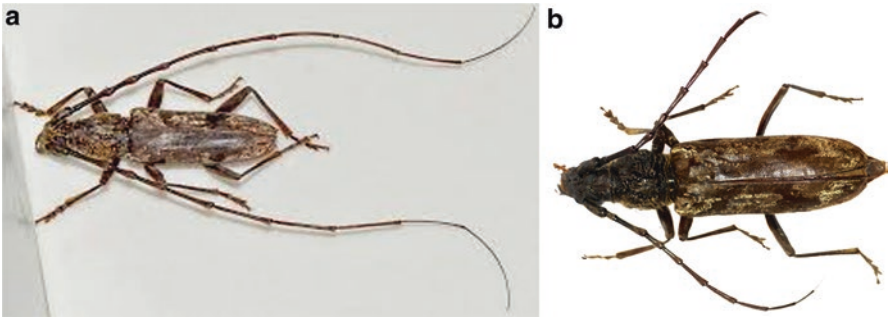

Fig. 30 *Aeolesthes holosericea* (**a**) male (**b**) female (Source: nbair.res.in)

Damage

The nymphs and adults are sucking the sap from growing shoots and leaves. The infested plants are showing yellowing and curling of leaves along with wilting of terminal shoots. Development of fruits slowed down due to the shortage of nutrition and fell off prematurely. The attack in the spring season is most deleterious.

Predators: *Scymnus* sp., *Chilomenes sexmaculatus*, *Chrysoperla zastrow* Sillemi, preying mantids, predatory mites.

Management

(a) Removal of weeds from the vicinity.
(b) Spray of dimethoate (30 EC) @ 2*l* in 1250 liter water.

Cherry stem borer, *Aeolesthes holosericea* (Coleoptera: Cerambycidae)

Widely distributed in India, Srilanka, Bangladesh, Myanmar, Malaysia, and Thailand. It is a polyphagous and destructive pest of cherry and also attacks apple, guava, apricot, crab apple, mulberry, peach, pear, plum, walnut (Ambethgar 2003; Ahmed et al. 2004; Stebbing 1914).

Biology

Adults are dark brown, 38–45 mm in length, yellowish spots present on elytra. Antennae of male is 1.5 times greater than their body size (Fig. 30a). Antennae of female is about equal to the body length (Fig. 30b). Grubs are yellowish with hairs on the body and 70–80 mm long. Beetles emerge from the tunnels of the host trees from May to October and active throughout summer. A female lay about 100 eggs on dry wood, cracks, and crevices of bark. Eggs are white, elliptical and measure about 2.5 mm in length. The incubation period is about 7–12 days. Larval development completes in 27–32 months. Pre-pupal period remains for 3–150 days. Pupation occurred in October within the tunnels and carried for 40–100 days and remain from winter to spring season. Its life cycle completes in 3 years (Gupta and Tara 2013).

Damage

Frass comes out of the holes in the branches or in the main trunk in which grub is located. Newly hatched larva first feed on bark and makes zig-zag galleries during feeding on the sap of wood.

Management

(a) Collection and destruction of grubs and beetles.

(b) Insert cotton swab soaked with dichlorvos @ 0.1% or dimethoate @ 0.03% or methyl demeton @ 0.025% and seal with mud.

Walnut

Insect Pests of National significance

Stem borer: *Aeolesthes sarta* Solsky (Coleoptera: Cerambycidae)
Flat-headed borers: *Chrysobothris mali* Eschscholtz (Coleoptera: Buprestidae)
Long horned walnut beetle: *Bactocera horsfieldi* (Coleoptera: Cerambycidae)
Defoliating beetle: *Protactia neglecta* (Coleoptera: Scarabaeidae)
San Jose-scale: *Quadraspidiotus perniciosus* Comstock (Hemiptera: Diaspididae)

Regional Significance

Green peach aphid*: Myzus persicae* Sulzer (Homoptera: Aphididae)
Tent caterpillar*: Malacosoma indica* Walker (Lepidoptera: Lasiocampidae)
Root borer: *Dorysthenes hugelli* Ridt (Coleoptera: Cerambycidae)
Peach fruit fly: *Bactrocera zonata* (Saunders), *B. dorsalis* Hendel (Diptera: Tephritidae)
Green capsid: *Lygus pabulinus* Franz and Wagner (Heteroptera: Miridae)

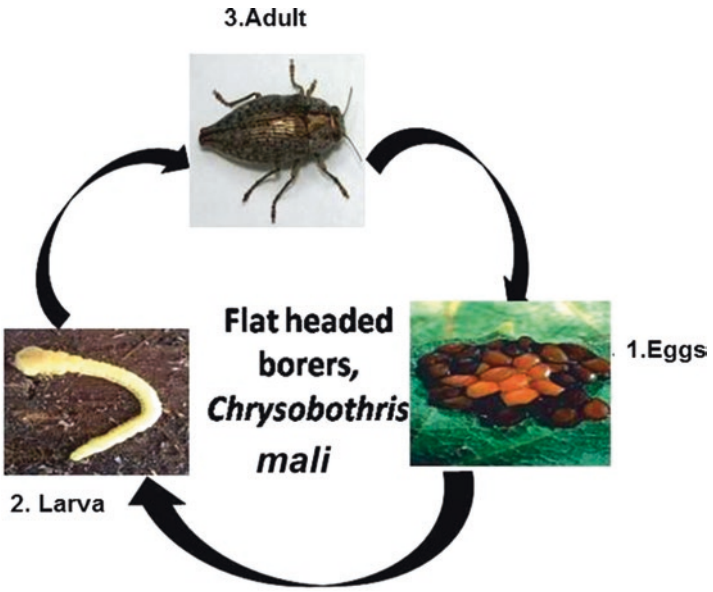

Fig. 31 Life cycle of flat headed borer, *Chrysobothris mali* Source: Vikaspedia

Codling moth: *Cydia pomonella* Linn. (Lepidoptera: Tortricidae)

Flat headed borer, *Chrysobothris mali*

Biology

Adults are metallic wood-boring with distinct copper spots on elytra, 6–11 mm long. Larva is yellow whitish to yellow and 15–188 mm long (Fig. 31). Thoracic segments are greatly enlarged and flattened. The abdomen is bent backward making as a hook-like structure. Larval stage overwinters inside the tree and emerges as adult beetles in June–August. The pupa is translucent white and then dark bronze at the emergence time. Pupation may occur in June and July. Females lay eggs on bark crevices, and the newly hatched larvae immediately bore into the bark to feed in the phloem layer. It completes one generation in a year.

Damage

They are attracted to diseased or injured parts of trees like affected by scale insects, bacterial canker, or major pruning cuts. Newly hatched larvae bore tunnels deep into the heartwood of the tree, and this turns wood into sawdust. Affected area appears as a wet spot on the bark and leads into the expose of mines. Its excessive feeding may cause the death of the infested trees. This borer can be particularly damaging to new grafts in established orchards.

Parasitoid: Tachinid fly

Walnut weevil, *Alcidodes porrectirostris*

It is a serious pest in Kumaon, Kulu, and Kashmir. It is a monophagous pest of walnut and found at the altitude of 1000–2450 m.

Biology

Adults are pitch black, 10 mm, snout directed downward. Adult stage passes overwinter under stones, the bark of trees and in cracks and crevices in the ground. Weevils become active in April, which coincides with the appearance of flower buds. Weevils puncture fruit and lay about 15 eggs per fruit. Eggs hatching occurs in 1 week. Grubs are legless, pale brown head and 15 mm in length. They feed deep into the kernel. Grub becomes full grown in 13–22 days. Larvae are legless with pale brown head and measures about 15 mm in length. Pupation takes place inside the fruit. The pupa is creamy white and transformed into adult in 9–17 days. They complete two generations in a year.

Fig. 32 *Batocera horsefieldii* (Source: www.biolib.cz)

Damage

Adults feed upon buds and flowers. Fruit with spots of dark brown or black resinous excretion harbor grubs inside. Grubs feed inside fruits and cause premature dropping and also feed upon the green twigs, petioles, flowers, and young fruits.

Management

(a) Collect and destroy infested fallen fruits in May–June.
(b) Spray carbaryl (50 WP) @ 2 kg in 1250 l of water/ha at fortnight intervals.

Long-horned walnut beetle, *Batocera horsfieldii*

Biology

It is a serious pest of walnut in Darjeeling, Kullu valley, Kumaon and Shimla hills. Adults are 45–65 mm long, black with fine ashy or yellow-grey pubescence (Fig. 32). Antennae of males are longer than the body size while equal in females. Adults emerge in June and July, live for about 4 months. Female lays 55–60 eggs singly in her lifetime. Eggs are brown and oval, lasts 8–15 days. Larval development completes in 20–25 months. Grubs are pale yellow and 90–150 mm long. They remain in the pre-pupal stage for 50–182 days. Pupal period lasts for 40–90 days. Life cycle completes in 23–32 months, spread over 2–3 winters.

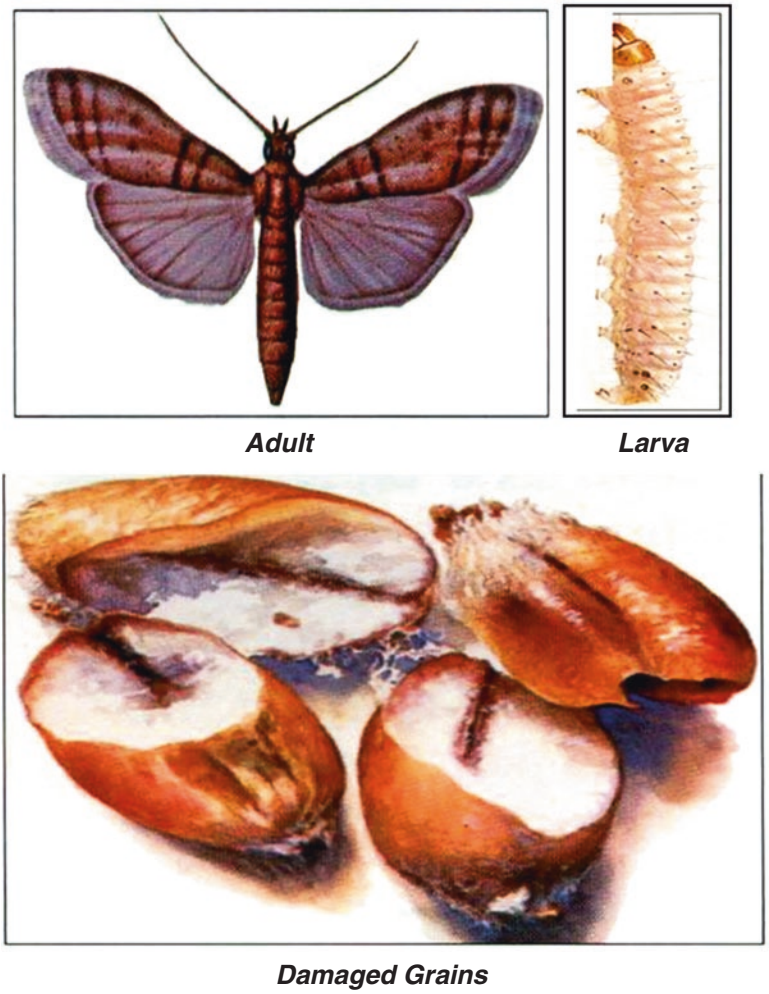

Fig. 33 *Cadra cautella* (Source: http://agropedia.iitk.ac.in)

Damage

Grubs feed on the inner side of bark making zig-zag tunnels. Grubs then bore down to the surface of sapwood and reach into the center of the wood.

Management

(a) Collection and destruction of infested fallen fruits.
(b) Insert the cotton swab soaked in kerosene or petroleum and seal it with mud on the entry hole.

Almond

Insect Pests of National Significance

Almond moth, *Cadra cautella* (Lepidoptera: Pyralidae)
Red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae)
Khapra beetle, *Trogoderma granarium* (Coleoptera: Dermestidae)

Almond moth, *Cadra cautella*

Biology

Adults are 13–20 mm long with 20 mm wingspan (Fig. 33). Wings are bicolor; top portion of wing is dark tan while grey or brown scales are present near the distal end of wing with dark lines. Female lays about 300 slightly greyish eggs. Life cycle completes in 25 days. Larvae are white colored with white spots. Pupal period completes in 12–15 days. Adults are non-feeding and short lived stage. It is active particularly at dusk and dawn. Biology of almond moth is influenced by warm and humid climate (Burges and Haskins 1965). It has round shape black pinacula (Solis 2011).

Damage

Larvae attack wide range of stored food products and may cause about 60% loss.

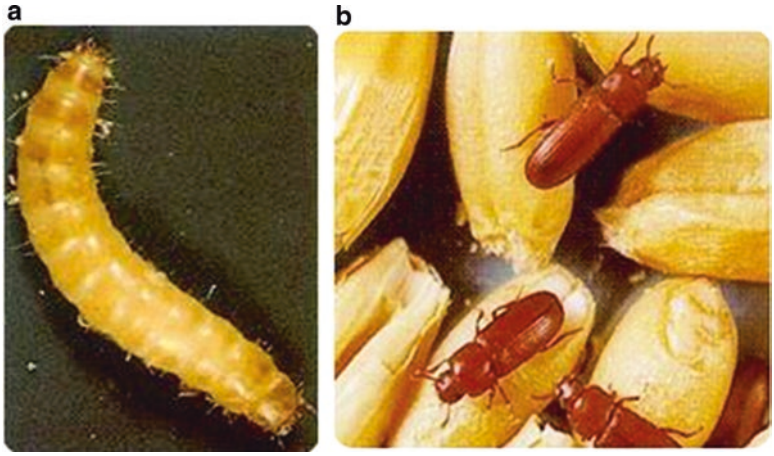

Fig. 34 Red flour beetle, *Tribolium* castaneum (**a**) larva (**b**) adult (http://vikaspedia.in)

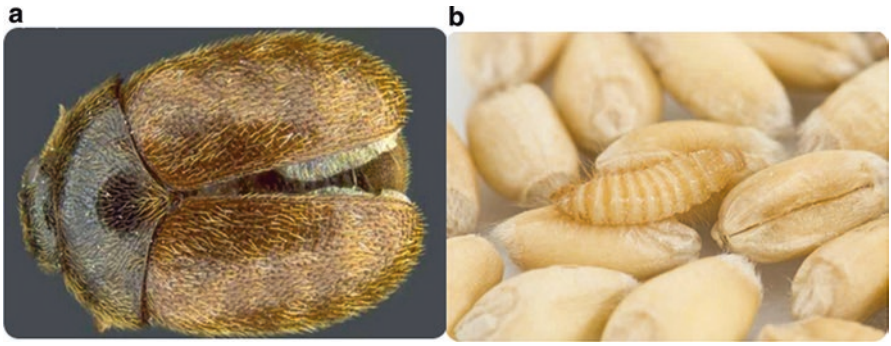

Fig. 35 Khapra beetle, *Trogoderma granarium* (**a**) adult (**b**) larva (http://vikaspedia.in)

Red flour beetle, *Tribolium castaneum*

Biology

Adults are reddish brown and have flat body (Fig. 34b). They have capitate antennae. Male have setiferous patch on the distal end of the fore femur whereas it is absent in female. Female lays white, small cylindrical eggs. Incubation period is 4–7 days, but it varies with the temperature. Incubation period is 3 days report by Beeman et al. 2012 at 30 °C and 2 days at 34 °C. Eggs are 0.54–68 mm in length. Larvae are creamish white to yellowish white (Fig. 34a), slender, covered with fine hairs (Devi and Devi 2015).

Damage

Its distribution is worldwide. It attacks particularly seeds and grains. The adult and immature are secondary pest, which feed on seeds or grains, which are previously damaged. They scratched the surface and form galleries. Shape of almond becomes deformed due to their infestation. Sometimes it also consider as primary pest due to its ability to initiate the injury in intact almonds.

Khapra beetle, *Trogoderma granarium*

Biology

Adults are reddish brown, convex and oval in shape (Fig. 35a). Female lays eggs on grains or crevices. Grubs are straw colored with dark bands on each segment of the body and the posterior end of the body is covered with tuft of long hairs (Fig. 35b).

Damage

Only grub damage the produce by scratching surface and consume germ portion of the seed and convert the seeds into frass.

Management

Fumigation of storage container with phosphine under atmospheric pressure 1–3 g/m^{-3} for at least 7 days or methyl bromide @ 80 g/m^{-3} for 24 h.

In India, Vegetables are Grown Throughout the Year in Three Seasons:

1. Kharif season (June to September): cucurbits, brinjal, okra
2. Rabi season (October to January): cabbage, cauliflower, beet, peas, tomato, cucurbits
3. Summer season (February to May): brinjal, chilli, cluster beans, cucurbits

Insect Pests of National Significance of Cucurbitaceous Crops

Fruit fly: *Bactrocera cucurbitae* (Coquillett), *B. dorsalis* (Hendel) (Diptera: Tephritidae)

Pumpkin beetles: *Raphidopalpa foveicollis* (Lucas) (Coleoptera: Chrysomelidae)

Snake gourd semilooper: *Plusia peponis* (Fabricius) (Lepidoptera: Noctuidae)

Leaf miner: *Liriomyza trifolii (Burgess)* (Diptera: Agromyzidae)

Bottle gourd plume moth: *Sphenarches caffer* (Zeller) (Lepidoptera: Pterophoridae)

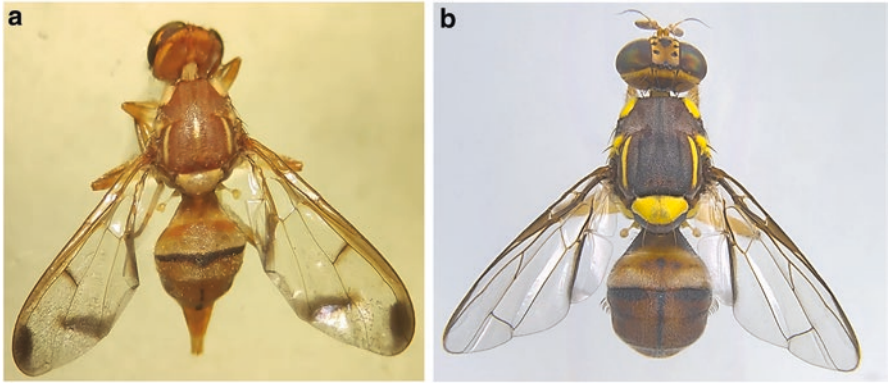

Fig. 36 (**a**) *B. cucurbitae* (Photo by: Irsad) (**b**) *B. dorsalis* (Source: nbair)

Fruit flies, *Bactrocera cucurbitae*, *B. dorsalis*

Fruit flies are serious insect pests reported to feed over 300 host plants from 40 families of fruit and vegetable crops. Among different species of the fruit fly, the *B. cucurbitae* (melon fruit fly, Fig. 36a) is responsible for causing 30–100% damage in cucurbits (Clarke et al. 2005; Shooker et al. 2006; Ryckewaert et al. 2010). This species is native to the Indo-Malayan region and extensively distributed in the Africa, Asia, and Pacific islands. It was introduced in Hawaii, the Mariana Islands, Tahiti (Drew and Raghu 2002), Japan, UAE, North America and Oceania (Anonymous 2018a; McQuate and Teruya 2015).

Biology

Fruit fly remains active in all seasons on the main or alternate hosts. During extreme of winters, they hide under the dried leaves of trees while in the hot and dry season, the flies migrate to cool places and feeds on honeydew produced by aphids. Freshly laid eggs are shiny white and slightly curved in shape. The incubation period of *B. cucurbitae* is 16 h (Dhillon et al. 2005a, b).

Three instars completed the development in 4.5 days, pre-pupal and pupal period for 0.8 and 8.4 days, respectively. Pre-oviposition and oviposition periods ranged

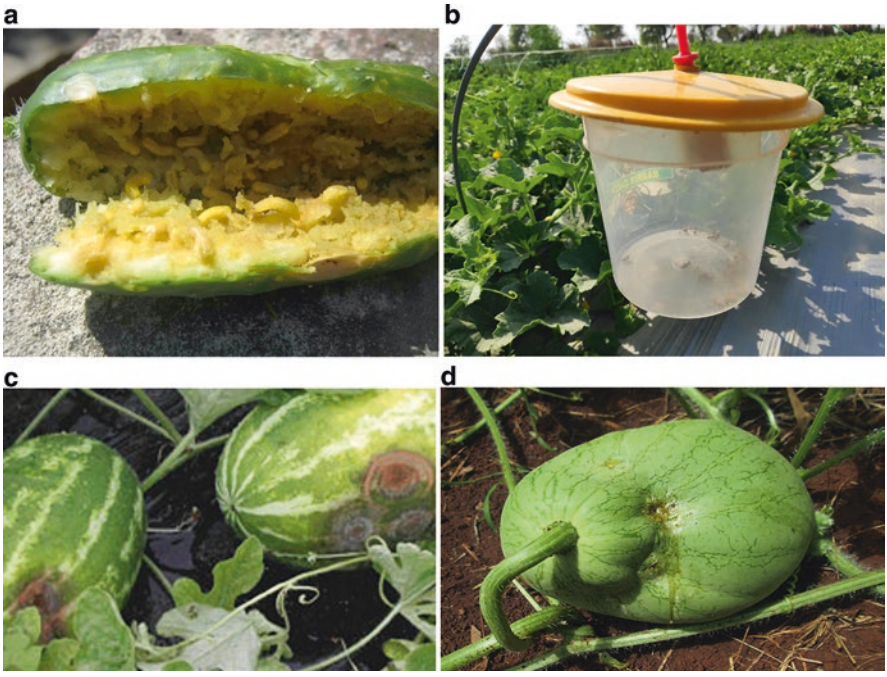

Fig. 37 (**a**) Damage by fruit fly larvae (**b**) A fruit fly trap (**c, d**) Fruit fly damage on watermelon (Source: Infested cucurbit fruit, greenidiom.com, Fruit-fly trap: Surendra S Shekhawat)

from 10 to 15 and 12 to 28 days, respectively. Fecundity is 58–92 eggs, longevity of male is 30–52 days and female for 30–60 days (Mir et al. 2014). The egg period of *B. dorsalis* (Fig. 36b) is 1.5 days; larval period lasts for 8.5 days, pupal and pre-oviposition period is for 12 days. Male and female lived for 52 and 70 days, respectively. Female lays about 371 eggs in her life span (Sunil Naik et al. 2017). Female *B. cucurbitae* depends strongly on the vision to locate their host fruits. It is capable of locating the oviposition site in papaya using sensory modalities of olfaction and vision alone or in combination (Pinero et al. 2017).

Damage

The female fly punctures the soft and tender fruits with her ovipositor and lays eggs below the exocarp of the fruit (Fig. 37c, d). After hatching, the maggots (Fig. 37a) start feeding on the placenta and pulp. Both fruit flies are considered as national and international quarantine pest (Back and Pemberton 1917; McQuate and Teruya 2015). These puncture mark reduces the quality of the produce (Weems and Heppner 2001) and feeding of maggots later results into secondary contamination of pathogens and vinegar fly, *Drosophila melanogaster* which is a scavenger (Dhillon et al. 2005c). ETL: 1.46 adults per trap per day (Srinivas et al. 2018).

Management

(a) The watermelon varieties; Asahi Yamato, AHW/BR-16, and Thar Manak are resistant to *B. cucurbitae* (Verghese et al. 2012; Haldhar et al. 2015).
(b) Remove the infested fruits and destroy.
(c) Bitter gourd var. IC 248282 and Karela collection-1 are also less susceptible to fruit fly (Panday et al. 2012).
(d) Pumpkin var. Arka Suryamukhi found resistant to the fruit fly (Anonymous 2014a, b).
(e) Cuelure pheromone trap (Fig. 37b) is effective for trapping of male fruit flies (Maharjan et al. 2015). Mashed sweet gourd (MSG) in bitter gourd crop attracts large numbers of fruit flies and reduce 40–65% fruit fly infestation and damage (Nasiruddin et al. 2002).
(f) Bait trapping with secufon + cucurbit chop also declines infestation of fruit fly (Sarkar et al. 2018).
(g) Protein baits GF-120 Fruit Fly Bait® containing spinosad in the area wide management of melon fruit fly in Hawaii (Prokopy et al. 2003, 2004).
(h) Spray of nimbecidine 5 ml/l (Sharma and Punam 2016) and two application of spinosad (200 ml/ha) and azadirachtin (0.03%) effective soon after ovipositor marks observed and subsequent sprays at an interval of 12 days (Shivangi and Swami 2017).

Insect Pests of National Significance of Solanaceous and Malvaceous Crops

Chilli

Chilli thrips: *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae)
Pod borers: *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae)
Tobacco caterpillar: *Spodoptera litura* (Fabricius (Lepidoptera: Noctuidae)
Mite: *Polyphagotarsonemus latus* Banks (Trombidiformes: Tarsonemidae)

Chilli thrips, *Scirtothrips dorsalis*

Scirtothrips dorsalis is a significant pest of chilli in India and other parts of the world. It is a polyphagous pest of various economically important vegetables, ornamental and fruit crops in southern and eastern Asia, Oceania and parts of Africa (Ananthakrishnan 1993; Seal et al. 2006). It has been reported to produce losses from 61% to 74% (Patel et al. 2009). It is native to the Indian subcontinent and reported to feed on more than 100 host plants (Mound and Palmer 1981). It was also reported in Florida (2005) infesting chilli, groundnuts, mango, beans, cotton, brinjal and *Casia fistula* (Kumar et al. 2012).

Biology

Eggs of *S. dorsalis* are kidney-shaped, glossy white and embedded in plant tissue. The incubation period ranged from 3 to 5 days. Duration of first and second instar larva ranged from 1 to 2.25, 2.25 to 3.75 days, respectively. In pre-pupal stage: antennae become shorter, laterally directed, and duration ranged from 0.75 to 1.50 days where pupa is dark yellow with pinkish eyes and antennae projected

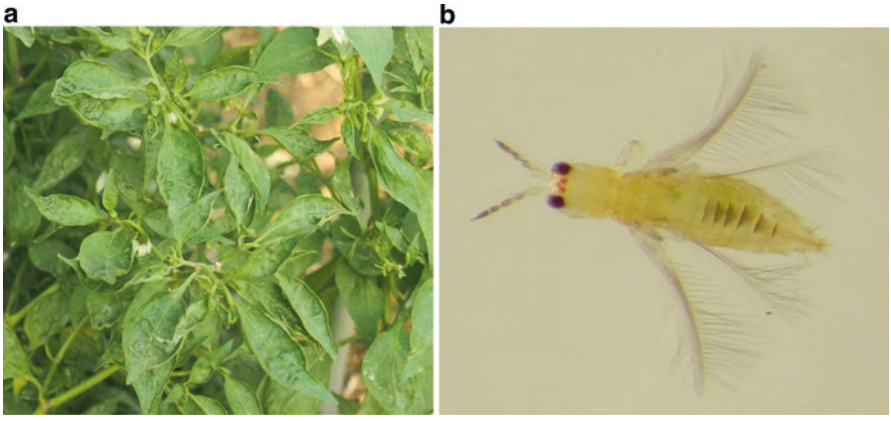

Fig. 38 (**a**) Damage by thrips on chilli (Photo by: Surendra S Shekhawat) (**b**) Adult chilli thrips (Source: TREC University of Florida)

backwards over the head. The pupal period ranged from 3.25 to 4.75 days. Pre-oviposition period of a newly emerged adult is range from 1 to 2 days and oviposition period 1 to 3 days. The adult (Fig. 38b) lays on an average 2–3 eggs per day and survives for a period of 3–7 days (Duraimurugan and Jagadish 2011). There are 18 generations per year in tropical and subtropical areas (Nietschke et al. 2008).

Damage

Chilli thrips feed on tender shoots, buds and flowers resulting in the curling of leaves (Fig. 38a), shedding and brittling, subsequently fall (Ramakrishna Ayyar and Subbiah 1935; Tatagar 2010). The *T. tabaci* is the most severe pest of onion and garlic crops in the tropical areas. With the help of rasping and sucking type of mouthparts, thrips suck up the fluids mostly from young plants (Chisholm and Lewis 1984). Damage produces silvery streaks which reduces the area of photosynthesis. A lot of water loss occurs, and wounding can permit the entry of pathogens easily. The crop ripens prematurely causing significant reduction in the yield (Gill et al. 2015). ETL: 2 thrips/leaf.

Management

(a) IPM module with barrier crops as outer row of maize and inner row of wheat restricts the entry of thrips.
(b) Spray of imidacloprid + fipronil 80WG 144 g/ha, chlorfenapyr 50WDG 240 g/ha, chlorfenapyr 36SC 240 ml/ha and imidacloprid 200SL 600 ml/ha (Din et al. 2016).
(c) Spinosad 0.2 ml/l and thiamethoxam 25WG @ 0.025% recorded the highest reduction of *S. dorsalis* population (Patel and Kumar 2017; Samota et al. 2017).

Mites, *Polyphagotarsonemus latus* and *Tetranychus urticae*

Phytophagous mites are pests of several crops that cause quality and economic losses. Mite infestation is stressful for plants, hindering the development and interfering with photosynthesis and storage organs. In order to combat such damage, plants utilise molecular and physiological modifications, as well as the production of mite-inhibitory compounds (Blasi et al. 2015). Red spider mite (*Tetranychus urticae*, Fig. 39a) and chilli mite (*P. latus*, Fig. 39b) are non-insect, polyphagous pests of vegetables (okra, brinjal and chilli). Due to its high reproductive potential, it can reach damaging densities within a short time.

Biology

The incubation period of *T. urticae* is reported 3–4 days, larval period 3–5 days, nymphal period 4 days and life cycle completed in 17–20 days. Pre-oviposition period ranged from 1 to 2 days, oviposition period 5 to 8 days and female survives

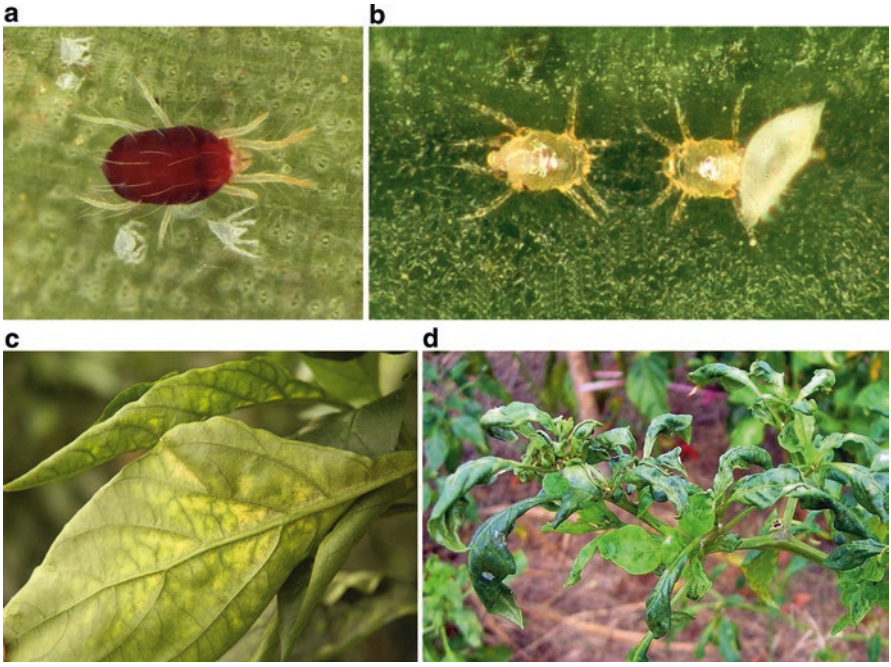

Fig. 39 (**a**) Spider mite, *Tetranychus urticae* (Source: nrcb.res.in) (**b**) Broad mite: *P. latus* (Source: alchetron.com) (**c**) Damage by spider mite on eggplant (Source: Yuan-Min Shen, Taichung District Agricultural Research and Extension Station) (**d**) Broad mite damage on chilli (Source: pestnet.org)

for 8 to 11 days. Average fecundity ranged from 41 to 52 eggs/female (Nain et al. 2017). On chilli pepper (*Capsicum sp*.) the developmental period of the egg to adult is 4.1 days for both males and females at 25 °C. The longevity of male and female are 11.4 and 15.3 days, respectively. The female lays about 25 eggs underside of leaves and flowers (Ho 1991).

Damage

Infestation of mite results into discolouration of tissues, downward cupping and deformed or unmatured fruits (Fig. 39c, d). Symptoms remain over a long period even after the treatment. *P. latus* disperses through other insects living on plant. Females of *P. latus* have a phoretic relationship with *B. tabaci* on *Phaseolus vulgaris* and found attached to the tarsi and tibiae of *B. tabaci* (Flechtmann et al. 1990; Fan and Petitt 1994). *Polyphagotarsonemus latus* is a severe pest of tea, chilli pepper and aubergines in China (Li et al. 1985). It has been reported to cause damage 50% on the bean crop in New Guinea and of the lemon crop in parts of South Africa. It is also a pest of cotton in tropical Africa and Brazil (Gerson 1992). Damage by *P. latus* can attain 100% on sweet peppers (*Capsicum* sp.) grown in a greenhouse (Liu et al. 1991). ETL: 1 mite/leaf.

Management

(a) Chilli mite can be managed effectively by the application of diafenthiuron (300 g a.i./ha), milbemectin (3.5 g a.i./ha) and propargite (1000 g a.i./ha) (Chakrabarti and Sarkar 2014).
(b) Fenazaquin (100 g a.i./ha) and spiromesifen (96 g a.i./ha) also proved better in controlling mites (Reddy and Pushpalatha 2013).

Insect Pests of Potato of National Significance

Potato tuber moth: *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae)
Aphids: *Myzus persicae* Sulzer, *Aphis gossypii* Glover (Homoptera: Aphididae)
Sweet potato weevil: *Cylas formicarius* (Fabricius) (Coleoptera: Curculionidae)

Potato tuber moth, *Phthorimaea operculella*

It is a cosmopolitan, found especially in warm temperate and tropical potato growing provinces (CABI). It has a widespread distribution in India, Bangladesh, Japan, Yemen, Israel, S. Africa, USA, Brazil, Peru, Argentina, Australia and New Zealand.

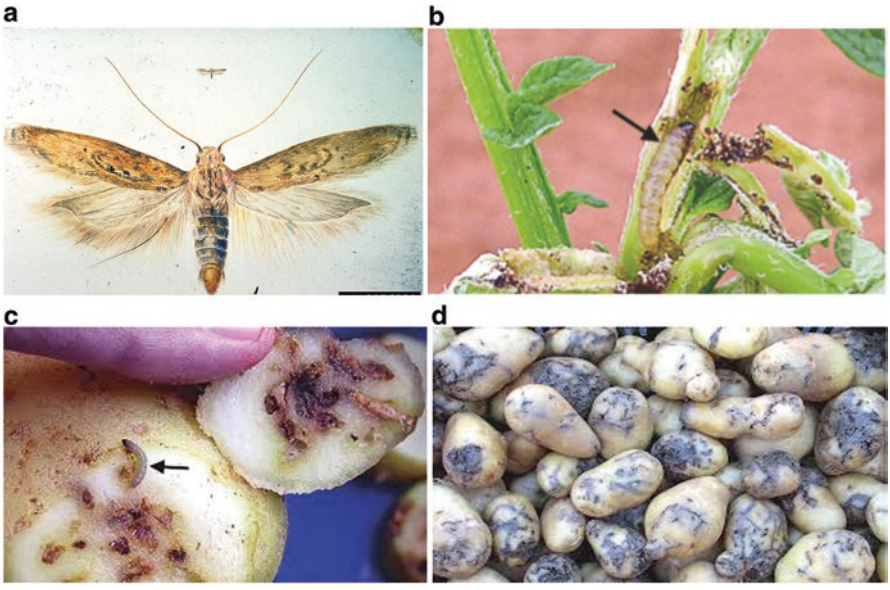

Fig. 40 (**a**) Adult PTM (**b**) Larva feeding inside stem (Source: Central Science Laboratory, Harpenden, British Crown, Bugwood.org) (**c**, **d**) Damage by PTM on potatoes (Source: Dr Diedrich Visser, Agricultural Research Council)

In India, it predominantly found in Himachal Pradesh and Meghalaya states (Anonymous 2018b; Trivedi and Rajagopal 1992).

Biology

A single female lays 40–290 eggs, singly or in batches on the leaves which takes 3–15 days for hatching. Eggs cannot tolerate low temperatures (Langford and Cory 1932). After hatching, larva mines into the petiole, or a young shoot or main leaf vein. Later it bores into a tuber, making a large irregular gallery. On stored tubers, feeding begins immediately. The larval stages last for 13–33 days. Pupation occurs in the soil and lasts 6–29 days. Adults (Fig. 40a) are most active at night and attracted towards the light and live up to 10 days. There could be 13 generations per year in India (Mukherjee 1948). The life cycle completes in 17–125 days.

Damage

The potato tuber moth (PTM), *Phthorimaea operculella* (Zeller), is one of the most damaging pest of potatoes in field and storage (Hanafi 1999). Mining of the leaves with weak stem shows the larval presence (Fig. 40b). However, in tubers, the larva can be detected after cutting when galleries found within the potato (Fig. 40c, d). It accounts for 16% of the crop losses of potato in the world (Oerke et al. 1994), and decline the tuber yield 30 to 70% (Raman and Radcliffe 1992; Kroschel and Schaub 2013). ETL: two galleries per plant or 5% leaf damage (Thakur and Chandla 2013).

Upon hatching from eggs laid on leaves, the larva can drop to the ground and burrow through cracks in the soil to a tuber, entering it through the eye. This is common after vine desiccation. Another common way is that the female PTM lays its eggs directly on exposed tubers at or near the eye. When the larva hatches, it just enters to the tuber through the eye, making a slender tunnel along the surface or deep into the tuber (Maharjan and Jung 2012).

Management

(a) Healthy seed tubers are a perfect preventive measure to manage the infestation.
(b) Three parasitoids, *Apanteles subandinus*, *Copidosoma koehleri* and *Orgilus lepidus* can be released for successful biological control (Sankara and Girling 1980).
(c) Pheromone traps @84/ha reduced the captures within the field, indicating that the mating disruption techniques can be effective for controlling this pest (Ortu and Floris 1989).

(d) Under the storage condition, abamectin is most effective followed by fenitrothion, *B. thuringiensis* and granulosis virus (Bhatia 2008).
(e) Spray indoxacarb 15% EC @ 50 ml/200 l water (El-din et al. 2016).

Insect Pests of Tomato of National Significance

Serpentine leaf miner: *Liriomyza trifolii* (*Burgess*) (Diptera: Agromyzidae)
Fruit borer: *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae)
Whitefly: *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae)
Tomato pinworm: *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

Tomato pinworm, *Tuta absoluta*

Tomato pinworm (Fig. 41c) is native to Peru and widespread in South America, Africa, Europe. In India, it is first reported in 2014 from Maharashtra infesting tomato (Sridhar et al. 2014). It is also known as tomato leaf miner and mainly feeds on Solanaceous crops.

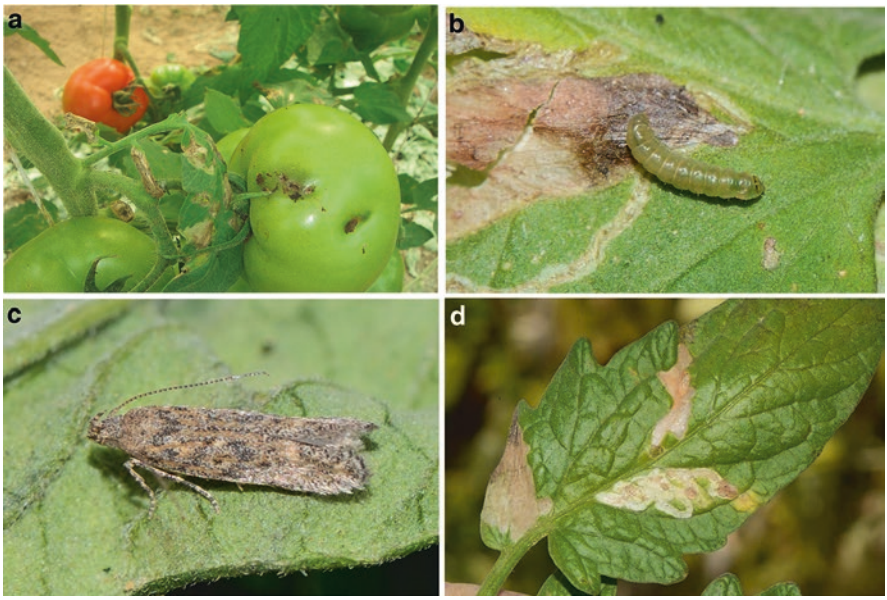

Fig. 41 (**a**) Damage symptoms on fruit by *Tuta absoluta* (Source: blog.plantwise.org) (**b**) Larva (**c**) Adult (**d**) Leaf mining by early instar (Source: nbair.res.in)

Biology

The female lays about 211 eggs mostly on the upper side of the leaf. The incubation period is 13 days; the larval (Fig. 41b) period is 12 days and pupa survive for 8 days. The longevity of male is 9 days while female lasts for 18 days and total life cycle completes in 30 days (Shiberu and Getu 2017).

Damage

Damage occurs by direct feeding of leaves, stems, buds, young or ripen fruits and infection of pathogens which got enter through the wounds caused by this pest (Fig. 41a, d). It can yield up to 90% loss and inferior fruit quality under greenhouses and field conditions. Currently, *T. absoluta* in India is an alarming pest because it is oligophagous and can outbreak on several suitable solanaceous host plants (Illakwahhi and Srivastava 2017; Anonymous 2019a, b, c, d).

Management

(a) Use sex pheromone (tetradecatrienyl acetate and tetradecadienyl acetate, 91:9) as mating disruptant @ 30 g/ha in greenhouses and fields (Vacas et al. 2011).
(b) *Trichogramma achaeae*, mirid bugs, *Nesidiocoris tenuis* and *Macrolophus pygmaeus, B. thuringiensis* (*Bt*) found effective to manage *T. absoluta* (Molla et al. 2009).
(c) Flubendiamine 480 g/l and neem are recommended for IPM (Nyandi 2017).
(d) Triflumuron 30 CC, abamectin 100 CC, chlorfenapyr 50 CC, and *B. thuringiensis* (*Bt*) 350 g/100 l water can be integrated into IPM (Virgala et al. 2017).

Fruit borer, *Helicoverpa armigera*

It is a widespread pest, distributed throughout Africa, Asia, Australia, USA and Europe (Cunningham and Zalucki 2014). It is a highly polyphagous and invasive agricultural pest (Queiroz-Santos 2018).

Biology

A female lays on an average of 412 eggs singly on upper and lower surface of leaves. The average duration of first, second, third, fourth, fifth, and sixth instar larva is: 2.50, 2.60, 3.60, 4.40, 4.70 and 4.20 days, respectively. The prepupal and pupal period lasted for 2.10 and 13.80 days, respectively. The mean pre-oviposition, oviposition and post-oviposition periods of fruit borer is for 2.90 days, 5.50 and 1.60 days, respectively. Longevity ranged from 8 to 10 days in males, while 10 to 14 days in females (Herald and Tayde 2018).

Fig. 42 (**a**) Larval feeding inside (**b**) infested fruits (**c**) boring of fruits (**d**) early feeding (Photo by: Surendra S Shekhawat)

Damage

Helicoverpa armigera is considered as the world's most damaging, cosmopolitan polyphagous insect pest which feeds on about 181 crops. The losses due to this pest alone is over rupees 6000 crores in India (Birthal and Sharma 2004) It is a destructive pest of fruits and vegetables, which makes the product unfit for human consumption and causing extensive crop loss up to 55% in yield (Selvanarayanan 2000). Neonate larva of *H. armigera* feeds on the terminal leaves (Wang et al. 1997) where old larva bores and remains inside (Fig. 42a–d) the tomato fruit (Hussain and Bilal 2007). ETL: 8 eggs/15 plants or 1 larva/plant or 1 damaged fruit/plant.

Management

(a) Pheromone traps @ 70/ha + neem seed kernel extract 2 ml/l + *T. chilonis* @ 50,000/ha and *Bracon hebetor* @ 1200/ha and Pheromone trap + *Ha*NPV @ 0.4 ml/l of water and *Bt* @ 2.0 g/l of water significantly effective (Rahman et al. 2016).
(b) Application of *Ha*NPV @ 1.5×10^{12} POB/ha, *B. thuringiensis* var. kurstaki (Delfin®) 25 WG @1 kg/ha, spinosad 45 SC @ 75 g a.i./ha, indoxacarb 14.5 SC @ 75 g a.i./ha and neem (neemazol) 1.2 EC @ 1000 ml/ha against *H. armigera* successfully lowers down the fruit damage (Levin 2004).
(c) The highest marketable yield recorded in case of flubendiamide 480 SC @ 200 ml/ha followed by spinosad 45 SC @ 200 ml/ha and Beta-cyfluthrin 2.5 SC @ 750 ml/ha (Jat and Ameta 2013).

Insect Pests of Brinjal of National Significane

Shoot and fruit borer: *Leucinodes orbonalis* Guenee (Lepidoptera: Pyraustidae)
Epilachna beetle: *Henosepilachna vigintioctopunctata* Fab. (Coleoptera: Coccinellidae)
Mealybug: *Coccidohystrix insolita* (Green) (Homoptera: Homoptera)
Red spider mite: *Tetranychus urticae* (Trombidiformes: Tetranychidae)

Fruit and shoot borer, *Leucinodes orbonalis*

It is a most devastating pest of the eggplant, *Solanum melongena* in Asian countries (Rashid et al. 2008), contributing more than 80% yield loss (Raju et al. 2007) and it was first reported in India (Dhankar 1988).

Biology

The average incubation period is 5.93 days; I instar 1 day, II instar 1.16 days, III instar 1.48 days, IV instar 2.63 days, V instar 4.46 days, pupa 11.2 days. Longevity of female is 4.14 days and male 4.31 days. The pre-oviposition period is 1.19 days, oviposition period for 2.71 days and post-oviposition period 3.75 days. Fecundity per female has been recorded 123 and 207 eggs. Life cycle completed in 28.17 days (Onekutu et al. 2013).

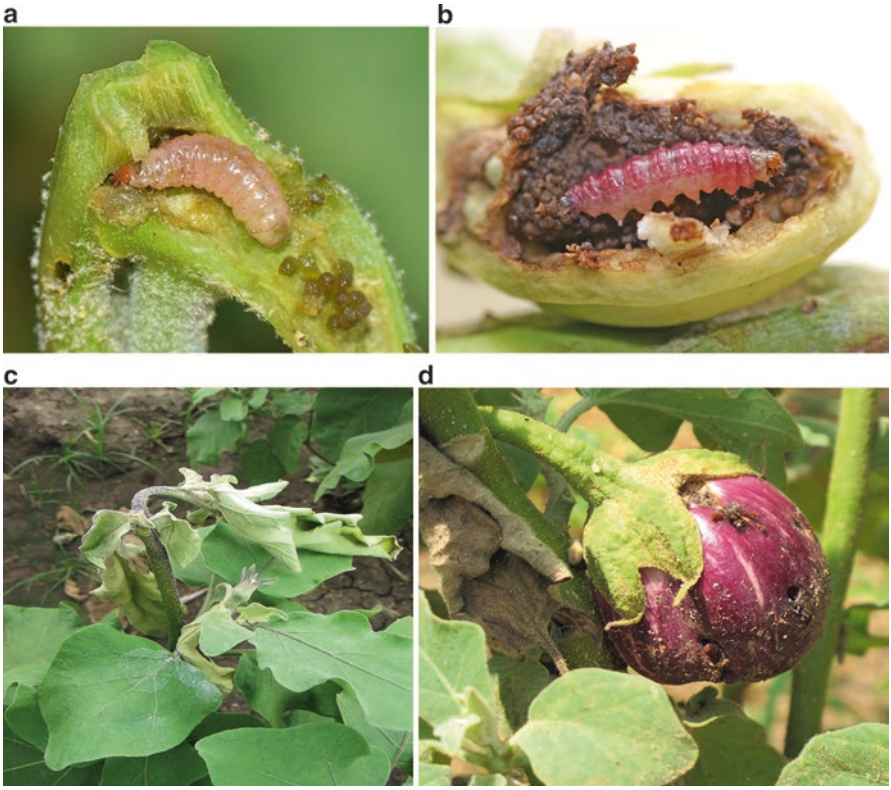

Fig. 43 (**a**, **b**) Larval feeding of *Leucinodes orbonalis* upon the fruit (Source: nbair) (**c**) Infested plant (**d**) Entry holes made by the larvae of *L. orbonalis* on infested fruit (Source: TNAU Agritech portal)

Damage

Larva cause damage by feeding inside the fruit and made large exit holes when undergoing for pupation (Fig. 43a–d). These holes reduce the market value of the fruits and make them unfit for human consumption (Alam et al. 2003) which later leads to secondary infection by certain bacteria causing further dilapidation of the fruits (Islam and Karim 1994). ETL: 0.5–5% shoot and fruit damage.

Management

(a) Install 4–5 pheromone (100:1 of E11-16: Ac and E11-16: OH) traps/acre @75 ft and change the lure after 2–3 weeks (PPQS).
(b) Two applications of flubendiamide (480SC) @ 80g a.i./ha or rynaxypyr (20SC) @ 50g a.i./ha or emamectin benzoate (5EC) @12g a.i./ha, and neem seed kernel extract (NSKE) @ 7ml /*l* gave satisfactory impact.

(c) Spinosad (Tracer 240SC) @ 60 ml /acre was proved most effective insecticide (Yousafi et al. 2015). A spray of bioneem 0.3 EC @ 1.5 ml /*l* at pre-fruiting stage and Tracer 45 SC at fruiting stage decreases shoot infestation in brinjal. Chlorantraniliprole @0.4 ml /*l*, spinosad @0.5 ml /*l*, *Beauveria* @2.5 g/l and *Bt* @2g /*l* was found effective against borer (Tripura et al. 2017).

Mealybug, *Coccidohystrix insolita*

It was a minor pest but attained the status of significant pests especially in cotton, fruits and vegetables. In India, it is a severe threat in Punjab, Rajasthan, Maharashtra and Gujarat (Tanwar et al. 2007).

Biology

It is cottony in appearance, small oval, soft-bodied sucking insects (Downie and Gullan 2004). Adults are found on leaves, stems and roots and covered with white mealy wax. First instar period range from 3 to 6 days, second and third instar for 4 to 6 days, respectively. The pre-oviposition period is 6–9 days, oviposition period for 12–15 days and post-oviposition period of 2–5 days. The longevity of adults is 21–30 days and total life cycle completed in 33–44 days (Singh and Kumar 2012).

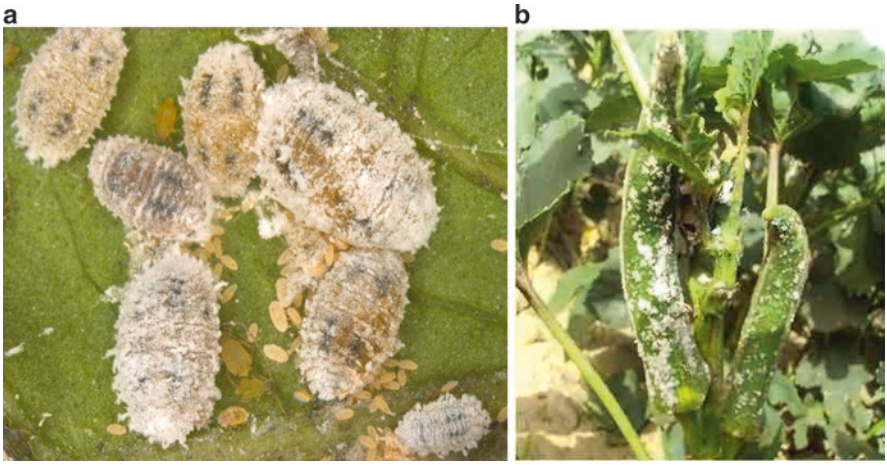

Fig. 44 (**a**) Life stages of *C. insolita* (Source: nbair). (**b**) Damage on okra (Source: krishisewa.com)

Damage

Nymphs and adults (Fig. 44a) both suck the sap from leaves and stems with the help of piercing/sucking mouthparts, deriving essential nutrients from the plant (Fig. 44b). The excess sap is excreted known as honeydew which attracts ants and develops sooty mould reducing the photosynthetic area. The host range of the mealybug is Malvaceae and Solanaceae families including okra, tomato, brinjal and cotton. *Phenacoccus solenopsis* found infesting okra and cotton in October and potato and tomato in February (Singh and Kumar 2012).

Management

(a) Release of *C. montrouzieri* @ 10/tree or @ 5000 beetles/ha, two times in a season especially during August–September and December–January. Khuksha leaves extract showed the best performance against the pest attack compare to other extracts (Azad et al. 2012).
(b) Chlorpyrifos 48% EC @ 5.00 cm^3/l, imidacloprid 35% SC @ 0.75 cm^3/l, and pyriproxyfen 10% EC @ 0.50 cm^3/l significantly effective in managing mealybug population (El Mageed et al. 2018).

Insect pests of Okra of National Significance

Fruit borer: *Earias vitella* (Fabricius), *E. insulana* (Boisduval) (Lepidoptera: Noctuidae)
Gram pod borer: *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)
Whitefly: *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae)
Mealybug: *Phenacoccus solenopsis* (Tinsley) (Homoptera: Pseudococcidae)

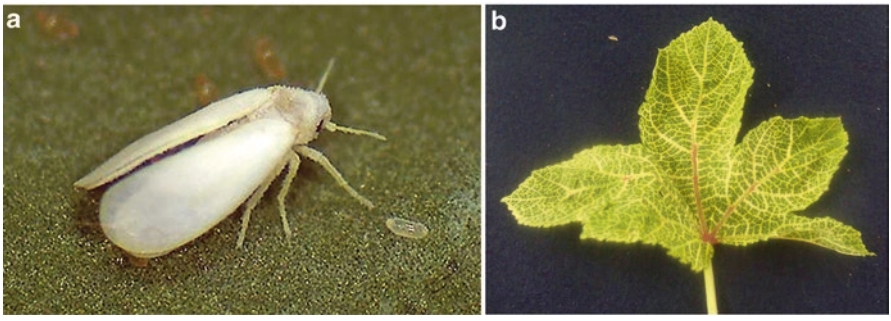

Fig. 45 (**a**) Adult whitefly (Source: Vivek Kumar)(**b**) Virus infection symptoms on okra (Source: eagri.org)

Whitefly, *Bemisia tabaci*

The whitefly is polyphagous and cosmopolitan pest native to India (Cuthbertson and Vänninen 2015). The Middle East-Asia Minor 1 (MEAM1) species of whitefly is aggressive coloniser and is a capable vector of many viruses, whereas the Mediterranean (MED) is strongly resistant to certain novel insecticides (Jones et al. 2008; McKenzie et al. 2009). It is a vector of vein clearing disease of okra (Fig. 45b), leaf curl of sesame and leaf curl disease of potato. Most biotypes of *B. tabaci* can transmit 60 plant viruses of genera Geminivirus, Closterovirus, Nepovirus, Carlavirus, Potyvirus and a rod-shaped DNA virus (Markham et al. 1994; Alegbejo 2000). Whitefly-transmitted Begomoviruses, formerly geminiviruses are agriculturally important, causing yield losses up to 20–100% (Brown and Bird 1992; Cathrin and Ghanim 2014). Begomoviruses have emerged as a severe threat to the variety of vegetable crops especially in the tropical and subtropical regions (Morales 2009). Whitefly-transmitted potato apical leaf curl virus disease causing upward or downward curling of leaves which became a new problem in potato growing areas of north-western plains in India. The first report of PALCV was made from Hissar around 2000 (Chandel et al. 2010).

Biology

Male and females are separate. The body is covered over by waxy white powder. Two pairs of wings are present; the hind wings are more prolonged than the fore wings. Females abdomen is bigger and broader than males. The anus opens on the dorsal surface of the last abdominal segment—known as 'vasiform orifice' provided with operculum as ligula. Eyes are crimson red coloured and antennae black in colour. A female lays 50–300 eggs, singly, pediculate usually in circular shape underside of leaves. Hatching occurs after 5–7 days. Nymphs are pale yellow. After first moult legs and antennae degenerated, remain anchored by its proboscis at one place and feeding in the phloem. The immature period is completed in 9–14 days and 2–8 days in the pupal stage to become adult. Whiteflies species can be identified through characters of the pupal stage. The first and second instar period is 3–4 days, third instar lasts up to 2–5 days and pupa 3–7 days. Total life cycle completes in 23 days on tomato and okra, 28 days on chilli and 19 days on brinjal. Female longevity is 12–19 days and male live for 10–12 days (Ahmad and Rizvi 2014). The life cycle is completed in 14–122 days and 11 generations/year.

Damage

Whiteflies (Fig. 45a) imbibe the sap from plant phloem by piercing and sucking type mouthparts. Feeding results in the loss of plant vigour and secrete honeydew, which develops sooty mould that interferes with photosynthesis and may lower harvest quality and quantity. In some host plants, whitefly releases toxins during

feeding that cause plant disorders like the silver leaf of squash (*Cucurbita pepo*) (Brown et al. 1992) and irregular ripening of tomato (Schuster et al. 1990). ETL: 3 nymphs/leaf or 4 adults/leaf

Management

(a) Parasitoids, *Encarsia formosa* (Polaszek et al. 1992), *Isaria fumosorosea* (Eslamizadeh et al. 2013) coccinellid predator from India, Predatory mites *Amblyseius limonicus*, *A. swirskii* and *Transeius montdorensis* are effective in reducing *B. tabaci* (Li et al. 2011; Cuthbertson 2014).
(b) Vertical and cylindrical yellow sticky traps are most attractive (Idris et al. 2012).
(c) Seed treatment with thiamethoxam 70 WS and imidacloprid 70 WS 5 g/kg following a foliar spray of spinosad 45 SC is sufficient for management of whitefly (Manju et al. 2018).
(d) Application of imidacloprid, 17.8% 1.25 ml/5 l, acetamiprid, 3.0% w/w 15 ml/5 l, dinotefuran, 20.0% w/w 2.5 g/5 l and cyantraniliprole, 10.26% 6.5 ml/5 l effectively declines the population (Zaini 2017).
(e) Soil application of carbofuran 3% CG is effective to whitefly in tomato without deterring natural enemy population and causing a phytotoxicity effect (Anandkumar and Hemalatha 2018).

Insect Pests of National Significance of *Allium* Crops

Onion thrips: *Thrips tabaci* Lindeman (Thysanoptera: Thripidae)

Onion thrips, *Thrips tabaci*

The native place of onion thrips is thought to be the Mediterranean region but now present throughout the world (Boateng et al. 2014). It is polyphagous pest reported to feed upon onion, garlic, crucifers such as cabbage, cauliflower and broccoli, asparagus, sugarbeet, melon, pumpkin, marrow and cucumber, strawberry, potato, tobacco, cotton and many fruiting and ornamental plants (Anonymous 2018c).

Biology

The incubation period of *T. tabaci* is 3–5 days and eggs are embedded in plant tissue, I instar duration is 1–2 days, II instar 2–3 days, Pre-pupa 1.25–1.7 days and Pupa lasts for 1 day. Total immature period ranged from 9 to 12 days. Oviposition period 7–19 days with a total life span of 15–35 days. (Moraiet et al. 2017). Antennae are seven segmented, I instar is creamy white and adult is light brown. Eyes are crimson red in nymphs and black in adults. The female inserts her saw-like

ovipositor into plant tissues and lay eggs under the epidermis. The eggs are white at first, turning orange later, and hatch in 4–5 days. Males are scarce in the population.

Damage

The larvae are white or yellowish and suck the sap from the plant tissues. Feeding of onion thrips causes yellowing of leaves and flower parts. Severe infestation results in stunted growth, white blotches, silvery whitish spots and a chlorotic yellowish appearance with greyish color along all large veins. The onion thrips is also a vector of certain plant viruses viz. iris yellow spot virus, strawberry necrotic shock virus, tobacco streak virus and tomato spotted wilt virus (Srinivasan et al. 2012; Reitz 2014). It also transmits *Alternaria porri*, which causes the purple blotch and a bacterial pathogen, *Pantoea ananatis*, to onion (Dutta et al. 2014; Gill et al. 2015). Onion thrips, *T. tabaci* is most serious pest and can cause 34–43% yield loss (Krishna Kumar et al. 2001).

Management

(a) Intercrop onion with cotton, tomato, chilli and okra to reduce the thrips population (Khaliq et al. 2016). *Amblyseius cucumeris* is proven successful biocontrol agent for *T. tabaci* in greenhouse cucumber (Gillespie 1989).
(b) Spinosad and *Lecanicillium muscarium* (Mycotal) with blue or white sticky traps results in a significant reduction in the population density of onion thrips (Al-Karboli and Al-Anbaki 2013).
(c) Spray thiamethoxam 25 WG (0.01%) or imidacloprid 17.8 SL (0.08%) or 1 ml/l water (Das et al. 2017).

Insect Pests of National Significance of *Brassicaceous* Crops

Diamondback moth: *Plutella xylostella* Linnaeus (Lepidoptera: Yponomeutidae)
Cabbage butterfly: *Pieris brassicae* (Linnaeus) (Lepidoptera: Pieridae)
Tobacco caterpillar: *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae)
Cabbage aphid: *Brevicoryne brassicae* Linnaeus (Homoptera: Aphididae)
Cabbage green semilooper: *Trichoplusia orichalcea* (Fabricius) (Lepidoptera: Noctuidae)
Leaf Webber: *Crocidolomia binotalis* (Zeller) (Lepidoptera: Pyralidae)

Diamondback moth, *Plutella xylostella*

Diamondback moth is the most destructive pest of crucifer crops in countries, such as India and South East Asia (Smith and Sears 1982). It developed resistance against several classes of insecticides. DBM found cause up to 52% losses on cabbage and cauliflower in India (Reddy and Zehr 2004). Brassica plants contain glucosinolates which play a significant role in oviposition, mating, and larval feeding. The toxic product in Brassica, mainly isothiocyanates are released during larval feeding. The DBM's gut lumen contains an enzyme called 'glucosinolate sulfatase' converts into desulphate glucosinolates through hydrolysis reactions that are harmless and ineffective to larval feeding (Ratzka et al. 2002; Müller et al. 2010). Because of the broad range of enzyme's detoxification, the DBM has many hosts to choose from Brassicaceae family (Sarfraz et al. 2006).

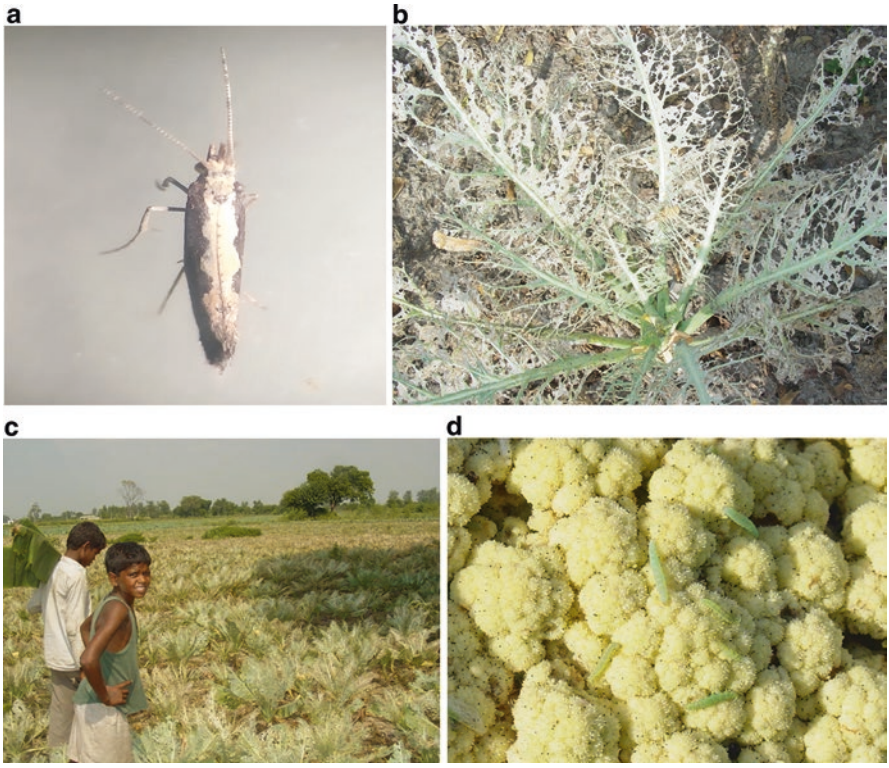

Fig. 46 (**a**) Adult DBM (**b**) damaged plant (**c**) Outbreak in field condition and (**d**) DBM larvae on cauliflower (Photo by: Tufail Ahmad and M. Shafiq Ansari)

Biology

Female can lay up to 175–200 eggs preferably on the lower surface of leaves (Gowri and Manimegalai 2016). Oviposition period of *P. xylostella* is 4.5 days in field while, 6.5 days in laboratory condition. It has four larval instars, the larval period of I, II, III, IV instar is about 9.5, 16.5, 7.5 and 7.5 days, respectively. The pupal period lasts for 10 days. Adult (Fig. 46a) longevity of males and females is 12.5 and 20 days in field condition. In laboratory condition, the larval period ranges from 5, 3.5, 4 and 3.5 days and the pupal period ranged from 4.50 days, the adult longevity of males and females 8 and 11 days in the lab. The life period of males and females is average 64.5 and 72 days in field condition while, in laboratory condition 32.5 and 35.5 days (Tufail et al. 2008; Ahmad and Ansari 2010).

Damage

Newly emerged larvae feed inside the leaves as leaf miners and second instar become surface feeders and feeds on the lower leaf epidermis and later instars appear on the surface of the leaves (Fig. 46b–d) and continue to consume foliage including the waxy surface (Talekar and Shelton 1993; Hasan and Singh 2008). ETL: 2 larvae/plant at 1–4 weeks after transplanting or 5 larvae/plant at 5–10 weeks after transplanting.

Management

(a) Cabbage intercropped with onion and tomato (5:1) helps to minimise the damage (Asare-Bediako et al. 2010).
(b) *Bacillus thuringiensis* and Novaluron (IGR) at 6–12 oz/acre significantly reduce DBM attack in cabbage (Seal 2003). Use overhead sprinkler irrigation to reduce pest population (Facknath 1998).
(c) Egg parasitoid, *T. bactrae*, conservation of *Cotesia plutellae* were effective (Krishnamoorthy 2004).
(d) After catching 8 moths/trap/night release the parasitoid *C. plutellae* (250,000 adults/ha), the predator *Chrysoperla carnea* (2500 eggs/ha), spray nimbecidine (625 ml/ha), *B. thuringiensis* (500 m1/ha), and phosalone (2.8 l/ha) (Reddy and Guerrero 2000).

Tobacco caterpillar, *Spodoptera litura*

The tobacco caterpillar, *S. litura*, is a polyphagous and very significant pest of cabbage, chilli, tomato and okra. It is extensively distributed throughout the tropical and temperate Asia, Australasia and the Pacific Islands (Feakin 1973; Kranz et al. 1977).

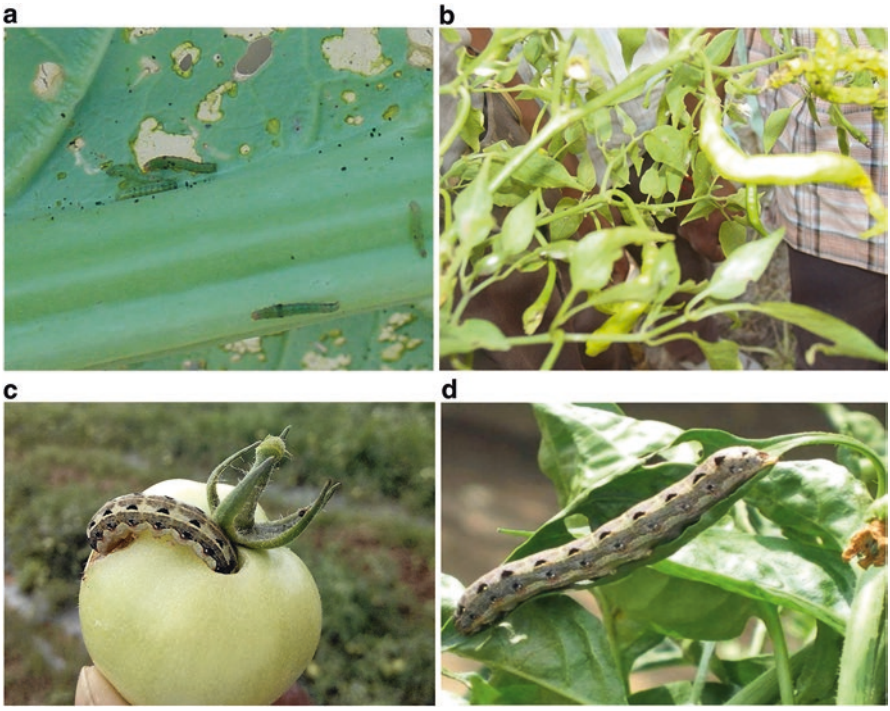

Fig. 47 (**a**) Damage by *S. litura* on cauliflower (**b**) Damage in chilli fruit (Photo by: Tufail Ahmad and M. Shafiq Ansari) (**c**) Infested tomato fruit (Source: Farmnest.com) (**d**) *S. litura* on chilli leaf (Source: Dr. Prabhakar Mahadevaiah)

Biology

A female can lay 350–800 eggs arranged in 3–4 layers covered by brown hairs. The incubation period is 3 days. Duration of I, II, III, IV, V and VI instar is 1.2, 2.2, 2.3, 3.2, 4, and 1.3 days, respectively. The total larval development period is ranged 12–15 days with an average of 13.5 days. The pre-pupal period is 1–2 days and the pupal period recorded 8–10 days. The longevity of male ranges for 5–7 days and female is 7–9 days. Life cycle completes in 33–35 days (Shekhawat et al. 2019).

Damage

The larva is a damaging stage; I instar larva scraps on the green matter of leaves gregariously. Older larvae feed voraciously on different plant parts causing defoliation and boreing into fruits (Fig. 47a–d).

Management

(a) *Pseudomonas fluorescens* results into reduced food consumption index, relative growth rate, approximate digestibility, the efficiency of conversion of ingested food, and the efficiency of conversion of digested food of *S. litura* (Sahayaraj et al. 2018).
(b) Spinosad 45 SC at 200 g/ha at 15 days interval is most effective in reducing larval population of *S. litura* (Jat et al. 2017), emamectin benzoate 5 SG @ 0.025, indoxacarb 14.5 SC @ 0.007 and thiodicarb 75 WP @ 0.075 found significantly superior for management of *S. litura* (Rabari et al. 2015).
(c) Eco-friendly management module comprised of trap cropping with mustard (one row of mustard as border crop), application of neem-based formulation neemazol 3000 ppm @ 0.0004%, application of *B. thuringiensis* @ 1.5 kg/ha, spraying of *Ha*NPV @ 450 LE/ha, release of *Chrysoperla* @ 10,000 larvae/ha proved effective (Somnath et al. 2015).

Important Insect Pests of Vegetables in Storage

(a) Potato tuber moth: *P. operculella*
(b) Fruit fly: *B. cucurbitae, B. zonata, B. dorsalis*
(c) Tomato fruit borer: *H. armigera*
(d) Okra fruit borer: *E. vitella*, *E. insulana*
(e) Sweetpotato weevil: *Cylas formicarius* (Fabricius)

The infestation is continuing to the storage or market after harvesting of already infested product. Post-harvest loss can range between 30% and 50% according to FAO. Hidden infestation leads to huge economic loss due to rejection of shipments and reduction of quality. Hidden stage of these insects and marketing of product without screening or grading can be main reason of the post-harvest loss by insects. Sometimes immature stages are so small and unable to recognish by producer. For example: fruit fly oviposits in tissues of ripening fruits and oviposition marks are generally avoided at the time of packing after harvesting. These oviposition marks ignored by the farmers and leds to further increase in infestation, production loss and economic loss. Study found the average loss due to fruit fly infestation via rejections at the farm is 24–60% (Caroline 2012). Other insects like potato tuber moth in potato, sweetpotato weevil, fruit borer in okra, thrips in onion and head borer in cole crops cause significant damage. Storage and marketing loss in potato is 5% and 19–22% (Urge et al. 2014).

Potato tuber moth, *P. operculella* is a major pest of potato in fields or storage, potentially cause the total crop loss in the form of discards or unfitness of tubers for seed. Larva can mine the leaf and infest tuber during season and even during the storage. Migration is attributed primarily due to movement of tubers carrying the pest into storage facilities further north. Farmers suffering from lower market value

for damaged potatoes. Tuber infestation and rotting is found to be positively correlated (Sileshi and Teriessa 2001).

Helicoverpa armigera early instar feeds on leaves by scraping green matter and later it migrates to fruit through boring and feeds internally. After fruit is completely damage it starts rotting and producing bad smell. Infested fruits are unmarketable causing great loss in quality and quantity.

Sweetpotato weevil is the most serious pest of sweet potato in field and storage throughout the world. It completes the life cycle in 35–40 days and remains active throughout the year. A single female can lay 75–90 eggs in her life span, deposited singly in root or stem. The larvae feed inside the tuber by making tunnels filled with fecal matter and pupates there in. It can cause damage from 5% to 97%. The infested tuber is often riddled with cavities, spongy in appearance, and dark in color. In addition to damage caused directly by tunneling, larvae cause damage indirectly by facilitating entry of soil-borne pathogens (Capinera 2018).

Management

This infestation results into various types of post-harvest losses; qualitative, quantitative and secondary infection of pathogens which also have great significance overall.

(a) Regular inspection of field and observation of symptoms of infestation
(b) Adopt control measures at early stage of infestation
(c) Screening and grading of harvested product
(d) Sanitation and keep soil moist for sweetpotato weevil
(e) Use of pheromone trap and spray of *Beauveria bassiana*
(f) In storage; fumigation, controlled atmospheres (low oxygen and high carbon dioxide)
(g) Entoletres

IPM

Integrated management defined as “applied pest control which combines and integrates biological and chemical control” (Stern et al. 1959). IPM was introduced by R.F. Smith and R. van den Bosch in 1967, considering the negative effect of insecticides on the ecosystem and human health, IPM was formulated into National policy in February 1972 when President Richard Nixon directed federal agencies to take steps to advance the application of IPM in all relevant sectors. In 1979, President Jimmy Carter established an interagency IPM Coordinating Committee to ensure development and implementation of IPM practices (Acosta 1995–2006). Perry Adkisson and Ray F. Smith received the World Food Prize in 1997 for encouraging the use of IPM.

Among total pesticide used and per hectare consumption in India, insecticide shares highest. In 2014–2015, pesticide consumption was 0.29 kg/ha (GCA), Although, per hectare use of pesticides in India is very less as compared to other countries like China (13.06 kg/ha), Japan (11.85 kg/ha), Brazil (4.57 kg/ha) and other Latin American countries (Anonymous 2017d; Subhash et al. 2017). Among different vegetable crops, the maximum pesticide usage is in chilli (5.13) followed by brinjal (4.60), Cole crops (3.73) and okra (2–3) (Rai 2015).

Pesticides have been widely used and their traces can be easily detected from air, water and soil. Despite the ban on DDT and HCH in India, they are still being used in both domestic and agricultural settings (Yadav et al. 2015). Fruits and vegetables use a significant share of agrochemicals and they account for 18% of the cropped area (Devi et al. 2017).

Country	Annual pesticide consumption (millions of kilograms)
China	1806
United States	386
Argentina	265
Thailand	87
Brazil	76
Italy	63
France	62
Canada	54
Japan	52
India	40

Source: https://www.worldatlas.com/articles/top-pesticide-consuming-countries-of-the-world.html, 2017

Kumari et al. (2002) found that samples taken from the market are 100% contaminated in a miserable amount and about 23% of the samples contaminated with organophosphorus compounds above their respective MRL. Health risks were found to be associated with methyl-chlorpyrifos, ethyl-chlorpyrifos, and omethoate in tomatoes and methyl-chlorpyrifos, ethyl-chlorpyrifos, dichlorvos, monocrotophos and omethoate in eggplant. Routine monitoring of these pollutants in food items is required to prevent, control and reduce the pollution and to minimise health risks (Darko and Akoto 2008).

Pesticide and chemical residues are a chief cause of concern for Indian agricultural exports. Indian food exports are sometimes rejected due to residues found that are higher than Maximum Residue Limit (MRL) of importing nations. Lack of awareness amongst Indian farmers regarding the judicious and timely use of chemicals has been a significant impediment (Anonymous 2019a, b, c, d). Farm products such as mangoes, table grapes, okra, peanuts, curry leaves, chillies, shrimps, prawns, and tamarind have faced rejections in markets of USA, Vietnam, EU, Saudi Arabia, Japan and Bhutan due to the presence of high level of chemical residues,

pest and bacterial infestation. According to the EUROPHYT portal's notifications, between 2005 and 2017 (May 31, 2017), Indian exports faced 1324 interceptions as compared to 452 for Brazil, 602 for China, 114 for Turkey and 922 for Vietnam. A majority of interceptions for India were raised in the years 2012 and 2013, and these pertained to eggplant, mangoes, snake gourd, bitter gourd and taro (Arabi), among others. It led to a ban on the entry of mangoes and these vegetables (Arpita et al. 2017). The EU had an imposed ban on India's premium Alphonso mangoes, brinjal, bitter gourd, snake gourd and the taro plant by stating that it had found contamination of fruit fly and other quarantine pests in 207 consignments (Anonymous 2014a, b).

Principles and strategies in IPM:

(a) Monitoring, pest surveillance and forecasting of insect pest population are essential tools in IPM which help in deciding for management.
(b) Injury level concepts: ETL (Economic threshold level) and EIL (Economic injury level) should be followed to judicious use of insecticide considering their impact on the environment (Alston 2011).
(c) Integration of pest management practices by adopting the proper compatible tactics and blending them so that every component complements each other. The policy of applying pest management tactics should be similar to that of human medicine. i.e. Preventive practice and curative practice both.

Preventive methods of IPM are:

(a) Use of natural enemies
(b) Selection of resistant varieties
(c) Apply cultural control
(d) Use regulatory control or plant quarantine

Curative methods of IPM include:

(a) Physical and mechanical methods
(b) Inundating method releasing biocontrol agents
(c) Chemical insecticides, IGR

Precautionary methods or preventive measures can be adopted, irrespective of the pest incidence. It can be followed as a routine, even if the pest population at a low level.

(a) Curative methods should be followed only when the pest population reaches the economic threshold level
(b) Field sanitation and clean culture
(c) Deep summer ploughing
(d) Use of healthy seed or planting material
(e) Removal of weeds and alternate hosts
(f) Apply the recommended dose of fertilisers and micronutrients
(g) Regular field scouting and pest monitoring
(h) Installation of yellow pan water or sticky traps for sucking insects
(i) Installation of light/pheromone traps for nocturnal insects

Do ecological engineering for pest management: Flowering plants belonging to family Compositae, Leguminaceae, Umbelliferae and Brassicaceae etc. (French bean, marigold, carrot, sunflower, buckwheat, mustard, castor, maize, alfalfa, chrysanthemum, dill) that attracts natural enemies as well as plants that repel pests can be grown as border/intercrop. Agro-ecosystem based analysis (AESA) IPM Packages of 87 crops are available at NIPHM and 85 at DPPQS website.

International Institute supporting IPM are: IPMWG, FAO, CABI, ICIPE Global IPM facility (1992)—Sponsored by FAO, UNDP, UNEP and World Bank.

National:

1. Farmer's portal: https://farmer.gov.in/
2. Department of Agriculture Cooperation & Farmers Welfare: http://agricoop.gov.in/
3. National Institute of Plant Health Management: https://niphm.gov.in/IPMPackages.html
4. Directorate of Plant Protection, Quarantine & Storage: http://ppqs.gov.in/
5. TNAU Agri-tech portal: http://agritech.tnau.ac.in/
6. National Centre for Integrated Pest Management: http://www.ncipm.res.in/

Conclusion

Losses caused by insect pests are very high besides the production. Therefore, there is need to adopt more bio-intensive management tactics and IPM policies to bring down those losses to below economic threshold. Extension services and Information technology (IT) sector should co-operate in coherent manner for dissemination of knowledge of advance management tactics to the farmers. By utilizing these resources, farmers would be able to increase the production as well as their economy. The malnourished and hunger population of the world could be supplied with required amount of fruits and vegetables in their diet.

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Insect Pest Infestation During Storage of Cereal Grains, Pulses and Oilseeds

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Abstract Due to the increasing population day by day, the food security is the most global concern in order to fulfil the food demand for both developed and developing nations across the world. In developing countries, cereal grains are the staple food and nearly 70% of the population depends upon agriculture. In India the most challenging issue is the storage of these cereal grains. A number of insect pests deteriorate stored grains. Insect and pest infestations are major contributor to quality deterioration of stored food stuff such as cereals grains, pulses, and oil seeds. Tropical climate of India provides favourable condition for the continuous growth of insect/pests throughout the year. During storage, pests infest the grains and therefore, fulfil their food and shelter requirements by causing qualitative as well as quantitative losses of stored products. These insect pests impose the damage on stored products by direct feeding and affect the farmers because their infested grain may have a significant effect on the value of marketing, consumption, or planting. This chapter provides an overview of different types of stored grains and their infestation by major pests, and also describes various techniques for prevention of these infestations.

Keywords Stored products · Cereals · Pulses · Oilseeds · Loss · Pests

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Introduction

Insect and pests are major threat to quality deterioration of stored food stuff such as cereals grains, pulses, and oil seeds. In different parts of the world, a variety of insect species have been associated with stored products but a few are considered as significant pests (Srivastava and Subramanian 2016). Among them majority of storage grain pests are belong from order coleoptera and lepidoptera (Khare 1994). Loss of the nutritional and physical properties of stored food stuff due to infestation by weevils, bruchids and other insects and pests is very common. Deterioration of quality and quantity of the food stuff is greatly affected by physical, biological, chemical and engineering factors. "A grain saved is a grain produced" concept must be taken into concern from the time of harvest itself. Role of technologists and scientists to minimize the wastage of stored products at farm level is therefore, the need of the hour. Contamination sources of cereal grains are mostly insects and microbial sources which make it totally inedible. Infestation of insect pests also results in secondary contamination of moulds. Increased humidity without adequate ventilation, mould growth develops "caking" causing severe losses. Post-harvest losses are surveyed to be about 10–20% and sometimes it may be extent of 30% in storage which turns out to be huge taking in consideration the consumption level (Rajasri and Kavitha 2015). Cereal grains contribute to major food sources to humans as well as animals. Manual inspection at farm level is done by sieves, while industrial techniques include acoustic detection, uric acid measurement, carbon dioxide measurement, X-ray and IR spectroscopy methods. Industrial methods are more effective and less time consuming (Neethirajan et al. 2007).

Stored Products

After harvest surplus amount of produce which is to be stored for supply during off season, seed, preservation, export, marketing and processing.

Cereals

Cereal grains are plant fruits obtained from the grass family (Gramineae), include rice, wheat, corn, barley, oats and millets which serve as staple foods in most areas of world. Nearly 80% of the produce is cultivated in Asia, America, and Europe. India is the largest producer and exporter of cereals in the world. During 2015–2016, India produced 104.32 million tonnes rice, 21.8 million tonnes maize and 8.08 million tonnes bajra and exported of 8078.85 Million USD (Anonymous 2018a, b). Cereal are good source of CHO, protein, fat, fibre and micronutrients such as vitamins of group B, calcium and iron. It fulfils about half of the calorie intake of total

energy requirement. Cereals are stored easily for a long time because of low moisture content, providing variety of acceptable products and also stored depending on market demand, size of production and the farmer's needs. Storage is the most important and critical post-harvest operation. Deterioration of the grain quality during storage can be due to improper storing conditions, which leads to contamination with fungi or insect infestation.

Common Insect Pests of Stored Cereals (Table 1)

Insects cause extensive damage to stored cereals and this may amount to 5–10% in the temperate geographical zone and 20–30% in the tropical zone (Chomchalow 2003). Therefore, there is a need for diverse kinds of safe control measures to prevent insect pests attack on food grains (Hikal et al. 2017). Quality of food grains is affected by insect infestation that represents severe and continuing problem at the grain and milling industry. Under the provisions of the Grain Standards Act, establishment of Federal standards for grain, is important for supervisors and inspectors to identify the various species of insects that cause damage to stored grains. There are five primary insect pests which attack stored grains namely Granary weevil, Rice weevil, Maize weevil, Grain borer. Angoumois grain moth spoils wheat and corn. Other insects that are found in stored grains are beetles, moths, and meal-worms.

- In stored grains most common insect pest are:
  - Rice weevil *Sitophilus oryzae* (L.)
  - Angoumois grain moth *Sitotroga cerealella* (Olivier)
  - Lesser grain borer *Rhyzopertha dominica* (Fab.)

Granary Weevil (*Sitophilus granarius* Linnaeus)

The granary weevil, *Sitophilus granarius* (Linnaeus) is small polished red brown to black beetle with no wing cover and well-marked thorax with oval pits (Gorham 1991). The head emends with a long pair of distinct snout of stow mandibles or jaw at the end; these two characteristics distinguish granary weevil to rice weevil and wheat weevil. It's long about 1/8″ to 1/4″. It is oldest insect pest which prefers a temperate climate and has been found all over the world. Adult is live about 7–8 week and during this period female lays about 50–250 eggs. Female weevil uses her strong mandibles to chew the grain kernel and make a small hole, and deposit eggs in the hole and cover it with gelatinous fluid. It takes about 5 weeks for development of egg to adult in warm weather while in cold weather it takes prolong periods. After few days the eggs hatch into a white, soft legless fleshy grub that feeds in the interior of the grain kernel. The grub develops to naked white pupa eventually into an adult beetle. Since the granary weevil cannot fly because it lacks

Table 1 Important insect pest of stored products (Chomchalow 2003)

Species	Common name	Classification	Stored products attacked
Acarus siro	Flour mite	Sarcoptiformes: Acaridae	Cereals, cereal products, dried fruits, tobacco
Acanthoscelides sp.	Pulse weevil	Coleoptera: Bruchidae	Many pulses including kidney bean
Callosobruchus chinensis	Pulse beetle	Coleoptera: Bruchidae	Many pulses including soybean except kidney bean
Callosobruchus maculatus	Pulse beetle	Coleoptera: Bruchidae	Many pulses except soybean except kidney bean
Carpophilus hemipterus	Dried fruit beetle	Coleoptera: Nitidulidae	Dried fruits, groundnut
Corcyra cephalonica	Rice moth	Lepidoptera: Galleridae	Rice, maize, soybean, groundnut, cacao, dried fruits, copra, flour
Cryptolestes ferrugineus	Rusty grain beetle	Coleoptera: Laemophloeidae	Maize, wheat
Cryptolestes pusillus	Flat grain beetle	Coleoptera: Laemophloeidae	Maize
Caedra cautella	Tropical warehouse moth	Lepidoptera: Phycitidae	Rice, maize, mung bean, soybean, groundnut, flour, dried fruits, copra
Latheticus oryzae	Long-headed flour beetle	Coleoptera: Tenebrionidae	Maize
Oryzaephilus mercator	Merchant grain beetle	Coleoptera: Silvanidae	Oilseeds, groundnut, maize, dried fruits
Oryzaephilus surinamensis	Saw-toothed grain beetle	Coleoptera: Sylvanidae	All cereals, pulses, spices, tobacco, dried fruits, flour
Plodia interpunctella	Indian meal moth	Lepidoptera: Phycitidae	Rice, wheat, maize, sorghum
Rhyzopertha dominica	Australian wheat borer	Coleoptera: Bostrichidae	Paddy, rice, maize, sorghum, root crops
Sitophilus granarius	Granary weevil	Coleoptera: Curculionidae	Rice, wheat, maize, sorghum
Sitophilus oryzae	Rice weevil	Coleoptera: Curculionidae	Rice, maize, wheat, sorghum, pulses
Sitophilus zeamais	Maize weevil	Coleoptera: Curculionidae	Maize, also other cereals
Sitotroga cerealella	Angoumois grain moth	Lepidoptera: Gelechiidae	Paddy, wheat, maize
Tribolium castaneum	Red flour beetle	Coleoptera: Tenebrionidae	All cereals, starch, pulses, oilseeds, spices, dried fruits
Tribolium confusum	Confused flour beetle	Coleoptera: Tenebrionidae	Flour, wheat, maize
Trogoderma granarium	Khapra beetle	Coleoptera: Dermestidae	All cereals

meta-thoracic flight wings which are well developed in *S. zeamais* and *S. oryzae*., it is mostly found in storage (Mason and McDonough 2012; Egbon and Ayertey 2009).

Rice Weevil (*Sitophilus oryzae*)

In Rice, insects cause economic losses because of ability to adverse effects of milled rice, paddy and the by-products of milling material. Rice weevil is a most destructive insect pest of cereals in the world which affects crops including wheat, rice, and maize. Adult rice weevil is around 2 mm long with a long snout and almost brown to black in colour. The wings usually cover four orange/red spots. Rice weevils have the ability to fly but are similar to granary weevil in the form however having different colour and wings. Rice weevils are known to be the worst pests for stored grain since ancient times and found all over the world. It's particularly abundant in warm weather country and breeds incessantly and damage the quality and quantity of unprotected stored grain. The life of adult rice weevil has average 4–5 months and during this period female lays around 300–400 eggs (Mason and McDonough 2012). Its early life stage is similar to granary weevil, the egg, larva and pupal stages passes to 26 days at hot weather and this is prolonged in cold weather. For killing of rice weevils in all stages of development freezing infected food below 17.7 °C (0 °F) for a period of 3 days or heating to 60 °C (140 °F) for a period of 15 min (Catsberg and Kempen-van Dommelen 2013).

Maize Weevil (*Sitophilus zeamais*)

The maize weevil (*Sitophilus zeamais*) is a species of the family Curculionidae (Cowley et al. 1980). It attacks standing crops and stored cereal products, such as rice, wheat, barley, sorghum, oats, buckwheat, rye, cottonseed and peas. Maize weevils are as similar appearance to rice weevil except they are 3–3.5 mm length, darker (reddish brown) with long snout and have reddish spot on the elytra defined clearly (Hidayat et al. 1996; CABI 2018). The maize weevil's thorax is densely and uniformly pitted with round punctures. Adults can fly from the granaries to the field and start infestations after harvest and destruct the storage grain. It reduces the kernels to dry powder and hulls. The life cycle of maize weevil is similar to rice weevil, 3–6 months and more (Devi et al. 2017; Ojo and Omoloye 2016). Adult females lay eggs in hole of the grain and sealing with mucus plug. The development rate is slower and requires minimum 30 days for development from egg to pupal stages (Clutton-Brock 1991). The maize weevil and rice weevil have similar look, but maize weevil is found in corn and is larger and darker as compare to rice weevil. *Sitophilus zeamais* is more resistant to starvation than *S. oryzae* (Maceljski and Korunić 1973).

The *Sitophilus* spp. are distributed throughout the world in temperate and tropical counties. These are internal feeders, both grubs and adults known to produce damage. Pupation also takes place inside grain and the adult exit by making a large

irregular hole. It is estimated about 5–10% loss in market value due to infestation by only *Sitophilus* spp. (Kumar and Kalita 2017).

Lesser Grain Borer (*Rhyzopertha dominica* F)

Lesser grain borer (*Rhyzopertha dominica* F.) is thought to be native of Indian subcontinent but now cosmopolitan. It is smallest around (2 mm), dark- brown to black coloured, slender cylindrical beetle, reported to major pest of wheat, rice and corn in storage and also found on olive trees in Sicily (Flinn et al. 2004; Buonocore et al. 2017). Both larva and adult bores into the grain and cause damage (Kargbo 2013). The female can lay 200–500 eggs on outside kernel, and after hatching larva bores into the grain. At 34 °C, incubation period is 2 days, the larval lasts about 17 days, and the takes 3 days to complete development. It takes 25 days to complete the life cycle from eggs to adult. The adults are strong flier and fly to spread infestation (Mason and McDonough 2012). In India, it is reported to cause up to 12% of post-harvest losses (Ajaykumara 2015).

Grain Moths

Grain moths are belonging from order lepidoptera known to cause enormous loss in storage of agricultural crops by destroying unbroken kernels and milled products. Immature larva is damaging stage which is known as caterpillar. Among four, two (Angoumois moth and Almond moth) are internal feeder and rest two (Indian meal moth and Rice moth) are external feeders. They are:

Angoumois Grain Moth: *Sitotroga cerealella* (Olivier)

The Angoumois grain moth *Sitotroga cerealella* (*Olivier*), is small in size about 1/8″ long. It is buff or yellowish in color, fully developed larvae is usually yellowish-white with a yellowish brown head (Beshir 2011). Grains infested with this insect have unpleasant smell by larvae. It affects cereal grains all over the worlds and attacks in field and stored grains. The ovipositional period, incubation period, larval period, pre-pupal period and pupal period are reported for 3.67 days, 5.5 days, 25.2 days, 3.0 days and 5 days, respectively. Male lives for 8 days where female longevity is lasts up to 10 days. The eggs are glued to the kernel. When the eggs are laid, they are white subsequently change to reddish colour (Akter et al. 2013). After hatching, larva crawls to grains kernel and spin into a cocoon that assists it in penetrating the hard kernel however, inside the grain larvae continues to feed the endosperm or the germ until maturation. When mature, eats outside of the seed channel and forms a weak fastened flap at the exit by cutting the shell of one-half to three-fourths of the circumference of a circle (Ignjatović et al. 2018). After that larva spun

into a silken reddish-brown cocoon and pupate within the kernel, adult moth pushes the flap back on the kernels.

Management

Preventive Methods

- Maintain sanitation and ventilation at field and storage
- Clean and disinfect storage structure, gunny bags, bins followed by fumigation to avoid carry forward of infestations
- Filling of cracks, crevices and burrows
- Drying of grains at minimum moisture (<8%)

Physical Methods

- Sieving or physical exclusion
- Vacuum sealing or controlled atmosphere conditions: lower oxygen level (1–2%) and increase CO_2 at room temperature
- Temperature control and cooling: 0 °F for 4 days usually kills most of the species
- Use of diatomaceous earth (DE, silicon dioxide): penetrates pest's cuticle, resulting in death by dehydration (Barbercheck 2018)

Pheromones: can be implicated for mass trapping in IPM:

- Male of *S. orzyae* produce an aggregation pheromone ((4S,5R)-5-Hydroxy-4-methylheptan-3-one)
- Hexane extracts of Tenax®-trapped volatiles from males held on wheat were attractive to both sexes (Faustini et al. 1982)
- For *Rhyzopertha dominica*: Dominicalure-1 (D1) and Dominicalure-2 (D2), (Bashir et al. 2003)
- For Angoumois Grain Moth:(Z,E)-7,11-Hexadecadienyl acetate (9) (Z,E)-7,11-Hexadecadienal (1)
- For Indian meal moth: (Z,E)-9,12-Tetradecadienyl acetate (1) (Z,E)-9,12-Tetradecadien-1-ol (1)
- For Almond moth: (Z,E)-9,12-Tetradecadienyl acetate (7) Z)-9-Tetradecenyl acetate (1)
- For Rice moth: 6,10,14-Trimethylpentadecan-1-ol (F) E,E-Farnesal (M), (Swords and Van Ryckeghem 2010).

Insecticide Control

- Spray storage surface with *Bacillus thuringiensis* (*Bt*) to control lepidopterous pests
- Treat the storage with pyrethrum

- Use insecticides viz., malathion (5% @250 g/quintal), dichlorvos and deltamethrin
- Place aluminium phosphide (celphos 3 tabs/tonnes of grain) in storage
- Treatment of grains with 2 ppm of Spinosad

Pulses

Pulses belong to legume family which is dried edible seeds of certain plants having rich source of protein and fibre and low fat content. Pulses are valuable sources of minerals such as iron, zinc, phosphorus; folate and vitamin B (Vaughan and Geissler 2009). According to the nutritional profile it helps in improving health, especially in developing countries and the main characteristics of pulses are less expensive proteins as compared to animal proteins. Protein content in soybean is 36%, soy chunk 52%, lentil 9%, moong bean 26%, Bengal gram 20% and peas 26%. Pulses are referred to as "poor man's crops", as "poor man's protein", or as marginal products of marginal lands. A survey reveals that about 68% people in India are lacking in protein. Consumption of pulses as whole or split can help to reduce this malnutrition. Fresh beans or peas are not included in pulses. Soybeans and peanuts are classified as oilseeds because of their higher content of fat (Singh 1997). The total production of India in 2018 was 24.51 million tonnes and require import to meet the domestic consumption of 29.92 million tonnes (Anonymous 2018a, b).

In Asia a broad characterization of the current situation has following observation:

- India is the largest consumer, and also has moved from an import to in an import/export regime.
- Second largest consumer is China, and has moved to a two-way trade regime. (Ortega et al. 2011)
- Myanmar and Australia, characterized by inter seasonality of supply, moved to export regimes.
- Thailand moved from an export regime to a two-way trade regime.
- There are signs that pulses moved from negative income elasticity to positive income elasticity in India and Pakistan (Deaton and Drèze 2009).

Oil Seeds

Oilseed crops are an important and economically grown world widely for extraction of edibles oils. In Europe, oilseed crops are being attacked by six bugs that frequently require control by cultivators to ensure seed yield, are: the cabbage stem bug creepy crawly, dust bug, cabbage seed weevil, cabbage stem weevil and brassica case midge. These damage the product progressively at different development stages and harm diverse parts of the plant. They are on the whole far reaching yet

their relative significance differs with nation and year. Push-pull (attractant-repellent) systems are being produced that utilization have plant inclinations and conduct reactions to semiochemicals to impact bug and regular adversary appropriations on the product (Verma 2000). There is likewise potential for common pest protection through change of inside field trim farming practices just as, on the scene scale, through territory and ecological control to support vegetational decent variety of the agro-ecosystem consolidating hedgerows, cover crops, blossoming preservation headlands and field edges to give shelter, sustenance, overwintering locales and elective prey or has for characteristic enemies. Now a day's demand of oilseeds is increased because of their health benefits such as healthy vegetable oils, pharmaceutical properties, livestock feeds, biofuels and other oleo chemical industrial uses. In last 30 years according to increasing interest of oilseed, there is an 82% expansion of oilseed crop in cultivation areas and around a 240% increment in total world production (Deininger and Byerlee 2011). Fulfilling the increasing demand of oilseeds over the world, sustainable oil production, through the classic breeding with biotechnological approach expands oil yield per unit area. Genetic engineering of oilseeds improves sustainable production of crops and also improves nutritional quality as well as enhancement of quality for industrial purpose. High grade yielding edible oil producing oilseed crops are sunflower, soybean, safflower, groundnut, sesame, castor and linseed are included in non-edible oil-producing species (Murphy 2007). For limit of infestation, insect pest management and possible control techniques are important for quality and quantity of the products. Varieties of pest attack oilseeds and significant losses of farms and storage products. These insect pests show significant effect on the economics of oilseed production. Pest Management is done by different types of preventive methods such as cultural control, physical control, biological control; host plant resistance and chemical control.

Storage Pests of Pulses and Oilseeds

Pulses are annual and seasonal crops which are stored for several months hence it has higher risk of damage due to insect pests which can cause post-harvest losses up to 25–50%. These losses occur due to insufficient and poor storage facilities, lack of proper knowledge of advanced technology in post-harvest pulse management and harsh climate in developing country like India. During storage, pulse beetles; *Callosobruchus chinensis* Linn and *Callosobruchus maculatus* Fab that causes about 5–10% loss of pulses during storage (Ngamo et al. 2007). Infestation of this pest leads to the loss of germinative ability and nutritive value of the seed (Sharma 1984). *C. chinensis* is the most primary and destructive pest found in India. Infestation of the pulses starts from the field and continues up to storage. The month July to October has recorded to have maximum damages of stored products. Pulse beetle not only causes qualitative but also nutritive loss that makes the pulse grains unfit for marketing and also less nutritious for human consumption.

Callosobruchus chinensis Linnaeus

It is commonly known as Chinese bruchid, Adzuki bean weevil, and pulse beetle found in world wide. *C. chinensis* has major pest in chick-peas (Pandey and Singh 1997), lentils, green gram, broad beans, soybean (Srinivasacharyulu and Yadav 1997).

Life Cycle

C. chinensis adults are about 2.0–3.5 mm long. The elytra are red brown with yellow marking, antennae and yellow legs. The antennae are serrate in female and pectinate in the male. Larva is about 5 mm long yellowish-white with brown head and reduced legs. This pest is raised annually seven to eight generations. Female lays about 50–100 eggs on smooth legumes pods, and larva immediately enter into the pods to feed. Several larvae occur within the same seed. Several weeks are required for full developments (Mulatu and Gebremedhin 2000).

Prevention and Control

- Intercropping with cereals and before pest attack, early harvest of the legumes. They should be stored in hygienic condition.
- Seeds are heated at 50 °C for 1 h which kills the eggs and larvae.
- Some legumes varieties with thick and hairy walls are resistant to beetle infestation
- Many plants extracts and oils are used for prevention of oviposition deterrents and for beetle control such as Organophosphates and neem compounds
- Certain parasitoids of the families Braconidae and Pteromalidae are used to attack
- *C. chinensis* in various parts of the world. The mite *Pyemotes* is also used as a parasite of the pest (Mulatu and Gebremedhin 2000).

Callosobruchus maculatus (Fabricius)

Callosobruchus maculatus is commonly known as cowpea weevil and important pest of pulses throughout the world. It attacks leguminous grains, such as cowpeas, black gram, green gram, and lentils (Raja et al. 2000). Infestations of this pest starts in the field and continue to the storage, sometimes it can cause the total destruction of the seeds even within a period of 3–4 months (Barde et al. 2013).

C. maculatus adult body is 3 mm to 4.5 mm length, reddish brown, with block spots on the elytra and prothorax. The antennae of both male and female are slightly serrate. The last segment of abdomen extends out from under the short elytra, on black spots. The males are sometime shorter and lighter than females. It has two forms, one is flying form and other is flightless form (Raja et al. 2000).

Life Cycle

The female lays up to 200 eggs on the seed coat in the field and storage. The larvae burrowed inside the seed where the development is complete for the expense of grain endosperm and embryo, that's are responsible for cowpea damage. Larvae cannot move among seeds they are restricted to a particular seed that is chosen by their mother for them. It requires 14 °C for threshold development and 435 days are required for generation completion. It takes 4–5 week for completion of life cycle and overlapping six to seven annual generations. These beetles mostly live for 1–2 weeks. In India, the insects mostly breed from March to November and hibernate in the larvae stage in winters. Maximum damages were estimated during February to August due overall developmental stages that exist simultaneously. Infestation level in storage is influenced by the type of storage structure employed and seed variety (Ojimelukwe et al. 1999). Moisture content of seeds and temperature of storage influence the infestation level in local stores (Singh 1997).

Prevention

- Hygienic stores play an important role for limiting the infestation by these insect species.
- Harvesting crops early and intercropping maize with cowpeas is also effective.
- Storage area of freezing for 6–24 h at −18 °C will kill both adults and larvae
- Solarization (sun drying and heating) is also useful for control from infestations without affecting seed germination (Mohemed and Ismail 1996).
- Prevention from infestation through insect legume pods with hairy and thick walls is very effective.
- Several plant extracts such as neem, together called botanical biological pest control agents that are used against the pest control.
- Various *Hymenopterous parasitoids* include *Anisopteromalus calandrae*, *Uscana mukerjii* and *Dinarmus* spp. specifically targeted to *Callosobruchus* species.
- *Dinarmusbasalis* attacks small larvae and also limits their damage, even their presence still makes the beans unfit for sowing and human consumption. *Uscana mukerjii* is used as an egg parasite which prevents egg hatching.

Indian Meal Moth: *Plodia interpunctella*

Host range: Maize, cereals, dry fruits, groundnuts and cereal products and milled products.

The Indian meal moth received its title from the US where it was reported to feed on meal made of "Indian corn". Adults 12.7 mm long with wing span of 16–20 mm. Distinctive bicoloured wings—dark reddish brown with a copper cluster on rear half of the wing and whitish grey on the inner body ends. Larvae have four pairs of legs, in which three pairs are true legs and fourth one is abdominal leg.

Life Cycle

Each female moth lays up to 400 eggs singly or in groups on food stuff. Hatched larva is small about half an inch long and varying in colour dirty white, greenish and pinkish. It creates webbing as they feed on the grain podium, dried fruits and nuts. The larva spins a silken cocoon and transforms into a light-brown pupa, from which the adult emerges later. At 18–35 °C, the Indian meal moth may complete all stages in 6–8 weeks. Mechanism of action—firstly larvae enter into kernel and releases massive amounts of silk over the grain surface. Webbing which happens by moth disrupt the air movement, which can cause grain heating leads to mould growth. It decreases the fumigation effectiveness (Fasulo and Knox 2015).

Cigarette Beetle (Tobacco Beetle) *Lasioderma serricorne* (Fab)

Family: Anobiidae; Coleoptera.

Hosts: Cigarettes of tobacco, Cocoa beans, groundnut, peas, cottonseed and beans, many stored grains, flours and foodstuffs (alternative). Adult Cigarette beetle is small, stout, oval, reddish-yellow or brownish red beetle of about 2.5 mm length. Head is projected downwards with concealed antennae that appears hump. Beetle founds in subtropical regions, infesting the tobacco and some others storage grains (Bhargava et al. 2007).

Life Cycle

A single female can lay about 100 eggs in folds and crevices of food material. Eggs are elliptical, ovoid, whitish that become opaque and dull in colour just before hatching. It occurs in 5–6 days. Larval stages last for 25 days followed by 5–7 days of pupal periods. Newly develop larvae is about 1 and less mm in length and cover with fine hairs and antenna is serrate. Larval head is yellowish with a semi-transparent and whitish body. After larval period, it constructs a smooth lined cell in which it pupates. Newly pupa formed is glossy white in appearance and later changes to reddish brown colour. Males are slightly shorter than female. It mostly prefers higher temperature and attacks the wide range of food stuffs. It can be controlled by temperature and humidity below 19 °C and 30% RH ceases development (Srivastava and Subramanian 2016).

Khapra Beetle (*Trogoderma granarium*)

It is a significant storage pest of wheat and groundnut (main), jowar, rice, maize, sorghum, oilseeds and pulses. The Khapra name of this beetle has given of its habit of congregation in cracks and crevices of bricks, masonry and wood storage. This beetle found in hot and dry and tropical/subtropical regions. It prefers low humidity

and high temperature. Adult Khapra beetle has oval in shape and grey and pale brown markings. Head is essentially hidden beneath hood like pronotum.

Life Cycle

Adult female lays about 100–120 eggs on grain surface or crevices. Growth and development of beetle depends upon the temperature and humidity and requires 4–6 days incubation period at 35 °C (Bhargava et al. 2007). Larvae have brownish white colour, body cover with bundle of long, brownish red movable and erectile hair on the posterior segments and form a sort of tail in the posterior end. In the first stage larvae feeds on broken grains and debris resulting from the feeding of old larvae as its do not attack the whole grains. Developmental period from egg to adult is ranged 39–45 days at 30 °C and maximum 220 days at 21 °C (EPPO 2013; Athanassiou et al. 2019). These are highly resistant to starvation. Larva have ability to survive without food for few years, under abnormal condition (Setyaningrum 2015). This beetle damaged almost all parts of the grains, but prefer mostly germ position and also the viability of seed is lost long before any quantity damages occurs. Larva is a destructive stage because adult doesn't feed and its life is very short.

Almond Moth *Cadraicautella* (Lepidoptera: Phycitidae)

It is also known as fig moth, widely spread in the tropics and subtropics. It mainly attacks the figs, rough rice, dry fruits, wheat, barley, sorghum, soybean, and oilseeds etc.

Life Cycle

Adult almond moth has a dark band on their forewings and its length is three fourth of the size of rice moth (*C. cephalonia*) and has greyish body. Adult females lay about 200–250 eggs per individual, randomly scattered in stores, in cracks, on grains or another surfaces. Eggs are less than 1 mm, and hatch within 3–4 days (Jacob 2012). The young larvae spin silk profusely and at maturity these form small silken tubes among in the grain in which they remain lodged and grow. Larval stage known is the damaging stage. Fully developed larvae are white with pinkish shade. Its colour and habit of spinning tubes in food material are most prominent diagnostic characters. About 7–10 days is pupal period. Generally, moths are more abundant during rainy and humid seasons.

Miscellaneous Invertebrates

Rodents are important pests of storage found all over the world, there are 1700 species of rodents, although 5–10% of species are major pest that infest the stored grain. These consume substantial amounts of stored grains. In developing countries farmers consider rodents as the main impediment to higher yields (Makundi and Massawe 2011). Every year in Asia, rats consume about 200 million people's feed consumed for an entire year (Singleton et al. 2005). In South America, native rodents damage the crops varying between 5 and 90% of total production. During storage it is necessary to control the insect pest infestation in grains, pest included birds and rodents that contaminate the stored grains. Rodents damage both stored grains and storage structure, their droppings, hairs and urines are main factor for contamination of the grains. The main species of rodents which causing damage are the house mouse, (*Mus musculus*) (Meehan 1984), the brown rat (*Rattus norvegicus*) (Niethammer 1981) and the Pacific rat (*Rattus exulans*) (Poché 1980) and the brown rat (*Rattus norvegicus*) (Niethammer 1981). Weight loss was observed 10.3% in the dry season and 7.4% in the wet season (Brown et al. 2013).

Storage Types

Chomchalow (2003) identified the storage of products on three levels which are as follows:

Farmer Storage

On this level large proportion of harvested food grains are retained by traditional storage methods and use botanicals with other inert materials for prevention of pest and insect infestation. Farmers generally store the food stuff at the site of harvest. This includes underground cellars, straw bins, cement bins, wooden bins, mud bins, bamboo bins, etc.

Community Storage

In Thailand many Rice Banks and Seed Banks are established for storage of food stuff at community level. These are owned by farmer groups or farmer cooperatives. Storage is done in bulk or in bags with certain fumigation facilities.

Commercial Storage

Commercial storage covers millers, exporters, middlemen and industrial manufacturers. Carbon dioxide fumigation is used in large warehouses which is effective for prevention of insect and pest control as compared to conventional methods.

Management

Preventive Methods

Measures are adopted before the infestation to prevent the produce from storage pests; rodents, insects and microbes. Among these methods; cleaning and sanitation, selection of storage structure, rodent proofing includes doors, filling of holes and openings, and foundations and floors, vents and windows are important (Brown et al. 2006)

Cleanliness

- Keep the farms and storage area clean as possible
- Do not pile food or trash inside and outside of the storage building.
- Destroy safely all garbage and old food, at a distance from the storage area.
- Store the all food grains in covered containers
- Sweep out all dust, dirt, spilled food, straw, old cloth that rodents nest and hide in and destroy it immediately.
- If possible, to cover mud floors in storage area with a thin layer of mortar. This keeps rats from digging up through the floors.
- All around the storage area keep the grass cut in shorts due to rodent hide in tall grasses.
- Make sure the storage building have rodent proof. This means that the farmer has to construct the store in such a way where rodent cannot enter in the store.
- Drying platforms—The products should dry before storage should be placed at least 80 cm above the ground from the protection of rat jumps. And also use of barrier around the poles that's the rodent does not climb the poles. These types of barrier called rat baffles or rat guards that made of metal sheet or empty tins.
- Greater than ¼-in. in diameter of holes are allowed for rodent entry into the structures. To seal such types of opening with use of cement and metal collars. Greater than ¼-in. holes in diameter that's set aside entry of mouse into structures.
- At storage area make sure doors and grain outlets close tightly. A wooden door has a thick metal sheet at the bottom that's to stop rodents from eating through.
- Using heavy wire netting with an 8 mm mesh is a good size for cover of all windows and large openings.

Traps Setting

For catching of rodents' regular traps are very effective method and safer than poison. Generally, poisons are toxic for human beings and domestic animals; it could be transferred by the rodents to the stored products. So, traps are very effective if correctly used and placed (Brown et al. 2006).

Rodenticides

Rodenticides are poisons that kill rodents and rodents are mammals so it is also very poisonous to others mammals such as human beings, domestic animals and to wild animals. It is categorised into anticoagulants and non-anticoagulants (Tobin and Fall 2004) (Table 2).

- **Anticoagulant Poisons**—These rodenticides have properties to destroy the blood clotting of poisoned animals and after the use of this animals die from internal bleeding. The anticoagulant is relatively slow-acting, lethal dosing of its animal death after 3–5 days.
- **Non-anticoagulant Poisons**—It's also called single dose poisons. They act quickly and decrease rodent populations and also control anticoagulant-resistant populations.

Another disadvantage of rodenticides is that they are costly and not always in stock.

Prevention

1. **Sanitation**

   Infestation of grain mostly begins from the field. Mostly harmful pests are present in farm storage bin. For reducing the infestation of these insects, an effective sanitation is necessary.

Table 2 Some examples of oils used for storage protection (Campolo et al. 2018)

Storage product	Type of oil	Amount of oil	Effect
Cowpea	Peanut oil	5 ml/kg	Peanut oil protects the cowpeas against the Cowpea weevil infestation for about 6 months
Mung beans	Cotton seed oil	6 ml/kg	After 6 months only 3.5% of the seeds were damaged.
Mung beans	Rice husk oil	5 ml/kg	Prevents against damage for about 4 months
Cowpea	Maize Denettia oil	1 ml/kg	Denettia oil protects cowpea against Cowpea weevil for more than 3 months. Maize is also protected for a period of 3 months, even when only 2/3 of the given amount of oil is added
Beans	Neem oil	2–3 ml/kg	If well mixed this amount of neem oil protects the beans for about 6 months. Neem oil has an insecticidal effect as well

Equipment—It includes trucks, augers and grain driers. These should be cleaned from all old grains.

(a) **Empty bins**

The best strategy to protect stored grains from insect damage is to properly sanitize the bin before introduction of new grains to minimize the need of pesticides. Primary sanitization of bin involves the removal of old grains and dust from the corner, floor and walls. When infested grains present in the bin contaminate new grains so it is important to sanitize. After cleaning, attention should be given to repair all cracks, areas around doorways and other places where insect can hide and enter (Boxall et al. 1997). It is important that screening is done to eliminate broken kernels. After transferring the grains in clean bins, it should be checked regularly at 2-week interval during warm months and at 1-month interval in cold months for the presence of hotspots, mouldy areas, and live insects. If these conditions exist, the grain should be aerated to lower temperature and moisture levels. For grains that are stored for a long period of time it is important to use an approved insecticide. If infestation occurs, fumigation is necessary. Fumigation is toxic, so qualified pesticide personnel is necessary to perform it.

(b) **Near the bin**

It is important to remember that the surrounding of the bins always be clean. And always remember to take out and demolish all spill grains Control the weeds and grasses, since they harbour insects, rodents and pests. Carefully check the outside walls, the base and roof for damage that allow the pests and moisture to enter. Keep away stock feeders as far as possible from the bins.

(c) **The grain**

Store only clean and dry grain in the bins. The optimum moisture content of stored grain should be 12 and 13%. Inappropriate moisture levels pose a problem for insect infestation damage. Most grain inhibiting insects require 13–15% moisture for optimum metabolic activities and reproduction (Rajendran 2005). Optimum level of the grain should be filled in the bin that allows good air flow and maintain moisture and temperature. It also allows for proper inspection and treatment.

(d) **Temperature**

There are number of factors that affect the quality and quantity of stored grains such as temperature, time, and type of dryer. Ranging from 20 °C to 40 °C temperatures, the growth of insects is accelerated and above 42 °C and temperatures below 14 °C diminishes the reproduction and growth, although use of prolonged high temperature above 45 °C and temperatures below 10 °C could kill the insects. Heating the grains at 50 °C will be lethal but is not allowed, since the grains are affected and also lose their viability.

For example, grains kept at −5 °C for 12 weeks will control insect & pests at all life stages.

The temperature of the stored grains can be lowered by:

Mixing and transferring the infested grain from one bin or pile to other.

Transferring part of the crop to a truck and exposing a small pile of the grains to low temperature air and leaving it to cool before returning it to the bin.

Aerating the bin

Aeration systems are effective for reducing grain temperatures, as well as to reduce moisture migration.

2. **Pneumatic conveyer**

Cyclone-based grain pneumatic conveyers are also called pneumatic grain auger or grain vacs) which can be used for controlling insect infestations. Insects are killed by abrasive contact and impact as the grain and insects are moved through the discharge tube. Better control is achieved when there is a 90° bend in the tube which leads to greater abrasion of insects with the sidewalls of the tube (Gouda 2016).

Protection (Chemical)

Contact insecticides and fumigation are included in the chemical compounds that are used for protection from insect infestation in food grains approved by FAD/WHO (Bond 2007).The insecticides are used for the purposes which require prescribed dilution rates due to its low mammalian toxicity, and also using it at a non-hazardous level. Applying of this is comparatively safer than handling other pesticides that are approved and commonly applied for pre-harvest pest control.

Several factors important in assuring successful fumigation are

- For evenly fumigation, grains must be levelled in the bin
- Caking and crusting surfaces should be broken up and removed
- For proper vaporization, should maintain grain temperature 60 °F or higher (Tables 3, 4, and 5)

1. Insecticide Treatments

Commercial facilities should be observed with the Occupational Safety and Health Administration (OSHA) bin entry permits. For treating of empty bins following pesticides are available (Table 6)

Various insecticides are available for treating of cracks and crevice in empty bins or buildings and also uses other storage containers:

(a) Malathion

It is known as organophosphates is a pesticide used for control of insects on agricultural crops, and stored products. Since 1950, Malathion has been manufactured in the United States and used to kill many insects on many types of crops. The Food and Drug Administration (FDA) and the EPA permit a maximum amount of 8 parts per million (ppm) of Malathion residue to be present on specific crops used as foods (Matsumura 2012). Malathion has two forms: a pure form which is a colourless liquid and a technical-grade solution which is a brownish-yellow liquid, that contains Malathion (greater

Table 3 Recommended insecticide application rates (Tang et al. 2005)

Insecticide	Dust admixture with cereals (ppm)	Surface treatments (g/m^2)	
		Walls	Bags
Malathion	8–12	1–2	1–2
Perimorfs methyl	4–10	0.5	0.5
Fenitrothion	4–12	0.5	0.5–1
Chlorpyrifos methyl	4–10	0.5–1	0.5–1
Dichlorvos	2–20	0.5	
Methacrifos	5–15	0.2	0.4
Lindane	0.5		
Pyrethrin/piperonylbutoxide (1:5)	3	0.1	
Bioresmethrin (resmethrin)	2		
Phenothrin	5		
Permethrin	0.05–0.1	0.05–0.1	
Carbaryl	5–10	1–2	
Bendiocarb	0.1–0.2	–	
Dioxacarb	0.4–0.8	–	
Propoxur	–	0.5	–

Table 4 Pesticides available for treating empty bins (Arthur and Subramanyam 2012)

Insecticides labelled for use as empty bin treatments		
Active ingredient	Example brands	Comments/usage
Cyfluthrin	Tempo Sc Ultra Premise Spray®	Most effective residual as compared with malathion and chloripyrifos-methyl
Diatomaceous earth (DE)	Insecto, Protect-it®	Excellent empty bin treatment. Special grade required for grain use. Must use DE labelled for grain
Malathion	Malathion	No longer recommended for empty grain bins because of high insect resistance and rapid degradation in warm, relatively moist grain
Chlorpyrifos-methyl deltamethrin	Storcide II®	Can only be applied from outside of bin and sprayed downward into bin

than 90%) and some impurities in the solvent. The technical-grade Malathion smells like garlic. It is toxic for mammals, so should be applied by trained person (Tulloch 1972). It is important for:

- Incapacitation of insect's central nervous system (CNS).
- For best result. Used in the form of dust and liquid spray with the help of conveyer.
- Best applied as a liquid spray or dust as grain flows through an auger or conveyer.
- In oilseeds it is not recommended but formulation of malathion is registered for direct cereal grain treatment.

Table 5 Dust insecticides labelled for use as grain protectants (Nikpay 2006)

Active ingredient	Example brands	Comments/usage
Malathion	Big 6 Grain Protector®, Agri solutions 6% Malathion Grain Dust	Top-dress treatment. Insects are resistant in many areas. Millers resist purchasing grain with strong Malathion odour
Diatomaceous earth (DE)	Protect-It™, Insecto®	Can lower the test weight of grain and is expensive if it is applied to entire grain mass, so is best applied to empty bins and to the top and bottom layers of the grain mass

Table 6 Labelled insecticides for use as empty bin treatments (Matsumura 2012)

Active ingredient (A.I.)	Example brands	Comments/usage
Cyfluthrin	Tempo Sc Ultra Premise Spray®	Most effective residual as compared with malathion and chloripyrifos-methyl
Diatomaceous earth (DE)	Insecto, Protect-it®	Excellent empty bin treatment. Special grade required for grain use. Must use DE labelled for grain
Malathion	Malathion	No longer recommended for empty grain bins because of high insect resistance and rapid degradation in warm, relatively moist grain
Chlorpyrifos-methyl + deltamethrin	Storcide II®	Can only be applied from outside of bin and sprayed downward into bin.
Chloropicrin	Chlor-o-pic®	Empty bin fumigant, under false floor, aeration tubes, and tunnels
Methyl bromide	Brom-o-gas®, others	Empty bin fumigant; seldom used
Phosphine	Phostoxin®, others	Empty bin fumigant

(b) Diatomaceous earth

Diatomaceous earth has been used as a protector of grain. DE is low in mammalian toxicity and provides superior protection when food stuff is stored properly. DE is registered as a feed additive, in the USA and Canada. Amorphous silicon dioxide is considered Generally Recognized as Safe (GRAS), and registered as food additive in the USA and Canada (Korunic 1998). DE is useful to provide a preventive and structural treatment before grain storage and it is left behind as a residue on freshly harvested grain as it goes into storage. DE is ideal to use due to the long lasting protection. In addition to Australia, its registered as a protectant of grain or for structural treatment in Canada, Croatia, China, USA, Germany, and some other Asian countries.

- Mechanism of action of diatomaceous earth causing death through desiccation by absorbing wax which is coated on the skin of insect.
- During harvesting it is most effective when directly apply with dry grain into a bin.
- Treatment temperature should not be less than 20 °C for up to 6-week period
- It increases in grain friction and decreases auger flow rates by reducing test weight.

(c) Aluminium Phosphide (Phosphine) (Weaver and Petroff 2005)

- It is put in cereal grain as in the form of pellets with the help of probe.
- For the effective treatment completely sealed structures should be used.
- Aluminium phosphide reacts with water in the air to produce the gas phosphine.
- Moisture range is not more than 10 °C and temperature at which fumigation takes place for effective treatment should not be more than 50 °C.
- Doses should be 20 times higher than normal doses for controlling egg stage because it is more resistant than adult and larvae stage
- Disadvantage of phosphine—At high humidity and temperatures it causes corrosion of some metal e.g. gold, silver, brass, copper
- Prolonged and inappropriate use of phosphine in Australia and U.S.A has resulted in resistance development.

(d) Magnesium Phosphide (Phosphine) (White and Leesch 1995)

- As like aluminium phosphide, magnesium phosphide acts with water in the air to generate the gas phosphine.
- Magnesium phosphide produces phosphine that is faster than aluminium phosphide.
- Magnesium phosphide could not contact with the fumigated commodities.
- Magnesium phosphide is used as a plates, pouches or strips.
- It is mostly used for fumigation in empty structures.
- As like aluminium phosphide it's similar as humidity, temperature, and corrosion of metals.
- To make treatment effective with Magnesium phosphide, storage structure must be well sealed.

(e) Gaseous Phosphine

- Gaseous phosphine is fast in fumigation as compare to metal phosphides and is more precise dosage of phosphine
- Fumigation at minimum temperature of 0 °C, compared to metal phosphides requires a minimum temperature of 5 °C.
- Gaseous phosphine, similarly uses as aluminium phosphide

- Two formulations are used: ECO2FUME® is ready for application, VAPORPH3OS® its needs onsite dilution.

(f) Carbon Dioxide

- As a gas carbon dioxide apply to the stored products.
- Treatment will be effective when structure must be sealed.
- Doses: Maintain up to 60% for 4 days between 20 °C and 25 °C (White and Leesch 1995)

Inspection

Grain bin inspection gives important information of the general condition, moisture temperature, and pest activity of stored food stuff. It is also important for early detection of problems that's helpful for correct action before any severe damages. The commercially available "probe" traps should be safe and easy method for grab of various beetles they infest the bin (Phillips and Throne 2010).

The traps are hollow "plastic" tube along a string of downward sloping hole along the side. The top has flat cap and the bottom has pointed piece that screws in place. The insects crawl into the tube through the small holes where they are trapped. A nylon line is securely attached for easily rescue from the stored grain. Trap should be inserted in the grains via a long pole with a cup device attached at the end. This device is easily prepared with handle of paint roller extension and various "PVC" plumbing fixtures. Attached extension handle is a PVC "reducer" that one side of the screw and other side is a cup device (Levy and Carley 1989). This device will allow push the trap into the stored grain from internal ladder, an inspection hatch, or some other safe place, therefore avoid crossing the grain surface. The trap is reclaiming using a nylon line which was close to the trap before it was placed in the grain, and it is easily tied off some convenient location in the bin. It keeps the traps from being easily sucked into the stream of grain in case they are elapsed at unloading time.

Conclusion

Insect infestation of cereals, dried seeds, pulses causes quality deterioration in the stored products kept in humid and warm climates. Stored food products infestation caused by microorganisms (mainly fungi and bacteria), bruchids, weevils, mites, rodents and birds and other insects considerable causes physical and nutritional loss. It reduces the nutritional value, quality of the grain, grain weight, and germination of stored grain by Direct-feeding of the insect. It's unhealthy for consumption of animals or humans. Commercial buyers might be paying a low price because of insect contaminated grain. A good management program is required to be maintained for proper grain-handling, regular grain inspections and pest control.

Various procedures are used for the management of pests at storage facilities before and after storage, that diminishes pest attack into storage such as: Cleaning the bins, equipment used for harvest, sealing structures, remove the weeds, cleaning up grain spill on the grounds, insecticides used in empty-bin. About 5–10% is the estimated loss in stored product caused by insects worldwide.

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Recent Studies on Healthy Nutrients Changing in Fruit Juices Processed with Non-thermal Technologies

Erdal Ağçam, Burcu Dündar, Süleyman Polat, and Asiye Akyildiz

Abstract Fruit juices are the most preferred beverage around the world due to their high content of healthy nutrients and being source of antioxidants, such as vitamins, phenolic and carotenoid compounds. Fruit juices contain some vitamins, phenolic and carotenoid compounds having important antioxidant function that scavenge free radicals damaging cells with reacting structural molecules and reduce cardiovascular diseases. Therefore, they are unique for growth, maintenance and well-being of human life. Nowadays consumer demands have tendency around both safe to consume and minimal processed foods. Therefore, food processing industry has made an effort in order to improve processing technologies having potential to fulfill these consumer demands in final product. In the last decades, promising non-thermal food processing technologies, such as pulsed electric fields (PEF), high pressure processing (HPP), ultrasound processing (UP) and ultraviolet light processing (UVLP), have been alternatively developed to the traditional thermal pasteurization for extending shelf life and minimizing loss of healthy nutrients of fruit juices. In the present book chapter, effect of non-thermal technologies (PEF, HPP, UP and UVLP) on fruit juices, health related compounds (vitamins, phenolic and carotenoid compounds) were evaluated and discussed from the perspective of recent published research studies in the literature.

Keywords Non-thermal processing technologies · Fruit juices · Carotenoids · Vitamins · Phenolics

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Introduction

Consumer concerns about health issues resulted in changing of consumption demands and they began to increase their consciousness towards the foods containing high amount of antioxidants, minimally processed and without containing processing-induced detrimental substances. Fruit based products consumption is a marker of higher-quality diets and they must be taken daily to support healthy life. The consumption of high quality fruit juices, together with whole fresh fruit, is one of the alternative ways to fulfill daily fruit consumption goals (Francou et al. 2015). Therefore, fruit juices are among the most widely consumed ready to drink beverages due to important sources of health supported nutrients such as, phenolics, carotenoids, fibers, vitamins and minerals. A significant portion of those healthy compounds like phenolic acids, flavonoids, carotenoids, vitamin C, vitamin A and tocopherol shows antioxidant activity. Epidemiological studies indicated that antioxidant compounds are able to reduce risk of chronic diseases such as obesity, cardiovascular, cancer, inflammatory activities, diabetes, viral infection, stroke, Alzheimer's and oxidative stress-induced other malignancies (Akhtar et al. 2017; Netzel et al. 2007; Sevimli-Gur et al. 2013; Stinco et al. 2019; Temple 2000; Willett 2002). Similarly, in a research study, it was indicated that diet exposure with balanced fruit juice consumption (not exceed eight glasses/week) was associated with a lower risk of cardiovascular diseases and stroke (Scheffers et al. 2019).

Non-thermal technologies, defined as an alternative processing techniques to thermal pasteurization and sterilization, can be successfully used for both producing foods safe to consume and improving shelf-life of the foods by inactivating enzymes, spoilage and pathogenic microorganisms. Although achievement of traditional thermal processing is satisfied in extending shelf-life of fruit juices due to high inactivation of resistant enzymes, spores and microorganisms, it causes dramatic change in phenolic and carotenoid compounds, vitamins, taste and color of juices as well as increasing in undesirable substances such as furfural, hydroxymethylfurfural, furan and acrylamide. However, non-thermal processing technologies such as high pressure processing (HPP), pulsed electric fields (PEF), ultrasound processing (UP) and ultraviolet light processing (UVLP) are able to both adequately inactivate fruit juice enzymes and microorganisms and remarkably save health related nutrients and original flavor attributes of juices without or lower processing-induced detrimental substances (Agcam et al. 2014a, b, 2016; Al-juhaimi et al. 2018; Balasubramaniam et al. 2015; Dhakal et al. 2017; Dundar et al. 2019).

High pressure processing (HPP), an innovative non-thermal technology, preserves food products by using of pressures in the range of 100–800 MPa, with or without heat treatment assistance, for inactivating a variety of pathogenic and spoilage vegetative bacteria, yeasts, molds, viruses, enzymes and spores to ensure microbiologically safe foods. Those hydrostatic pressure levels have little effect on covalent bonds of the molecules existing in foods, and therefore, small molecules such as vitamins, phenolics, carotenoids, pigments and flavors do not undergo significant chemical transformation. The basic HPP system consists of high-pressure vessel, two end closures to cover the pressure vessel, headlock to close the vessel

off, pressure generation pump and intensifier to reach target pressures, a system for pressure and temperature control, material handling system to load and remove the products, and finally control/monitoring system. For food processing industry, high pressure pasteurization equipment is available in both horizontal and vertical configurations having an operation capacity between 35 and 525 L. Although HPP can generally operate with batch regime, the systems as semi-batch regime are also available. When high pressure is started to transmit through the vessel, at the same time, it is transmitted to the food product equally from all sides. Therefore, during high pressure exposure, the food products are not crushed owing to this equal pressure distribution (Rastogi 2010; Balasubramaniam et al. 2015, 2016).

Pulsed electric fields (PEF) processing, which is a promising alternative to classical thermal preservation processes for liquid foods like fruit juices containing heat sensitive volatile or bioactive compounds, has capacity to inactivate microbial cells combined with low to moderate temperatures (<50 °C). In PEF treatments, food matrix is passed between two electrodes and exposed to an electrical field in the form of very short (a few μs), high-voltage (kV) pulses. The electric field strength, which is created between the couple of electrodes, can be calculated by dividing the applied voltage by the distance between the electrodes (Buckow et al. 2013). An example of PEF treatment system used for orange juice pasteurization was shown in Fig. 1. The exposure of food matrix to an electric field of moderate intensity (0.5–10 kV/cm) and relatively low energy (1–10 kJ/kg), implemented in the form of recurrent very short voltage pulses (μs or ms), induces a permeability of cell membranes that makes possible the release of juice and valuable components from the internal parts of the cells (Bobinaitė et al. 2015). Fluid or pumpable foods as fruit juices, milk, smoothies, yogurt, sauces, wine and soup-based products can be pasteurized with PEF technology instead of traditional thermal techniques. These food matrixes comprise large quantity of water and dipolar molecules making them more conductive for transition of electrical currents compared to solid foods. The PEF system unloads a high voltage pulse uniformly throughout the food in a treatment

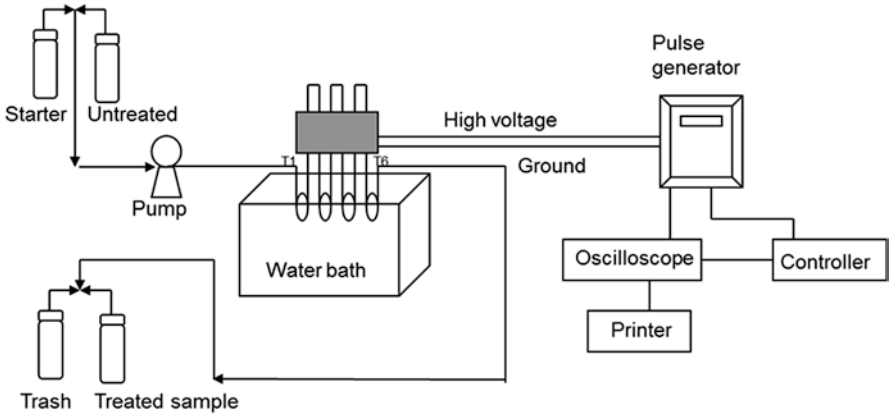

Fig. 1 Schematic diagram of pulsed electric fields (PEF) system (Agcam et al. 2014a)

chamber (Barba et al. 2017; Tiwari and Cummins 2013; Ricci et al. 2018; Toepfl et al. 2006; Ganessingh et al. 2018). Additionally, PEF treatment improves the extraction ratio of bioactive compounds like anthocyanins, carotenoids, betanines and polyphenols from foods and assists shorten extraction time and decreases solvent expenses and/or lower extraction temperatures (Puértolas et al. 2013; Boussetta et al. 2012). The main mechanism of PEF is based on the exchange or disintegration of cells exposed to sufficiently large external electric fields, which increases permeability and electrical conductivity of the cellular material (Martín-Belloso et al. 2014). The mechanism of PEF can be explained using the "electroporation" model in which the strong electric fields generate either reversible or irreversible perforation (permanent or temporary pores) of the cytoplasmic membrane causing cell leak depending on electric field intensity level (Ricci et al. 2018; Ganessingh et al. 2018). This destructive effect of PEF results with microbial or enzymatic inactivation, thereby providing the products for consumers with microbiologically-safe, high-quality (better flavor, color, texture, and high nutritional value) and enhanced efficiency in juice extraction process.

In food technology, ultrasound processing (UP) treatments mainly can be divided in two groups based on their intensity level: low and high intensity ultrasound. While low-intensity applications are generated by using small amplitude waves (at high frequency, >1 MHz) with no damage on food material and generally for analytical measurements, high intensity applications (20–500 kHz) can cause changing in microbiological or chemical properties of food (Kentish and Feng 2014). While ultrasound is generated, electrical energy is transformed to mechanical vibration by a transducer. In the laboratories and food industry, ultrasound treatments are applied in sonication water bath (Fig. 2a) or with a probe (Fig. 2b), a titanium cylinder consists of a transducer. During processing, a part of electrical energy converts to heat. Hence, ultrasound processing equipment should contain cooling systems as it can be seen in Fig. 2. Ultrasound probe can transfer high amount of energy directly to the medium, but the energy decreases while distance from probe increases. On the other hand, although an ultrasonic bath has more than one transducer at the base part of the device, generally provide lower ultrasound energy intensity to treat food material. The generated ultrasound energy causes a phenomenon in liquid foods called as "cavitation". After formation of low and high pressure regions in the medium, very small bubbles appear at the low pressure points. These bubbles can coalesce with each other; however when they reach the biggest size (also called the critical size), they collapse violently ending with temperature and pressure increasing up to 2000–5000 K and 300–1200 bar (Suslick et al. 1999).

The increasing consumers' tendency towards products with similar characteristics to fresh ones has led researchers to develop alternative processing techniques. One of the non-thermal technologies applied as an alternative to thermal pasteurization is UV-C irradiation. The inactivation mechanism of UV-C irradiation is based on the absorption of UV photons by genetic material and consequently the replication of the cell and the formation of dimers which inhibit transcription. The antimicrobial effect of UV-C light is well known and this method is used in surface disinfection of hospitals, water resources, drinking water, fruit juices and different fruits (Unluturk 2012).

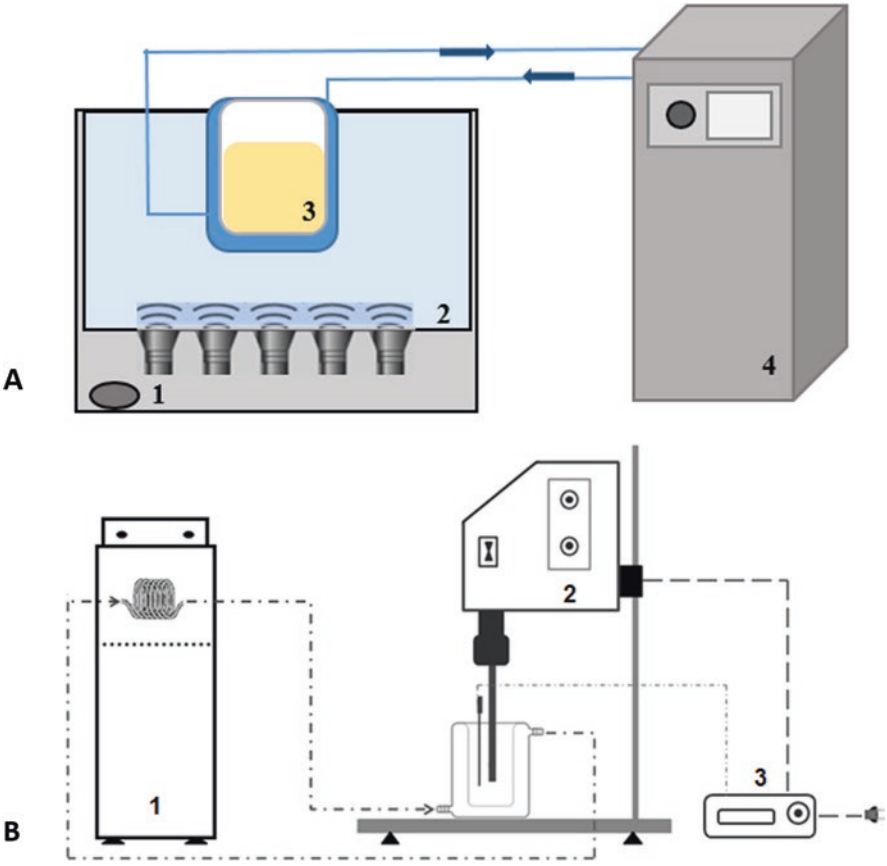

Fig. 2 Ultrasound applications with ultrasonic bath (**a**: (1) Ultrasonic bath with time controller, (2) water, (3) juice, (4) cooling water circulator for jacketed vessel) and probe (**b**: (1) cooler, (2) ultrasound equipment, (3) energy-and thermo-meter (Ağçam et al. 2017))

In the present chapter, effects of various non-thermal technologies that are the most promising for the juicing industry such as PEF, HPP, UP and UVLP on health related compounds like vitamins, phenolic and carotenoid compounds containing in fruit juices were evaluated and discussed from the perspective of recent published research studies in the literature.

Effect of High Pressure Processing (HPP) on Bioactive Compounds

The summary about bioactive compounds changing in different juice sources processed with HPP was given in Table 1. Fernández-Jalao et al. (2018) conducted a study in order to determine effect of HPP conditions on phenolic compounds of

Table 1 Short summary about bioactive compounds changing in different juice sources processed with high pressure processing (HPP)

Juice/ source	Bioactive compounds	Processing conditions	Highlights	Reference
Apple	– Ascorbic acid – Antioxidant activity	430 MPa, 7 min, room temperature	– The dramatic decrease determined in ascorbic acid during 34 days storage at 4 and 20 °C. – Antioxidant activity reduced significantly during storage periods.	Juarez-Enriquez et al. (2015)
Apple (Golden Delicious)	– Phenolic compounds – Antioxidant activity	400–600 MPa, 35 °C, 5 min	– The results showed that high pressure affected differently the phenolic compounds according to the origin of apples. – While higher antioxidant activity was determined with increasing processing pressure for Spanish-apples, lower antioxidant activity was determined for Italian-apples.	Fernández-Jalao et al. (2018)
Mango	– Ascorbic acid – Phenolic compounds – Carotenoid compounds – Antioxidant activity	400–550 MPa, 34 and 59 °C, 2–16 min	– Ascorbic acid degraded significantly at higher pressure, temperature and holding time. – Phenolic concentrations increased up to 34%. – Except violaxanthin, individual carotenoids remained unchanged. – Antioxidant activity showed increasing tendency up to 4 min processing time.	Camiro-Cabrera et al. (2017)
Strawberry	– Ascorbic acid – Anthocyanin contents	400–600 MPa, 20 °C, 1.5 or 3 min	– Ascorbic acid and anthocyanin contents were well preserved after HPP. – Thermally processed samples showed better storage stability than high pressure treated in terms of these bioactive compounds.	Aaby et al. (2018)

(continued)

Table 1 (continued)

Juice/ source	Bioactive compounds	Processing conditions	Highlights	Reference
Pineapple	– Phenolic and flavonoid content – Ascorbic acid – Antioxidant capacity	200–600 MPa, 30–70 °C, up to 20 min	– The combined high pressure-temperature conditions affected significantly bioactive components. – Phenolic contents increased up to 50 °C. – Flavonoid content was stable at the pressure levels, while decreasing after 50 °C processing temperature. – Processing temperature higher than 50 °C was responsible from ascorbic acid loss. – Dramatic decreasing was determined for antioxidant capacity of the samples processed at >45 °C	Chakraborty et al. (2015)
Pineapple	– Ascorbic acid	0.1–600 MPa, 30–95 °C, 0–15 min	– No ascorbic acid degradation was determined for processing conditions at 300–600 MPa-30 °C and up to 15 min. – Similar activation energy values for ascorbic acid degradation were calculated for both thermal and pressure-thermal treated samples.	Dhakal et al. (2018)
Orange	– Ascorbic acid – Phenolic compounds – Carotenoid compounds – Antioxidant activity	350–550 MPa, 41–68 °C, up to 10 min	– Higher ascorbic acid contents and antioxidant activity values were determined for most high pressure treated samples than untreated ones. – Phenolic compounds were not significantly affected by HPP. – Carotenoid contents of most samples processed at ≥56 °C decreased about 15% independent to applied pressure and time.	Escobedo-Avellaneda et al. (2015)

(continued)

Table 1 (continued)

Juice/ source	Bioactive compounds	Processing conditions	Highlights	Reference
Orange	– Phenolic compounds – Total phenolic, flavonoid and anthocyanin contents – Total carotenoid – Antioxidant activity	550 MPa,18 °C, 70 s	– HPP caused a significant increase in flavonoid content, while thermal pasteurization decreased anthocyanin ad flavonoid contents. – Both after processing and during storage, higher carotenoid contents were detected in HPP treated samples. – Most of bioactive compounds of HPP treated samples showed lower degradation rates than thermally treated samples during 36 days storage at 4 °C. – After treatments, HPP treated samples had higher antioxidant activity. And also, better antioxidant activity results were obtained for HPP treated samples.	Vieira et al. (2018)
Carrot	– Carotenoids – Phenolic content – Antioxidant capacity	550 MPa, 6 min	– Samples treated with HPP had higher bioactive compound concentrations than thermal treated. – Studied bioactive compound concentrations decrease significantly during 20 days storage at 4 °C.	Zhang et al. (2016)
Aronia berry	– Total phenolic – Total anthocyanin content – Antioxidant capacity	200–600 MPa, 21–33 °C, 2.5 and 5 min	– No significant decreasing was determined in bioactive compounds and antioxidant capacities of treated aronia purees. – During 8 weeks storage at 4 °C, no significant difference was obtained for total phenolic content and antioxidant capacity of the samples treated with 400 and 600 MPa-5 min, while a significant decreasing tendency was determined for total anthocyanin content.	Yuan et al. (2018a, b)

(continued)

Table 1 (continued)

Juice/ source	Bioactive compounds	Processing conditions	Highlights	Reference
Kiwi fruit	– Ascorbic acid – Total phenols – Chlorophyll	500 MPa, 25 °C, 10 min	– Ascorbic acid content of the HPP treated juices was remarkably higher than thermal treated (110 °C-8.6 s) juices. – The difference between total phenol values was not significant but HPP treated samples higher than thermal treated ones. – After HPP treatments, the chlorophyll contents were determined higher than double. – HPP treated samples showed better results of ascorbic acid and chlorophyll contents during 42 days-storage, while better results of total phenols were determined in that period for thermal treated samples.	Xu et al. (2018)
Gooseberry	– Ascorbic acid – Total phenols – Antioxidant activity	200–500 MPa, 30–60 °C, 1 s–20 min	– The loss in ascorbic acid for assistance of temperature and HPP varied from 0.3% to 15.4%, while that value was determined 34% for thermal treatment at 60 °C-20 min. – Ascorbic acid degradation rate ranged between 1.57×10^{-3} and 2.013×10^{-3} m^{-1} for temperature assisted HPP. – Up to 50 °C, total phenols and antioxidant activity increased with increasing processing pressure.	Raj et al. (2019)

(continued)

Table 1 (continued)

Juice/ source	Bioactive compounds	Processing conditions	Highlights	Reference
Sugarcane	– Ascorbic acid – Total phenols – Antioxidant capacity	300–600 MPa, 30–60 °C, 10–25 min	– Dramatic ascorbic acid reduction (25%) was detected at 600 MPa/60 °C/25 min. – Ascorbic acid degradation increased significantly accordingly with processing at higher pressure, temperature and time. – The highest total phenols were determined for samples processed at 600 MPa/50 °C/20 min. – Samples treated at 300–600 MPa/10–25 min and lower or equal to 50 °C showed higher antioxidant capacity than samples treated at higher temperature.	Sreedevi et al. (2018)
Mulberry	– Total monomeric anthocyanin – Anthocyanin compounds	400–500 MPa, 25 °C, 5–10 min	– At the end of storage (4 °C-30 days), the highest retention rates for total anthocyanin content and cyanidin-3-rutinoside were determined for juices treated at 400 MPa-5 min. – Higher processing time at the constant pressure caused significant reduction in anthocyanin compounds. – The juice samples processed with 75 °C-10 min lost significantly initial anthocyanin contents at the end of storage.	You et al. (2018)
Papaya	– Total carotenoids – Total phenols – Antioxidant capacity	350–650 MPa, 20 °C, 5 and 10 min	– Quality and microbiological results suggested HPP processing at 550 MPa/5 min. – Better retentions of total carotenoids, total phenols and antioxidant capacity immediately after HPP treatments. – HPP treated samples remarkably showed better stability than thermal process (110 °C-8.6 s) in terms of total phenols and antioxidant capacity during storage period (40 days/4 °C).	Chen et al. (2015)

apples collected from different regions. While the highest phenolic compounds were determined for Spanish-apples processed at 400 MPa/35 °C/5 min, for Italian-apples, it was determined at 600 MPa/35 °C/5 min. Generally, for apples from Spain, it can be said that there is a decreasing tendency in response to higher processing pressure for the most abundant phenolic compounds such as procyanidin B2, chlorogenic acid, epicatechin, phloridzin and Q-3-rhamnoside. However, the phenolic compound results for apples collected from Italy were detected as increasing response to higher processing pressure. In the same study, it was reported that significant positive correlations were found between all the antioxidant activity determinations and total phenolic in Italian and Spanish apples. Juarez-Enriquez et al. (2015) also studied shelf life stability of apple juice processed with 430 MPa/7 min high pressure conditions and results showed that ascorbic acid and antioxidant activity decreased remarkably during 34 days storage period at both 4 and 20 °C.

Effect of HPP conditions on mango pulp bioactive compounds (ascorbic acid, phenolic contents and carotenoid compounds) was performed by Camiro-Cabrera et al. (2017). According to the results, the phenolic content increased remarkably up to 34% as compared to initial concentrations at higher processing pressure. The researchers commented that high pressure is able to increase extractable phenolic compounds due to its destructive effect on cell wall and cell membrane. And also, they reported that after 8 min high pressure holding time, the phenolic content started to affect negatively. Ascorbic acid concentration started to degrade after 4 min holding time at 34 °C, while decreased significantly after all high pressure treatments at 59 °C. Comparing to untreated samples, mango pulp samples treated with high pressure showed the same carotenoid profile. However, pronounced degradation was observed for violaxanthin which is one of the most abundant carotenoids in mango pulp. Higher holding time at higher processing temperature resulted with higher carotenoid compounds degradation due to temperature sensitivity of carotenoids. The antioxidant activity of the treated samples was not affected or increased up to 39% in respect of untreated samples. Finally, the researchers suggested to process mango pulp at 550 MPa-moderate temperatures (<34 °C)-8 min in order to obtain mango pulp with the highest bioactive compounds and functionality.

Aaby et al. (2018) conducted a comparing study about effect of HPP and thermal pasteurization on strawberry puree and juice. Moreover, treated samples were followed for 49 days at 6 °C storage temperature. While thermally processed (85 °C-2 min) puree had higher ascorbic acid concentration than high pressure treated ones, juices processed with high pressure at 400 and 500 MPa had higher concentration than untreated and thermally processed juice samples. For the strawberry puree, the anthocyanin content results showed that high pressure applied purees contained higher concentration than untreated but lower than thermal pasteurized ones. However, pressurized strawberry juice, independent to the applied high pressure conditions, had higher anthocyanin contents than both thermal treated and untreated juice samples. Ascorbic acid and anthocyanin contents of treated strawberry puree and juice samples were remarkably reduced during 49 days stor-

age period at 6 °C. It can be observed from the presented results that ascorbic acid degradation rate is higher for thermal treated samples than high pressure treated. However, at the end of the storage, anthocyanin content of thermally processed strawberry samples was determined significantly higher than samples processed with high pressure. The reason for that situation was stated by the researchers that high pressure applied strawberry samples had higher residual oxidative enzymes, such as PPO.

The combined high pressure-temperature conditions were studied for pineapple puree and changing of quality attributes of the puree, such as ascorbic acid, phenolic and flavonoid contents and antioxidant capacity, was also reported. The findings showed that the pressure increasing from 400 to 600 MPa at the constant low temperature-time and processing time increasing from 10 to 20 min at constant pressure-low temperature did not meaningfully affect ascorbic acid content, while increasing temperature from 50 to 70 °C at constant pressure and time caused significant decomposition in ascorbic acid content. For example; ascorbic acid lost for samples processed at 200 MPa and 50, 60 and 70 °C was determined as 7.3, 12.5 and 23.5%, respectively. For all pressure-time combinations, instead of decreasing in total phenolic content at higher pressure conditions, it was found an increasing in total phenolic content up to 60 °C processing temperature. However, after that processing temperature, the phenolic content started to decrease remarkably. While flavonoid content was stable for processing temperature between 30 and 50 °C, it had a significant decreasing trend for higher values of pressure-temperature and processing time. Similar tendency was determined for antioxidant capacity of pineapple puree (Chakraborty et al. 2015). Consequently, in order to obtain pineapple puree with a source of bioactive compounds, HPP conditions up to 600 MPa at ≤60 °C were suggested by the researchers. Dhakal et al. (2018) conducted a research study on ascorbic acid degradation kinetics of pineapple juice subjected to different pressure (0.1–600 MPa), temperature (30–95 °C) and holding time (0–15 min). They reported that there was no ascorbic acid degradation for pineapple juice samples treated with high pressure between 300 and 600 MPa-up to 15 min at 30 °C. However, comparing to untreated juice samples, samples treated at 75 °C and 95 °C for 60 min lost 25% and 39% of ascorbic acid content, respectively. The researchers highlighted that ascorbic acid degradation increased in the samples treated with combined pressure-thermal processing. For ascorbic acid degradation, activation energy values of thermal processing at atmospheric pressure (0.1 MPa) and combined pressure-thermal processing were calculated in the range of 14–30 kJ/mol and 17.4–43.8 kJ/mol, respectively.

A comparing study was conducted to investigate effect of HPP (550 MPa-6 min) and thermal pasteurization on (110 °C-8.6 s) carrot juice phenolic contents, carotenoid compounds and antioxidant capacity. Moreover, treated samples were stored at 4 °C for 20 days. No difference was detected between control and treated samples for lutein content. In the HPP-treated samples, both of α-carotene and β-carotene were significantly detected higher than thermal pasteurized samples. Similarly, total phenolic contents were better preserved in the juices treated with HPP but remarkably decreased for thermal treated juices. The antioxidant capacity of carrot juice

samples reduced significantly after HPP and thermal treatments. However, in the samples processed with high pressure, the antioxidant capacity showed better retention than thermal processed. After 20 days storage at 4 °C, the decreasing in carotenoid contents of juices treated with HPP and thermal pasteurization were 66.7% and 72.9% for lutein, 16.2% and 26.8% for α-carotene, 11.1% and 16.6% for β-carotene, respectively. However, total phenolic contents were decreased 35.8% and 33.5% for carrot juices treated with HPP and thermal pasteurization at the end of the storage, respectively. Due to decrease in concentration of the carotenoids and phenolics, the antioxidant capacity of the treated carrot juices decreased linearly during storage period. The researchers also calculated degradation rate constant of the bioactive compounds for treated juices and, the results showed that carotenoid compounds (except lutein) and total phenolic of HPP treated samples degraded with higher reaction rate constants than thermally pasteurized samples during the storage (Zhang et al. 2016).

Yuan et al. (2018b) studied the effect of different high pressure levels (200–600 MPa) and holding times (2.5 and 5 min) on aronia berry puree bioactive compounds. High pressure treated samples had higher bioactive compound levels than untreated samples. According to the results, up to 400 MPa, the bioactive compounds had an increasing tendency but after that pressure, decreasing tendency was observed. Compared to untreated puree, total phenolic and anthocyanin contents of pressurized purees increased 3–13% and 6–17%, respectively. In addition, the researchers reported that the highest phenolic contents and antioxidant capacities were obtained at 400 MPa for 5 min. The same researchers conducted a storage study for similar product treated at 400 MPa and 600 MPa-5 min (Yuan et al. 2018a). They reported that total phenolic contents and antioxidant capacities had insignificant change during 8 weeks storage at 4 °C. However, significant reduction in total anthocyanin contents of aronia puree processed at 400 MPa-5 min was determined during the storage period. Taking into consideration the cost and energy efficiency of HPP, they suggested that the treatment at pressure of 400 MPa or 600 MPa and holding time of 5 min was effective to obtain an aronia berry puree having the lowest microbial counts with the highest bioactive compounds and antioxidant capacity.

Briefly, the results of the conducted studies by the research groups from all around the world (Table 1), associated with healthy nutrients of the various fruit juices clearly demonstrated that HPP is a promising non-thermal technology; (1) to extend shelf-life of fruit juices, (2) to obtain fruit juices closest to their initial fresh attributes and finally, (3) to produce fruit juices with high concentration of bioactive compounds. And also, it was reported that compared to traditional thermal pasteurization treated fruit juices, high pressure treated ones were nutritionally superior in terms of bioactive compounds for collected juices both immediately after processing and during shelf-life.

Effect of Pulsed Electric Fields (PEF) on Bioactive Compounds

Recently, consumers are demanding minimally processed, healthy, functional and high quality food products that have inherent flavor, fresh appearance and intense taste (Bisconsin Junior et al. 2015). PEF can be applied to tissue softening, increasing of extraction processes and pasteurization processes (Praporscic et al. 2007). Compare with to the use of heat treatments for pasteurization, PEF cannot cause protein coagulation or starch gelatinization. Moreover, covalent chemical bonds are not affected so the nutrients remain intact (Korma et al. 2016).

Results of recent studies about PEF treatment of different juices are summarized in Table 2 with regards to bioactive components. Lee et al. (2018), studied on the effects of H-PEF treatment on ascorbic acid concentration in mixed mandarin-hallabong tangor (MH) juice. An efficient pasteurization method was determined as H-PEF processing (at 70 °C (inlet temperature), 16 kV/cm–100 kJ/L) that preserves the ascorbic acid concentration, antioxidant capacity, total soluble solid, pH and also for inactivation of microbial and quality of MH juice. Bobinaitė et al. (2015) reported that the juice obtained from PEF pre-treated blueberries had a significantly higher antioxidant activity (31% increase), total phenolic content (43% increase) and total anthocyanin content (60% increase). However, PEF treatment with intensity higher than 1 kV/cm did not improve the qualitative characteristics of the blueberry juice significantly. García-Parra et al. (2017) was found the highest anthocyanins content in purees from plums pretreated with MIPEF (moderate-intensity pulsed electric fields), manufactured with ascorbic acid (AA) addition. However, the lowest contents were found in non-MIPEF pretreated, without AA addition and untreated purees. González-Casado et al. (2018) showed the significant increasing effect of the application of PEF as a pre-processing treatment on the concentration of total and individual carotenoids in tomato fruit. The PEF treatment intensity is found effective on the concentration of individual carotenoids of the product obtained from tomatoes after PEF application. The concentrations of phytoene and phytofluene were increased by 178% and 131%, respectively, tomatoes after PEF (30 pulses at 2 kV/cm, 2.31 kJ/kg) compared to untreated fruit. Also increase in lycopene concentration (4400–6072 μg/kg) was determined in tomato puree. The maximum lycopene concentration was found treated with the most intense PEF treatment (2.31 kJ/kg), leading to a 1.5-fold increase, according to untreated tomatoes.

Agcam et al. (2014a) reported the total phenolic concentration of the juices varied depending on the applied electric field intensity of PEF. The PEF treatment with 21.50 kV/cm electric field strength and 1206.2 μs ensured higher total phenolic concentration was obtained by during the storage (4 °C for 180 days) of orange juices. Untreated orange juice samples had a shelf-life of approximately 10 days, whereas both PEF and heat treated samples had a shelf life of 180 days at 4 °C. Hence, the application of PEF processing to orange juice seems to be a promising alternative to heat pasteurization in order to obtain an extended shelf-life and

Table 2 Effect of pulsed electric fields (PEF) studies on bioactive components of different juices

Juice/source	Bioactive compounds	Processing conditions	Highlights	Reference
Mixed mandarin-Hallabong tangor (MH) juice	– Ascorbic acid (AA) – Antioxidant capacity (AC)	16 kV/cm 100 kJ/L 12 kV/cm 150 kJ/L	AA and AC content of juice (H-PEF treated at 16 kV/cm–100 kJ/L) was insignificantly differ AA and AC content of juice (H-PEF treated at 12 kV/cm–150 kJ/L) was lower than control.	Lee et al. (2018)
Blueberry juice	– Total phenolic compounds (TP) – Anthocyanins (TAC) – Antioxidant activity (AA)	1, 3, 5 kV/cm 10 kJ/kg	– PEF pre-treatment significantly increased TP, TAC and AA values of blueberry (45.5–39.4% for TP, and 77.5–44.3% for TAC, 35.9–28.4% for AA).	Bobinaitė et al. (2015)
Red flesh and skin plums	– Anthocyanins – Total phenolic content	0.4 kV/cm (Moderate-Intensity Pulsed Electric Fields, MIPEF)	– Anthocyanins and the antioxidant activity slightly increased.	García-Parra et al. (2017)
Tomato pure	– Carotenoid compounds	0.4, 1.2–2 kV/cm 5, 18 and 30 pulses, 0.1 Hz	– The highest carotenoid concentrations in the tomato puree obtained in PEF-treated fruit (at 30 pulses, 2 kV/cm, 2.31 kJ/kg), 52% greater than in control.	González-Casado et al. (2018)
Orange Juice	– Phenolic compounds – Ascorbic acid	13.82 kV/cm-10.89 J-1033.9μs-31.88 °C 25.26 kV/cm-51.32 J-1206.2μs-42.60 °C	– Samples processed by PEF contained higher phenolic compound concentrations than processed by the heat. – The flavonoid and phenolic acid concentrations treated with PEF appeared to be highly stable than treated with traditional heat application during the storage (180 days). – The highest ascorbic acid content was detected at moderate PEF conditions. – Samples treated with extreme PEF conditions lost remarkably ascorbic acid content. – In juices treated at moderate PEF conditions, half-life values of ascorbic acid calculated significantly higher than thermal pasteurized ones (90 °C/10 and 20 s) during storage (180 days-4 °C).	Agcam et al. (2014a, b, 2016)

(continued)

Table 2 (continued)

Juice/source	Bioactive compounds	Processing conditions	Highlights	Reference
Exotic fruit juice	– Phenolic compound – Anthocyanin – Antioxidant capacity	25 kV/cm-32 kJ/kg-<35 °C 25 kV/cm-256 kJ/kg-<35 °C	– PEF treatment improved bioaccessibility of phenolic compounds (37.0%), anthocyanins (15.6%) and antioxidant capacity (29.4%, 26.5%, 23.5% for different antioxidant assays, respectively) compared to untreated juice.	Buniowska et al. (2017)
Blueberry juice	– Ascorbic acid (AA) – Anthocyanin	20, 25, 30, 35 kV/cm, 27, 54, 82 µs, conductivity 1.4 and 1.8 mS/cm	– The retention rate of AA in the blueberry juice after PEF was 87.87% (13.27% higher than that of the heated sample). After 30 days of storage, the anthocyanin retention of the blueberry juice samples after PEF treatment was 84.84%, which is 6.23% higher than that of the heated sample.	Chen et al. (2014)
Grapefruit juice	– Total phenolics – Total antioxidant capacity – Total anthocyanins – Total carotenoids	0, 5, 10, 15, 20, 25 kV/cm 1 kHz at 40 °C for 600 µs	– Total carotenoids content of PEF treated grapefruit juice were determined increase at field strengths of 5, 10, 15, 20 and 25 kV/cm, which was 1.92, 1.98, 2.04, 2.11 and 2.15 µg/mL, respectively, as compared to control (1.81) µg/mL. – Increase in percentage inhibition (DPPH radical), total antioxidant content and total phenolic treated with PEF.	Aadil et al. (2015a, b)
Fruit beverage	– Carotenoid compounds	35 kV/cm, 1800 µs 4 µs pulses at 200 Hz	– Increase in cis-violaxanthin + neoxanthin (16%), antheraxanthin (10%), lutein (23%) and zeaxanthin (28%).	Rodríguez-Roque et al. (2016)
Grape juice	– Ascorbic acid – Total phenolic content	15–70 kJ/kg, 1.5 kV/cm, 50 Hz, 20 µs pulse width	– Increase in ascorbic acid (19%) and total phenolic content (61%).	Leong et al. (2016)
Date juice	– Total phenolic content	35 kV/cm for 1000 µs, 100 Hz bipolar mode	– The highest phenolic content was observed after HIPEF treatment (569 mg/L) in comparison with untreated-control juice (483 mg/L).	Mtaoua et al. (2017)
Apple juice	– Ascorbic acid – Total polyphenol – Antioxidant activity	200, 300, and 400 pulses, 30 kV/cm	– The PEF processing, regardless of the number of pulses, did not significantly affect the content of AA and TP in apple juice. – PEF treatment and also the number of pulses affected antioxidant activity, which decreased just after process and also after 24 h of storage (with an exception of 400 pulses treatment).	Dziadek et al. (2019)

Grapefruit juice	– Lycopene – Anthocyanin content – Total carotenoid content – Total antioxidant capacity – Total phenolics (TP)	80 mL/min 20 kV/cm for 600 μs	– Increase in lycopene content during PEF (0.62 μg/mL), control (0.32 μg/mL). – Increase in anthocyanin content was significantly in PEF (1.58 mg/L) as compared to control (1.37 mg/L). – Total carotenoid contents were increased from 0.84 mg/mL (control) to 1.03 mg/mL. – Significant increase in total antioxidant capacity. – Significant increase in the total phenolics during PEF treatments of grapefruit juice as compared to control. – TP contents were increased to 701.1 mg/g as compared to control (640 mg/g).	Aadil et al. (2017)
Raspberries	– Total phenolics – Anthocyanins content – FRAP value	1 and 3 kV/cm 1, 6 and 12 kJ/kg 20 Hz 20 μs	– The total phenolics content of fresh raspberries was 1033.9 mg/L. – The total phenolics content of the juice was similar with control sample. – The juice obtained from frozen-thawed raspberries had significantly higher content of total phenolics (1219.3 mg/L). – Anthocyanin content and antioxidant activity values remained unchanged.	Lamanauskas et al. (2016)
Sweet cherries	– Anthocyanin compounds – Antioxidant activity	0.5–3 kV/cm, 10 kJ/kg	– PEF-assisted pressing (E = 1 kV/cm) led to a significant increase of juice yield (+40%), anthocyanins (+80%), and antioxidant activity (+27%) with respect to untreated samples. – PEF treatment intensity higher than 1 kV/cm did not significantly improved the quantitative and qualitative characteristics of juice.	Pataro et al. (2017)
Apple juice	– Total antioxidant capacity (TAC) – Total phenolic compounds (TPC) – Phenolic compounds	0–26.7 kV/cm 0–873.1 μs 10–40 °C 0–147 J/s	– The PEF treatment at 147 J/s energy level slightly increased both values to 0.029 mg/L and 50.33%. – Decrease in chlorogenic and sinapic acids. – No significant change was observed in (−)-epicatechin, caffeic acid, p-coumaric acid, ferrulic acid, quercetin, and gallic acid after PEF.	Evrendilek et al. (2017)

(continued)

a better preservation of phenolic compounds. Buniowska et al. (2017) studied on bioaccessibility of bioactive compounds after non-thermal processing of an exotic fruit juice blend sweetened with *Stevia rebaudiana*. They reported an increase in bioactive compounds bioaccessibility after PEF treatments, which improved bioaccessibility of phenolics (37.0%), anthocyanins (15.6%), and antioxidant capacity (29.4%, 26.5%, 23.5% for TEAC, ORAC and DPPH respectively). Chen et al. (2014) noticed that the PEF-treated of blueberry juice was compared with the control group, which appeared almost unchanged. After heat treatment, ascorbic acid and anthocyanin content of PEF treated blueberry juice sample was reduced by 14.78%, 3.64%, respectively. The anthocyanin content of the different treated blueberry juice dropped with the increasing of storage time. After 30 days of storage, the anthocyanin content of the control, PEF-treated and heated blueberry juice samples decreased by 22.55%, 15.15%, and 21.38%, respectively. Also, at the same storage period, ascorbic acid content of the control, PEF-treated and heated samples decreased to 30.21%, 13.96%, 25.39%, respectively.

Aadil et al. (2015a, b) suggested that PEF at 25 kV/cm could improve the quality of grapefruit juice. They determined a significant increase in percentage inhibition (DPPH-radical), total antioxidant content, total phenolics and total carotenoids in response to increase in electric field strengths, compared to control treatment. Rodríguez-Roque et al. (2016) reported a decrease up to 7.6–48.2% in the carotenoids bioaccessibility of fruit juice based beverages treated with PEF, whereas the carotenoids bioaccessibility diminished up to 63% in thermally treated beverages compared to the untreated beverages. Leong et al. (2016) evaluated the health-promoting properties of Pinot Noir grape juices obtained after PEF-treatment (15 or 70 kJ/kg). PEF pre-treatment on grapes were enhanced the release of the major anthocyanin compared to untreated grapes juice. Mtaoua et al. (2017) reported that applicability of HIPEF (35 kV/cm for 1000 μs using pulses of 4 ms pulses at 100 Hz in bipolar mode) to preserve the nutritional and physicochemical characteristics of date juice after treatment and during 5 weeks of storage (4–5 °C) by comparison to untreated juice.

Dziadek et al. (2019) reported PEF technology did not affect the content of bioactive compounds in apple juice. Moreover, PEF-treated juice did not show change in the amount of vitamin C and total polyphenols during for 72 h under refrigeration storage. Aadil et al. (2017) studied on effects of PEF on bioactive compounds of grapefruit juice. After PEF treatment, lycopene, anthocyanin, carotenoids contents and total antioxidant activity were increased from 0.32 μg/mL, 1.37 mg/L, 0.84 μg/mL, 177.48 (control) to 0.62 μg/mL, 1.58 mg/L, 1.26 μg/mL and 226.73, respectively. Lamanauskas et al. (2016) showed that mild-PEF pretreatment (1 kV/cm electric field strength and 6 kJ/kg total specific energy) was sufficient to achieve higher raspberry juice recovery and to enhance extraction of bioactive compounds from raspberry press cake left after the juice pressing. Moreover, juice recovery from raspberries was increased in the range of 9–25%, after PEF pretreatment and mechanical pressing (1.32 bar, 6 min). Press cake extracts contained significantly higher amounts of total phenolics (up to 22%), total anthocyanins (up to 26%) and higher ferric reducing antioxidant power, FRAP, (up to 24%) compared with untreated sample.

Pataro et al. (2017) reported that the application of a PEF pre-treatment expressly contributed to a further increase in the extraction of all the anthocyanin compounds of cherry fruits (cyanidin-3-rutinoside, peonidin-3-rutinoside, cyanidin-3-glucoside and pelargonidin-3-rutinoside) compare to untreated sample. Total anthocyanin content increased to 33%, 80% and 52%, PEF-treated at 0.5, 1 and 3 kV/cm, respectively. The antioxidant activity (FRAP values) of juice was increased 10.0%, 27.4%, and 15.2%, after PEF pre-treatments at 0.5, 1, and 3 kV/cm, respectively. The results demonstrated that the electroporation effect induced by PEF pre-treatment at relatively low field strength (E = 0.5–1 kV/cm) and energy input (WT = 10 kJ/kg) appeared to be sufficient for the improvement of juice yield as well as for the condensation of the anthocyanins extraction from both cherry fruits and their by-products (press cakes). Evrendilek et al. (2017) found that no significant difference was detected between the control and PEF-treated apple juice in terms of physical properties, organic acids, and polyphenols of (−)-epicatechin, caffeic acid, *p*-coumaric acid, ferrulic acid, quercetin, and gallic acid. PEF processing was also provided retention of quality characteristic and bioactive compounds without significant formation of furfural and hydroxymethylfurfural.

Finally, these promising results confirm the potential of PEF technology to improve the efficiency of the fruits conversion process to add value to food product and also enable the evaluation of food processing waste that leads to more product diversity. PEF could increase the extraction of bioactive compounds from fruit in this way increase their healthy potential. Furthermore, the use of PEF as abiotic stressor may be an appropriate strategy to increase the biological production of secondary metabolites in raw fruits and vegetables, thereby increasing their antioxidant potential (Yilmaz and Evrendilek 2017). Therefore, PEF technology has good prospects for commercial application provided that different PEF strategies are used to provide new healthy food for consumers. However, further research and development activities are needed to fully understand, optimize, and implement PEF processes (Elez-Martínez et al. 2017).

Effect of Ultrasound Processing (UP) on Bioactive Compounds

For a long time, traditional thermal treatments, sterilization or pasteurization, have been used to produce microbiologically safe juices. However, after the effects of heat on sensorial characteristics like taste or color, and bioactive properties of juice like antioxidant capacity or vitamin content, emerging technologies have started to be more popular. One of these technologies, ultrasound, is generally applied as a processing aid and pre-treatment, although ultrasound with the higher frequency levels can be effective on different features of foods.

The effects of ultrasound on bioactive components which are in fruit juices and have great importance for human health are shown in Table 3. The cavitation regulates various chemical or biological reactions including increase in the diffusion rates and disintegration of affected particles (Tiwari et al. 2009). Hence, bioactive

Table 3 Effect of ultrasound processing (UP) technology on bioactive components of different juices

Juice/source	Bioactive compounds	Processing conditions	Highlights	Reference
Pumpkin	– Carotenoid – Flavonoid – Antioxidant capacity	37 kHz, 150 W, 30 dk, 40–50–60 °C, thermosonication (TS) in ultrasonic bath	– TS-40 and TS-50 samples had a lower total carotenoid than the control. – Total carotenoid content increased with increasing temperature. – The total amount of flavonoid is higher than the heat treated samples (40, 50, 60, 70, 80 °C, 15 min). – The highest total amount of flavonoids was obtained in the TS-40. – The total amount of flavonoid decreased with the increase of temperature in the thermosonicated samples.	Demir and Kılınç (2018)
Grapefruit	– Total carotenoid – Total flavonoid – Antioxidant capacity	20, 30, 40, 50 and 60 °C, frequency (28 kHz), power (70%, 420 W), 30 and 60 min	– Maximum increase in total carotenoids was obtained in the samples processed at 60 °C during 60 min. – Total antioxidant capacity, total phenols, total flavonoids and total flavonols increased with the temperature (from 30 to 60 °C). – All compounds increased significantly at lower ultrasound processing (20 °C, 30 and 60 min).	Aadil et al. (2015a, b)
	– Carotenoid – Flavonoid – Flavonol – Lycopene – Anthocyanin – Phenolics	28 kHz, 600 W, 30 min, 20 °C, ultrasonic bath	– Increase in carotenoids. – PEF&US treatment showed higher values for carotenoids than individually treated UP and PEF juices. – PEF&US treatment appeared to be more adequate in retention of lycopene whereas US and PEF were more effective than control. – Combined treatment (PEF&US) could improve the antioxidant activity, total phenolics, flavonols, flavonoids, lycopene, and total carotenoids. – Significant increase in lycopene content was noted during UP. – Anthocyanin contents were increased significantly in UP.	Aadil et al. (2018)

Orange	– Carotenoid – Total flavonoid – Total phenolics – Ascorbic acid	1, 10, 20 and 30 min 24 kHz frequency, 105 μm, 33.31 W/mL, <46 °C	– Total carotenoids (a-carotene, β-carotene and lycopene) increased significantly in all sonicated samples. – Compared to the amount found in the control sample, a significant increase in flavonoids was found in all sonication treatments. – Sonication during 10 min at 43.4 °C gave the highest amounts. – Enhancement in most of the bioactive compounds observed in sonication of juice samples for 10, 20 and 30 min (43–45 °C) compared to control sample. – Sonication coupled with high temperature significantly enhanced orange juice quality.	Guerrouj et al. (2016)
Star fruit	– Carotenoid – Flavonoid – Ascorbic acid	15, 30, 45, and 60 min, 44 kHz, 35–40–45 °C	– Significant ($p < 0.05$) increase in antioxidant activity, total phenolic content, total flavonoid content, ascorbic acid content. – The amount of carotenoids levels were decreased slightly as the processing temperature increased to more than 35 °C. – Carotenoid levels were insignificantly increased as the sonication time increased.	Nayak et al. (2018)
Pear	– Flavonoid – Ascorbic acid – Phenolic	25, 45 and 65 °C, 10 min, 750 W probe sonicator, 20 kHz 70% amplitude	– Increase in flavonoids 17.7% (25 °C). – Decrease in flavonoid 8.9% (65 °C). – Increase in ascorbic acid content (25 °C). – 65 °C for 10 min treatment was the best in retention of ascorbic acid and other phenolic compounds. – Significant increase with ultrasound at 25 °C in ascorbic acid, total phenols and flavonoids. – The loss of compounds increased as the temperature of ultrasound treatment increased.	Saeeduddin et al. (2015)

(continued)

Table 3 (continued)

Juice/source	Bioactive compounds	Processing conditions	Highlights	Reference
Carrot	– Flavonoid – Phenolics – Ascorbic acid	15 °C, 2 min keeping pulse duration 5 s on and 5 s off 70% amplitude 20 kHz frequency	– US resulted in an increase in total phenols, total flavonoids and tannins. – Significant increase is observed in ascorbic acid. – Improved antioxidant capacity.	Jabbar et al. (2014)
	– Flavonoid – Ascorbic acid – Lycopene – Lutein	20 kHz, 70% amplitude 48 W cm^2 of ultrasonic intensity 20, 40 and 60 °C, 5 and 10 min using 5 s on/off pulse cycle	– Increase in carotenoid content. – Increase in ascorbic acid of juice treated with ultrasound at 20 °C (UP20-5 and UP20-10). – Maximum improvement in lutein, carotenoids and lycopene after thermosonication at 60 °C-10 min.	Jabbar et al. (2015)
	– Ascorbic acid – Carotenoid	40 kHz and 0.5 W/cm^2 ultrasound intensity 20, 40 or 60 min	– Total soluble solids, total sugars, total carotenoids and ascorbic acid contents were significantly improved ($p < 0.05$). – A significant increase ($p < 0.05$) in total carotenoids and ascorbic acid contents in the sample sonicated for 40 min compared to sample sonicated for 20 min and control (non-sonicated) sample. – Significant increase ($p < 0.05$) in total carotenoids and ascorbic acid contents in the sample sonicated for 40 min compared to control.	Zou and Jiang (2016)
	– Ascorbic acid – Carotenoid	24 kHz, 400 W, 22 mm probe, 50, 54, and 58 °C	– No significant difference on total carotenoid, phenolic compounds and ascorbic acid ($p < 0.05$).	Pokhrel et al. (2017)
	– Carotenoid – Ascorbic acid – Phenolic compounds	24 kHz, 120 µm amplitude, 50, 54 and 58 °C, 10 min acoustic power 2204.40, 2155.72, 2181.68 mW/mL	– Thermosonicated juice at 58 °C retained >98% of carotenoids. – Thermosonicated juice at 58 °C retained 100% of ascorbic acid. – Samples treated by ultrasound at temperatures of 50 and 54 °C retained 91.67% of the ascorbic acid content after 12 and 14 days of storage.	Martínez-Flores et al. (2015)

Plum	– Flavonoid – Total phenolic	20 kHz, 400 W, 2.5, 5, 7.5, and 10 min 40, 50, 60 and 70 °C	– A slight increase in the total phenolic content under some ultrasonic treatment conditions. – No dramatic trends were observed for the total phenolic compound content of the plum nectar. – Dramatically increased flavonoid content.	İrkilmez et al. (2017)
Acerola	– Ascorbic acid – Vitamins	18 kHz, 500 W, 13 mm probe, 1000, 3000 and 5000 W/L, 2.5, 5, 10 and 15 min, 40 °C	– Increased the availability of pro-vitamin A and vitamins B3, B5, C and E. – The retention of the major vitamins in acerola juice (vitamins A and C) was higher at lower temperatures (10–20 °C).	Santos et al. (2018)
Kiwi	– Ascorbic acid	10 and 30 min, 180 W, 40 kHz	– UP10 and US30 applied individually showed AA retentions of 84.30% and 79.96%, respectively. – No significant increase the ascorbic acid retention through storage time.	Tomadoni et al. (2017)
Blackberry	– Ascorbic acid – Total Phenols – Anthocyanin – Antioxidant activity	1500 W, 20 kHz, 80% amplitude for 25 min, with pulse duration of 4 s on and 2 s, 13 mm probe	– The ascorbic acid content decreased in thermoultrasonicated (24%) and in the pasteurized juice (9%) as compared to the control. – Higher levels of total phenols, anthocyanins; antioxidant activity by ABTS and DPPH in comparison to the control and thermally treated juices.	Manríquez-Torres et al. (2016)
	– Ascorbic acid – Antioxidant activity – Anthocyanins	20 kHz, 1500 W, 25 mm probe, 28 μm, 40–50 °C, 15–20 min	– Ascorbic acid, antioxidant activity and anthocyanin content of thermoultrasonicated juices were higher than pasteurized juice. – Total phenolic content of juices did not differ significantly ($p < 0.05$).	Cervantes-Elizarrarás et al. (2017)

(continued)

Table 3 (continued)

Juice/source	Bioactive compounds	Processing conditions	Highlights	Reference
Strawberry	– Ascorbic acid – Total Phenolic – Anthocyanin – Antioxidant capacity	0, 15 and 30 min, 20 °C and 25 kHz	– Ultrasonication (30 min) showed significant enhancement in bioactive compounds. – Ascorbic acid significantly increased in sonicated samples. – Anthocyanin level in strawberry juice samples showed significant increase. – Significant increase in antioxidant capacity and total phenolic content was determined.	Bhat and Goh (2017)
Strawberry	– Ascorbic acid – Total phenolic – Anthocyanin	150 W, 0.1–30 min, 25–75 °C	– High temperature and low ultrasound energy density combination must be applied for minimizing the change in ascorbic acid content. – Maximum total monomeric anthocyanin and total phenolic contents can be obtained at mild temperature and ultrasound energy density (~50 °C, ~230 J/g).	Dündar et al. (2019)
Apple	– Ascorbic acid – Total carotenoids – Phenolic compounds – Minerals	0, 30 and 60 min, 20 °C, 25 kHz amplitude 70%	– The contents of polyphenolic compounds and sugars significantly increased ($p < 0.05$) especially in the samples treated for 30 min. – Total carotenoids significantly increased ($P < 0.05$) in samples treated for 60 min. – Significant increase ($p < 0.05$) in chlorogenic acid, caffeic acid, catechin, epicatechin and phloridzin in sonicated apple juice. – Sonication treatments showed insignificant ($p > 0.05$) change in the total anthocyanins. – The concentrations of Na, Ca, K increased, while the concentrations of P, Mg and Cu decreased after ultrasonication.	Abid et al. (2014)

compounds are most likely affected by ultrasound. In the literature, it was showed that total carotenoid content of juice decreased in pumpkin juice while it increased mostly as in grapefruit, carrot, orange juice (Demir and Kılınç 2018; Aadil et al. 2015a, b; Martínez-Flores et al. 2015; Jabbar et al. 2015; Guerrouj et al. 2016). The ultrasound process is able to weaken the matrix of food and rupture cell walls, caused free carotenoid releasing. Also, the increase in lycopene content due to sonication treatment may be attributed to the cavitations which cause an increase in the rate of diffusion, chemical reaction and dispersing the aggregates. In ultrasound treatment, disruption of chromoplast membrane and collapse of cell-wall occurs due to the cavitation that results in release of more lycopene contents (Jabbar et al. 2014). The main reason of degradation of these carotenoids is by isomerization and oxidation. It is widely presumed that carotenoids in general undergo isomerization with thermal processing (Sánchez-Moreno et al. 2005; Shi and Maguer 2000; Van den Berg et al. 2000).

Fruits were considered as a potent source of phenolic compounds which have most importantly a vital role in human health (Aadil et al. 2015a, b). Even if the studies related with level of ultrasound treatments and juice type differ widely in the literature, the results showed that the total phenolic content of juices is generally increased slightly or significantly. The phenolic acids can be found in nature both free and bound forms. The bound phenolic acids remain bound to some structural carbohydrate and protein either through ester linkage with carboxylic groups or ether linkages with lignin through their hydroxyl groups in the aromatic ring or acetyl bonds. The increase in phenolic content may be related with the conversation of phenolic compounds to their free form. Also, hydroxyl groups formed during cavitation might be added to the aromatic rings (Bhat et al. 2011).

Aadil et al. (2018) suggested that combination of ultrasound and pulsed electric field technologies could be the best option to obtain the better results related to bioactive compounds in grapefruit juice. The increasing in ascorbic acid content may be related with the elimination of dissolved oxygen that is essential for ascorbic acid degradation during cavitation (Cheng et al. 2007). Ascorbic acid content has a direct influence on oxidative stability and its degradation caused by ultrasound processing mainly based on two pathways: thermolysis and reaction with hydroxyl radicals produced after sonolysis of water molecules found in juice (Feril and Kondo 2005). Aguilar et al. (2017) suggested that deaeration of juice before ultrasonication can be effective to reduce the ascorbic acid degradation.

Cervantes-Elizarrarás et al. (2017) showed that ultrasound can be alternative for production of microbiologically safe blackberry juice with high-quality. Abid et al. (2014) suggested that ultrasonication may improve the apple juice quality in terms of phytonutrients. Nayak et al. (2018) obtained similar results for star fruit juice after ultrasonication. Aadil et al. (2015a, b) claimed that thermosonication, the combination of ultrasound and heat, of grapefruit juice can be more preferable, because thermosonication can be applied temperature which was much less than the temperature required for a traditional thermal process. Also, Bhat and Goh (2017) showed that sonication improved the overall quality of hand-pressed strawberry juice. Therefore, it has a great importance that ultrasonication parameters (amplitude, temperature,

time and frequency) should be optimized with further studies to produce juices with higher quality than the juices thermally treated. Dündar et al. (2019) reveal the effect of not only the thermosonication but also the change of parameters, ultrasound energy density and temperature, on total monomeric anthocyanin, ascorbic acid and total phenolic content of strawberry nectar and optimized the thermosonication process conditions. While Jabbar et al. 2014 suggested that combination of blanching and sonication may be preferred in juice industry to produce high-quality carrot juice with reduced enzyme activity and protected nutritional value, Aadil et al. (2018) suggested the PEF and ultrasound combination for grapefruit juice.

In conclusion, researches about ultrasonication of juices clearly showed that ultrasound energy, which is non-toxic and environmentally friendly, mostly has a positive impact on total phenolic content, anthocyanins, ascorbic acid, flavonoids, lycopene, lutein and even minerals of fruit juices (Kentish and Ashokkumar 2011).

Effect of Ultraviolet Light Processing (UVLP) on Bioactive Compounds

Thermal pasteurization is the most common processing technique which is applied to make the fruit juices microbiologically safe to consume. However, thermal processing is known to have some adverse effects on the healthy nutrients and sensory quality of the product. The increasing tendency of consumers towards products with similar characteristics to fresh produce has led researchers to develop alternative processing techniques (Tahiri et al. 2006). Non-thermal processing techniques have been developed in order to accomplish those effects. One of the non-thermal technologies applied as an alternative to thermal pasteurization is UV-C treatment. UV treatment is a disinfection method that can be applied for the inactivation of microorganisms. The treatment includes the use of radiation from the electromagnetic spectrum (from 100 to 400 nm). It is classified as UV-C (200–280 nm), UV-B (280–320 nm) and UV-A (320–400 nm) (Bintsis et al. 2000; Unluturk 2012). The highest disinfectant effect is obtained between wavelengths of 250 and 280 nm. Thus, the applications and studies concentrated mostly on UV-C treatment and the wavelength at 254 nm is used for the disinfection of water, surfaces and various liquid food products such as fruit juices (Guerrero-Beltrán and Barbosa-Cánovas 2004).

UV-A treatment has a mechanism that inactivates microorganisms by damaging proteins and creating hydroxyl and oxygen radicals that destroy cell membrane and other cellular elements. In addition, the underlying principle of UV-C treatment is based on the prevention of replication and transcription of the cells by the dimers which are formed due to the absorption of UV light by the genetic material. The UV-C treatment has also an efficient effect on enzymes (Chatterley and Linden 2010; Unluturk 2012). UV-C treatment has been used in disinfection of water systems for many years and it has been reported to be effective in inactivation of bac-

teria, viruses, protozoa and algae (Begum et al. 2009). This treatment is also used for surface disinfection of foods (Pan et al. 2004; Nigro et al. 1998). There are also many applications of UV-C treatment on different fruit juices such as orange juice (Torkamani and Niakousari 2011), apple juice (Gabriel 2012).

The studies on fruit juices have been accelerated after the FDA permission in 2000 for the use of low or medium pressure mercury lamps to disinfection of fruit juices (Koutchma 2009). The advantages of the UV-C treatment that it does not leave any chemical residue due to being a physical process, thus it can be considered environment-friendly, economically attractive and easy to apply (Canitez 2002; Guerrero-Beltrán and Barbosa-Cánovas 2004). While the efficacy can be changed depending on the target microorganism species or intrinsic characteristics of juices (physical, optical and chemical) and intensities or doses applied juices (Koutchma 2009). It was reported that UV-C light cannot penetrate sufficiently in a highly absorbing environment such as cloudy fruit juice and also, some enzymes (such as pectinmethylesterase (PME), polyphenoloxidase (PPO) and peroxidase (POD)) which have effect on fruit juice quality cannot be inactivated at desired level by UV treatment (Tran and Farid 2004; Noci et al. 2008). The applied UV-C intensities or doses on juices are crucial factor to understand and compare the studies in bibliography. The variability of published researches may be explained by the differences in applied UV-C doses and UV-C systems (batch or flow). Recent studies about UV-C treatment applied on various fruit juices and effects of treatment on bioactive compounds are given in Table 4.

Unluturk and Atilgan (2015) was investigated the applicability of UV-C treatment in fresh grape juice as an alternative processing method to thermal pasteurization. They succeed 5.34 log CFU/mL reduction for *E. coli* and increased microbial shelf life of the juice by twofold. However, the ascorbic acid content was decreased significantly after the treatment. Nevertheless, they concluded that UV-C treatment can be used for extending the shelf life of fresh grape juice. Islam et al. (2016) studied apple juice with different UV-C doses (0–240 mJ/cm^2). Total phenolic content was well preserved regardless of the UV-C doses and total antioxidant activity decreased when UV-C dose reached 40 mJ/cm^2, but remained unchanged until 240 mJ/cm. The authors concluded that UV-C treated foods could be sold at a higher price than thermally-processed counterparts, because they have preserved their fresh-like properties. Bhat (2016) studied with fresh tomato juice and reported that UV-C treatment increased the total phenolic content compared to control samples. Total phenolic content was detected as 27.79 mg GAE/g in untreated samples, while 60 min UV-C treated sample was 36.22 mg GAE/g. Total lycopene and ascorbic acid content in samples exhibited decreasing trend depending on increased treatment time.

As can be seen Table 4, antioxidant activity and total phenolic content values of fruit juices after UV-C treatment are variable. The effect of UV-C treatment on those values was changed depending on juice type, settled UV-C treatment conditions and analysis method that applied to detect this type of bioactive compounds. Authors attributed the increase polyphenol contents in UV treated samples, degradation of conjugated phenolic compounds, accretion of polyphenolic compounds as a mean

Table 4 Effect of ultraviolet light processing (UVLP) studies on bioactive components of different juices

Juice/source	Bioactive compounds	Processing conditions	Highlights	Reference
Strawberry	– Antioxidant activity – Total phenolic content	UV-C doses 2.2 kJ/m^2, 25 °C, 0–60 min, static UV reactor	– Decrease in total phenolic content. Antioxidant activity did not change after 15 min exposure.	Bhat and Stamminger (2015)
Grape	– Ascorbic acid	– A continuous flow UV reactor – UV average intensity was 5.1 ± 2.8 mW/cm^2, 32.5 min, 9.92 J/cm^2 of UV dose was absorbed by the juice	– The dramatic decrease determined in ascorbic acid after treatment. – A significant ascorbic acid decrease was detected for control samples on the third day of storage, and continued to decrease after this time.	Unluturk and Atilgan (2015)
Mango	– Total carotenoid – Ascorbic acid – Total polyphenol content – Total flavonoid content – Antioxidant activity	UV-C doses 3.525 J/m^2 15, 30, 60 min at 25 °C static UV reactor	– UV-C treatment (15–30 min) increased carotenoid content. – AA content decreased depending on exposure time. – UV-C treated juice samples showed higher-total polyphenol, flavonoid content and antioxidant activity when applied 15–30 min. – UV-C treatment increased shelf life (4 weeks longer than freshly squeezed juice).	Santhirasegaram et al. (2015)
Tomato	– Antioxidant activity – Total phenolics content – Total lycopene content – Ascorbic acid content	Average dose 2.16 J/m^2, 15, 30 and 60 min, 25–26 °C	– Depending on exposure time antioxidant activity increased significantly. – UV-C treatment exhibited a significant increase in the total phenolics content. – Total lycopene content decreased slightly. – Ascorbic acid showed significant decrease only at 60 min of UV-C.	Bhat (2016)

Apple	– Polyphenols • chlorogenic acid, • phloridzin, • epicatechin • catechin – Polyphenolic content – Antioxidant activity	UV-C doses ranging from 0 to 240 mJ/cm, a low-pressure mercury lamp emitting at 254 nm	– Chlorogenic acid and phloridzin were decreased linearly with treatment. (−)-epicatechin content also decreased significantly as a function of UV dose, while catechin content showed a strict increase. – The total phenol content was almost stable (ranged from 9.79 (control) to 9.48 (treated) mg GAE/100 mL). – Antioxidant activity of control (18.94%) and treated juice (16.49%) showed only minor changes. – Increasing dose from 40 to 240 mJ/cm did not result in a significant change in antioxidant activity.	Islam et al. (2016)
Carrot (with yerba mate extract)	– Total polyphenol content – Total antioxidant activity	UV-C doses 2 kJ/L 15 min, 20–50 °C	– UV-C treated juice samples (at 50 °C) showed the highest total polyphenol content (720.2 ± 70.0 μg GAE/mL). – Polyphenol content and antioxidant activity of a high turbidity juice blend preserved during the storage (24 days at 4 °C).	Ferrario et al. (2018)
Melon	– Phenolic compounds – Antioxidant activity	UV intensity was 13.44 W/m^2, 20 min	– A positive impact on color maintenance detected. – Total phenolics content were not different in untreated and treated juices. – After 13 days of storage; the antioxidant activity of untreated juices decreased significantly. – The antioxidant activity was well preserved after treatment.	Fundo et al. (2019)

of defense against UV radiation, change in polyphenol oxidase enzyme activities. The decreasing level of ascorbic acid is attributed to the UV-C treatment conditions (was performed directly in air, long treatment time) and possibly could have generated very minimal heat and might cause initiation of oxidation process. Authors also concluded that the efficacy of UV-C treatment depends on nature of juices and exposure doses, the attained results displayed that this application can be considered as an alternative to heat pasteurization. However, most UV lamps containing mercury, which makes them very toxic to both environment and human, also most systems used in studies were batch.

Conclusion

Consumer concerns about health issues caused a dramatic change in consumption demands and they began to increase their consciousness towards the foods containing high amount of antioxidants, minimally processed and without containing processing-induced detrimental substances. Fruit juices, due to important sources of health supported nutrients such as, phenolics, carotenoids, fibers, vitamins, minerals, and antioxidative compounds, are among the most widely consumed ready to drink beverages.

Non-thermal technologies, which are alternative processing techniques to thermal pasteurization and sterilization, can be successfully used for both producing foods safe to consume and improving shelf-life of the foods by inactivating enzymes, spoilage and pathogenic microorganisms. On the one hand, traditional thermal processing is satisfied in extending shelf-life of fruit juices due to high inactivation of resistant enzymes, spores and microorganisms. However, it causes dramatic change in phenolic and carotenoid compounds, vitamins, taste and color of juices as well as increasing the level of undesirable substances. On the other hand, non-thermal processing technologies such as high pressure processing (HPP), pulsed electric fields (PEF), ultrasound processing (UP) and ultraviolet light processing (UVLP) are able to both adequately inactivate fruit juice enzymes and microorganisms, and remarkably save health related nutrients and original flavor attributes of juices without (or lower) processing-induced detrimental substances. Moreover, the findings of the various studies carried out by the different research groups from all around world generally indicated that the fruit juices treated with non-thermal technologies were especially superior in terms of health related compounds for the juices both immediately after processing and during shelf-life, compared to traditional thermal pasteurization treated ones.

Although the benefits and potentiality of the discussed non-thermal technologies were clearly demonstrated by many research studies, commercialization of them is still not enough. According to the general views, at least, those technologies will be used as assistant of traditional thermal processing in the near future. In order to ensure the use of non-thermal technologies with rapid spread in the fruit juice industry, the following critical points must be clearly evaluated:

- Investment costs of non-thermal technologies are relatively higher than classical technologies. In order to reduce investment costs to acceptable levels, governments and other funding agencies must support the universities to conduct the researches about non-thermal technologies; and industrial organizations relating with production of non-thermal technologies. Thus, the ways to produce non-thermal processors with lower cost can be increased.
- Immediately after processing and during shelf-life of the products, studies concerned with processing-induced detrimental substances formation such as furfural, hydroxymethylfurfural, furan, acrylamide, and reactive oxygen species are still limited. This point is very important to be sure that treated juices must be free of these substances. Therefore, the research must be focused on these undesirable substances formation in the fruit juices.
- Inactivation kinetics of enzymes, spores and microorganisms must be clearly characterized, in order to obtain minimally processed and safe to consume fruit juices.
- To achieve rapid inactivation of enzymes, spores and microorganism without causing significant composition changing, with/without heat assistance, combined effects of non-thermal technologies must be put forth with future studies.
- For non-thermal technologies, like thermal pasteurization norms, optimum processing conditions must be characterized as operation norms for each fruit juice.
- Regulations about non-thermal technologies must be developed accordingly with recent scientific findings. This will help to spread non-thermal technologies faster for products extended shelf-life without a threat to health.
- Although the researchers declared no-risk in consumption of the products processed with non-thermal technologies, the scientific studies associated with long term exposure must be conducted in order to collect toxicological evidences.

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Potential Industrial Use of Compounds from By-Products of Fruits and Vegetables

Faizan Ahmad and Shams Tabrez Khan

Abstract The global consumption of fruits and vegetables is rapidly increasing with the increasing world population and health awareness. Waste and by-products are produced in all the phases of the food life cycle i.e. during production, industrial processing, and distribution. It has been reported that in the food manufacturing industry 39% of the food is wasted and this is expected to rise to about 126 metric tonnes by 2020 if proper prevention policies are not in place. The by-products of these fruits and vegetables like peels can be wisely used for other industries. It has also been observed that a large portion of fruits and vegetables undergo a post harvest loss in the processing industry. For example, the pulp and the peel after the extraction of juices from fruits and vegetables go unutilized. The fruits, vegetables and their by products are rich in bioactive compounds like phenolic compounds, flavonoids, carotenoids, and anthocyanins. These compounds are known to have anticancer, antiviral, anti-tumour, antimicrobial and antioxidant activities; and can be used as pharmaceutical, nutraceuticals, cosmetic, chemical industries as well as for the development of functional foods. Since many of these bioactive compounds possess antimicrobial activity, they can also be used in food preservation and to control food-borne pathogens. Compounds from these by-products such as carotenoids, essential oils, and flavours can be included in food products to enhance their sensory properties and to improve their nutritional value and health benefits. The present chapter focuses on various types of bioactive compounds present in the by-products of fruits and vegetables, their extraction techniques, and their possible use as pharmaceuticals, cosmetics and in chemical industries. The antioxidant and antimicrobial properties of these bioactive compounds are also discussed.

Keywords Bioactive compounds · Byproduct of fruits and vegetables · Polyphenols · Flavonoids · Antioxidant activity

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Introduction

Fresh vegetables, fruits and the variety of phytochemicals obtained there from constitute a significant part of nutrients of our dietary intakes. Hence, the demand for these vital nutritional food items has expanded dramatically, due to the continuously growing population of the world and the changing life-styles and dietary habits (Sagar et al. 2018). Growing awareness and concerns relating to hunger, conservation of food resources, ecological and financial losses due to the food wastage has led to the initiatives for food conservation and the development of effective food distribution system. Both public and private sectors are making serious efforts to improve the utilization of readily available food and to effectively use the nutrients from the by-products instead of wasting them. Since significant amounts of food and its by-products are wasted globally during its utilization in the house hold and industry (Thyberg and Tonjes 2015).

The by-products or wastes form fruits and vegetables amount to around 20–60% both in solid and liquid forms, 42% of which originates from household units, while 38% is contributed by the food processing industries and the remaining 20% originates from other sources. Therefore, large amounts of vegetable and fruit wastes worldwide are contributed by various food processing industries (Wadhwa et al. 2013) (http://www.fao.org/docrep/018/i3273e/i3273e.pdf). A number of plant parts associated with vegetables and fruits are not consumed which varies from vegetable to vegetable and fruit to fruit. These include peels, husk, pomace, stems, seeds, leaves, cases, shells, hulls, stalks, grain, bran, press-cakes, and many more. After the extraction and processing of commercial food these plant parts are either treated as waste or are used for other purposes. Although these waste by-products contain valuable organic constituents and are rich in phytochemicals, antioxidants and other bio-active compounds that can be reused after successful extraction (Ribeiro da Silva et al. 2014; Sharma et al. 2016). For instance, a total of around 55 million tonnes of vegetables and fruits waste is produced by countries like China, United States of America, India, and the Philippines during the process of preparation, packaging, and circulation of the products before it reaches to the consumers. It has been estimated that more than 3.5 million tonnes of brewers-grain, 5 million tonnes of sugar-beet pulp, and a million tonne of onion peels are wasted every year (Galanakis 2012). Which clearly shows that the industrial revolution in various parts of the world is also resulting in an ever increasing volume of valuable wastes especially from the food industries (Mirabella et al. 2014). As indicated by the ongoing researches directed by UN's FAO (Xue et al. 2017), around 1.3 billion tonnes of food material worldwide, has been lost as waste every year, which represents 1/3rd of the aggregate food industry produce. To further complicate the problem the improper disposal of the waste is contributing to the environmental problems. For example, dumping of such waste in river streams or land-fills are resulting in environmental pollution and health hazards (Chaboud and Daviron 2017). Therefore to minimize this ever increasing wasting of food and to alleviate the environmental issues due to the improper disposal of these products it is important to develop

strategies for the recycling of these materials for other purposes. These approaches may either use the fruit and vegetable waste (FVW) directly or may require some processing before they can be finally used. For example, the waste can be used as feed for livestock or can be converted to products like manure through further processing. There are reports indicating the growing use of agricultural waste as animal feeds or as compost (Lin et al. 2013). Figure 1 indicates the percentage of global food waste of different commodities (Kummu et al. 2012).

As discussed earlier these wastes may also serve as a rich source of nutrients such as vitamins and minerals and therefore can also be developed as health supplements which also makes these waste materials economically important (Gowe 2015). These products can be used for dairy and poultry feeds or can also be used for human consumption if proper regulations and safety aspects are followed. Furthermore, these waste materials may be used for the extraction and preparation of other commercial products such as edible oils, essential oils, biogas, biodiesel (a fuel produced using vegetable oils and animal fats) and polyphenols. Other products from these can be adhesives from citrus oils, enzymes, therapeutics, biodegradable films, pigments, catalysts, anti-cancer drugs, antioxidant derivatives, single cell proteins, juices and vinegar produced from fruit peels (Balasundram et al. 2006). Bioactive compounds from such wastes are already reported to have antiviral, anti-cancer, anti-cardiovascular diseases, tumor reduction, and other biological activities (Deng et al. 2012; Spatafora 2012). However, one of the most important factors for the commercial success and viability of these products will be to find out effective and economic ways to purify the required components from the waste to high purity. Various methods are used for the purification of these compounds including conventional and nonconventional methods such as Soxhlet extraction, solid-liquid extractions, supercritical-fluid extraction (SFE), microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), ultrasound-assisted extraction (UAE) and pressurized-fluid extraction (PFE), pulsed-electric-field

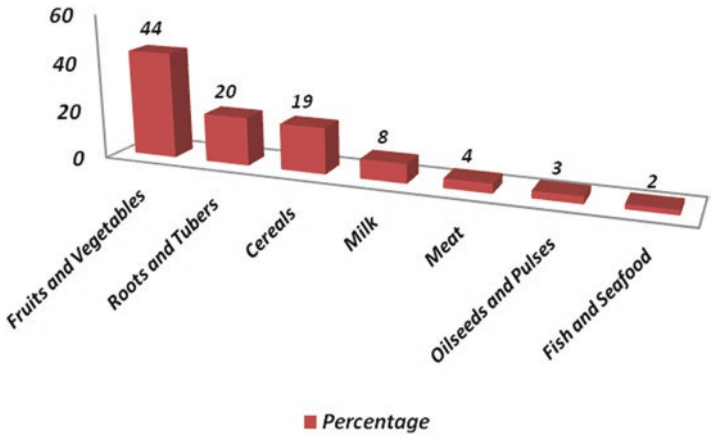

Fig. 1 Commodities of global food wastes

extraction (PEFE). The choice of method will depend on a number of factors including the type of waste, target compound to be isolated, economic feasibility and ease of execution etc. However, economic feasibility is one of the most important driving factor. Methods can also be modified or combinations of various approaches can also be used for making a given product affordable (Thyberg and Tonjes 2015). Scientific community is also trying to make these processes more economic and affordable with an aim of recycling the food waste into potentially nutritious or other valuable substances to feed the growing world population (Ayala-Zavala 2011; Wijngaard et al. 2009)

The main objective of this chapter is to review the progress made in exploring the potential of fruit and vegetable waste for the production of nutrients and bioactive compounds. This chapter discusses various methods that are used for the extraction and preparation of these compounds. It is evident from the literature discussed below that although some progress is made in this direction. But research in this area is still in its infancy and a lot needs to be done. These wastes hold great promise in the circular economy and can add great value to food and agriculture based industries.

Bioactive Compounds and Their Classification

The Bioactive compounds are secondary and sometimes primary metabolites with one or more bioactivities (Biesalski et al. 2009). These naturally occurring bioactive compounds can be obtained from a number of sources such as animals, plants, and microorganisms (Khan et al. 2017, 2011; Strobel 2003). For example, various kinds of fatty acids found in milk and dairy products may have some bioactivities (Korhonen 2009). But, plants are an especially good source of a variety of such bioactive compounds that are found in these plants in varied amounts. This diversity of the plant products is due to the biodiversity of the plants as well as the complex nature of the plants themselves (Guimaraes et al. 2014). It has been documented that the same plants growing in different geographical locations may also produce different types of bioactive compounds (Khan et al. 2018). Bioactive compounds or phytochemicals can be categorized based on various criteria, for example, according to Liu (2013), they can be grouped as nutrients, minerals, dietary fibres, and vitamins. Phytochemicals can also be classified based on their chemical nature such as amines, phenols, organic acids, terpenes, polysaccharides and organosulfurs. The phytochemicals present in food can be bioactive nutrients, saturated/unsaturated fats, vitamins, soluble and insoluble dietary fibres, peptides, phytosterols and flavonoids (Abdelkarim et al. 2014). Plant products are being conventionally used as medicinal plants for treating various diseases. But, the activities of compounds from plants are not only limited to treating diseases but can also help in maintaining good health and a robust immunity (Hooper and Cassidy 2006). Owing to the versatile bioactivities and other properties the plant products are being currently used in

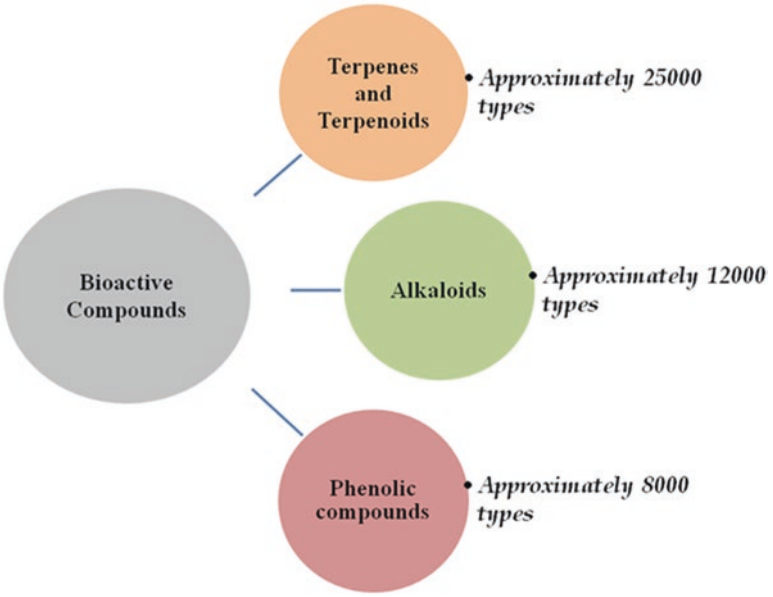

Fig. 2 Major classes of plant bioactive compounds

modern medicine and other industries such as agriculture, nanotechnology, food & flavouring industry, and cosmetics industry. Examples of modern medicine of plant origin include morphine and paclitaxel (Atanasov et al. 2015). As far as the bioactive compounds in fruits and vegetables are concerned a number of compounds are generally present in these plant products such as creatine, polyphenols, flavonoids, glucosinolates, polysaccharides, lignans, carnitine, carotenoids, kaempferol, taurine, dithiolthiones, ferulic acid, quercetin phytosterols, choline, phytoestrogens, and anthocyanins. For example, oranges contain phenols (bioflavonoids) and terpenes (limonoids and carotenoids). The plant's bioactive compounds are mainly grouped into three main categories as shown in Fig. 2 (Azmir et al. 2013).

Important Constituents of By-Products from Fruits and Vegetables

In fruits mainly the flesh and pulp are consumed, while various plant parts are utilized as vegetables. The consumption of these fruits and vegetables inevitably result in plant wastes like peels, seeds, and leaves that may contain a number of useful nutrients, bioactive compounds (for example terpenes) and other phytochemicals. Some of these plant waste materials are discussed below briefly.

Dietary Fibres

A number of plants and vegetable wastes may serve as a good source of dietary fibres, one of the most abundant sources can be onion that contains dietary fibres in varying degrees. Researchers have studied whole onion from the outermost skin to the innermost layer of three assorted species for the presence of dietary fibres. They found the highest content of the fibres in the skin of the onion. Insoluble filaments were additionally derived to be lower (11.6% DM) in the inner layers of onion contrasted with the (66.6% DM) inward parts (Benitez et al. 2011a). Benitez et al. (2011b) balanced out triturated onion waste (paste) as well triturated + squeezed onion cakes ("juice," fluid and "bagasse," solid wastes) by purification and sanitization. They saw that the industrial processes significantly affect the bioactive structures, creating items with various practical applications. Besides, sanitization was the most appropriate treatment to acquire safer products, with fructans and dietary fibres while cleansing resulted items rich in alkenyl cysteine sulfoxide (ACSO) (Benitez et al. 2011b).

Phenols

Phenols are secondary plant metabolites which when added to commercial product improve their organoleptic properties such as nutritive quality, aroma, taste, flavour, and colour. Phenolics are amongst the largest class of bio-active compounds exhibiting various important bioactivities. These compounds represent a diverse class of chemicals having one hydroxyl group in its aromatic ring (Ignat et al. 2011). Structures containing more than one phenolic ring will be referred to as polyphenols. Polyphenolic compounds are grouped into different categories, for example, phenolic acids, tannins, flavonoids (sub-classed into isoflavones, flavanones, anthocyanidins, flavanols, flavanonols, and flavones), lignans and stilbenes. The most widely found and important class of phenolic acids, are flavonoids, stilbenes, lignans, and tannins (Balasundram et al. 2006). Identification of specific phenolic compounds depends on the number of aromatic rings and other components present in the compound (Haminiuk et al. 2012).

The peels of citrus fruits are specifically rich in organic flavonoids. Therefore, the wastes acquired from the processing of citrus fruits also have a high content of phenolic compounds. Grape seeds and pomace are well known for their phenolic compounds contents, for example, epicatechin, procyanidins, gallic acid, and catechins. Apple pomace also contains polyphenols largely in the peels. The peels, skin, and seeds of leafy vegetables and fruits are also rich in polyphenols. Notably, the polyphenols constitute half of the total compounds obtained from the waste of vegetables and potato peels.

Carotenoids

Most of the naturally occurring colours in vegetable and fruits such as yellow, orange, red and others are due to the presence of different kinds of carotenoids. These carotenoids usually occur in trans conformation, but occasionally they are also found as cis isomers. It is estimated that more than 800 different types of carotenoids are found in vegetables and fruits. They can be mainly divided into two categories i.e. saturated and unsaturated carotenoids exemplified by lycopene, β-carotene and containing xanthophylls, respectively. Xanthophyll contain various functional groups such as -hydroxyl, -keto, -epoxy, and -aldehyde and are referred to as violaxanthin, canthaxanthin and β-cryptoxanthin.

Bioactive Compounds Present in Fruit and Vegetable By-Products

Fruit and vegetable by-products are rich in a number of bioactive compounds. The type and composition of these active compounds vary with the type of fruits and vegetable. In this section, the bioactive compounds present in some abundantly consumed fruits and vegetables and their by-products is reviewed (Table 1).

Apple By-Products

Apple is one of the largest grown and consumed fruit worldwide and is an important source of phytochemicals and antioxidants in the diet (Perussello et al. 2017). About 70 million tons of apples are produced worldwide per annum (Popescu 2012). Apples are not only consumed as fruits but are also used for the manufacturing of various products such as jam, vinegar, apple juice, and cider. The industrial manufacturing of these apple based products yields waste like peels, pomace, and seeds in high quantities (Xu et al. 2016). Disposal of these wastes may contribute to environmental pollution. But if used wisely these by-products may serve as a good source of bioactive compounds useful for human health and well-being. The apple peel, for example, is a rich source of cyanidin-3-galactoside, (−)-epicatechin and quercetin glycosides, chlorogenic acid, (+)-catechin, (−)-epicatechin, phloridzin, and rutin (Łata et al. 2009). These phenolic compounds help in preventing diseases such as asthma, cancer, cardiovascular disease, and diabetes. These compounds are especially known for their antioxidant activity. Apple pomace is also an abundant residue (about 1/4th of the total processed fruits) acquired after the extraction of apple juice (Bhushan et al. 2008). It is also a good source of polyphenols (Schieber et al. 2003) such as phloretin glycosides, quercetin glycosides, catechins,

Table 1 Bioactive compounds present in the by-products of apple, banana, mango, grape, and broccoli

By-products	Major classes of compounds	General use	Major compound	Activity	Reference
Apple peel	Phenolic compounds: cyanidin-3-galactoside, (−)-epicatechin and quercetin glycosides, chlorogenic acid, (+)-catechin, phloridzin, rutin etc.	Prevention in chronic diseases/used in the treatment of Alzheimer's disease	Rutin	Antioxidant, Antimicrobial, Antifungal and Anti-allergic	Al-Dhabi et al. (2015), Awad et al. (2000), Łata et al. (2009), Marks et al. (2007), Wolfe and Liu (2003)
Apple Pomace	Polyphenols: catechins, hydroxycinnamates, phloretin glycosides, quercetin glycosides, procyanidins, quercetin 3-glycosides, cyanidin 3- glycosides, Procyanidin B2, and 5-*O*-caffeoylquinic acid	Reduce the risk of cardiovascular disease, cancer, obesity, and diabetes,	Phloridzin	Antioxidant	Awad et al. (2000), Rana and Bhushan (2016), Schieber et al. (2003, 2001a, b), Xu et al. (2016), Yeap Foo and Lu (1999)
Apple seeds	Polyphenols: dihydrochalcones, quercetin, hydroxycinnamic acids, flavonols, proanthocyanin B2, flavan-3-ols ((+)-catechin and (−)-epicatechin), etc.	Antioxidants in food or cosmetics	Phloridzin	Antioxidant, Anti-diabetic	Ehrenkranz et al. (2005), Xu et al. (2016)

By-products	Major classes of compounds	General use	Major compound	Activity	Reference
Banana peel	Flavonols	Used as an antimicrobial, antifungal, and anti-allergic agent	Rutin	Antioxidant, Antimicrobial, Antifungal and Anti-allergic	Al-Dhabi et al. (2015), Passo Tsamo et al. (2015a)
	Hydroxycinnamic acids	Act as a preserving agent/skin protection agent	Ferulic acid	Antioxidant, Antimicrobial, Anti-inflammmatory and Anticancer	Ou and Kwok (2004), Passo Tsamo et al. (2015a), Srinivasan et al. (2007)
	Flavan-3-ols monomers	The resistance of LDL to oxidation	Gallocatechin	Antioxidant	Someya et al. (2002), Vu et al. (2018)
	Flavan-3-ols dimmers	Used to create bitterness	Procyanidin B2	Antioxidant	Rebello et al. (2014)
	Flavan-3-ols polymers	Used as medicinal agents for the treatment of burns	Tannins or Proanthocyanidins (prodelphinidin)	Antioxidant	Rebello et al. (2014)
	Catecholamines	Preservative agent/Parkinson's medicine	Dopamine	Antioxidant and Neurotransmitter	González-Montelongo et al. (2010), Kanazawa and Sakakibara (2000), Oh et al. (2018), Pereira and Maraschin (2015)
Mango peel	Xanthonoid	Provide protection against radiation-induced bone marrow deaths, sickness, and mortality	Mangiferin	Antioxidant	Jagetia and Baliga (2005)
Mango seed (kernels)	Phenolic compound	Used in viper bite treatment	Hesperidin	Antioxidant	Abdel-Aty et al. (2018)

(continued)

Table 1 (continued)

By-products	Major classes of compounds	General use	Major compound	Activity	Reference
Grape pomace	Anthocyanins monoglucosides	Used as color additives and natural antioxidants, in the food industry	Pelargonidin 3-*O*-glucoside and Malvidin	Antioxidant	Ramirez-Lopez and DeWitt (2014)
	Phenolic compounds	Use as antimicrobial agents and to be used in animal nutrition	Gallic acid	Antioxidant Antibacterial and cytotoxicity	Peixoto et al. (2018), Ramirez-Lopez and DeWitt (2014)
Broccoli (floret)	Glucosinolate	Helps in the treatment of cancer	Neo-glucobrassicin	Antioxidant and Anti-carcinogenic activity	Hwang and Lim (2015), Liu et al. (2018)
Broccoli (stem)	Glucosinolate	Provide protection against tumor and cancer	Glucoraphanin	Antimicrobial, Anticancer and Antioxidant	Bischoff (2016), Liu et al. (2018), Prakash and Gupta (2012)
Broccoli (leaf)	Glucosinolate	Use as food preservation and colouring agent	Neo-glucobrassicin	Antioxidant and Anti-carcinogenic activity	Jaiswal and Abu-Ghannam (2016), Liu et al. (2018), Prakash and Gupta (2012)

hydroxycinnamates, and procyanidins (Schieber et al. 2001a; Yeap Foo and Lu 1999). Another waste product is apple seeds that are equally rich in polyphenols and phloridzin is the major polyphenol present in the seeds (Ehrenkranz et al. 2005). The polyphenol of the seeds is composed of hydroxycinnamic acids, dihydrochalcones, flavonols and flavan-3-ols (Fromm et al. 2012, 2013). Other than its anoxidant activity, phloridzin also shows anti-diabetic activity by controlling intestinal and renal absorption of glucose and by inhibiting sodium-linked glucose transporters 1 and 2 (Dudash et al. 2004; Manzano and Williamson 2010). It has been found that the phenolic extracts from apple seeds have good commercial potential as a favourable antioxidant for the use in food and cosmetics (Xu et al. 2016). Some of the authors have reported that the seeds of apple have large phloridzin content as compared to peel and pomace (Diñeiro García et al. 2009; Xu et al. 2016). Hyperdin (quercetin 3-*O*-galactoside) and chlorogenic acids are the second and third major phenolic compounds, respectively in the seed (Xu et al. 2016). It was also observed that the quantity of chlorogenic acid in the seed is lower than the peel of apple (Łata et al. 2009). According to the results of different studies, it has been observed that apple seed extracts have higher content of total phenols than apple pomace extract (Bai et al. 2013; Schieber et al. 2003; Xu et al. 2016).

Banana By-Products

Banana is one of the second largest fruits produced all over the world. In the year 2016, the production of banana was nearly 113.28 million metric tons worldwide (https://www.statista.com/statistics/264003/production-of-bananas-worldwide-by-region/). Because of its high nutritional value and high productivity, it is considered that banana is one of the most popular and highly consumed fruits. The fruit can be consumed directly or after processing in the form of banana chips, ice-creams, milk shakes etc. Banana also acts as an important functional ingredient in the making of dry fruits, flour, and wine. Due to this high consumption of banana, large quantities of banana peels are generated as by products, about 33.33% of which is disposed into the landfills (Schieber et al. 2001b; Vu et al. 2018). Since the peel contains high levels of dietary fibre and phenolic compounds such as rutin, ferulic acid, gallocatechin, procyanidin B2, tannins, and dopamine, they can be used for various purposes (Al-Dhabi et al. 2015; Passo Tsamo et al. 2015a; Pereira and Maraschin 2015; Rebello et al. 2014; Vu et al. 2018). Among the peels from various fruits such as papaya, pineapple, watermelon and melon, banana peel has second highest content of phenolic compounds (Morais et al. 2015). The quantity of phenolic compounds present in the banana peel ranges between 4.95 and 47 mg GAE/g DM, which is 1.5- to 3-folds higher than its flesh, as reported by González-Montelongo et al. (2010) and Hernández-Carranza et al. (2016). Studies have identified more than 40 phenolic compounds from banana peels that can be classified into four subgroups namely flavan-3-ols, flavonols, hydroxycinnamic acids and catecholamines (Rebello et al. 2014). Among these groups, flavan-3-ols is found to be the most prevalent

group of phenolics, which includes monomers, dimers, and polymers. Among various flavan-3-ols the polymeric form represented by proanthocyanidins is the major component (3952 mg/kg), which is found as (+)-catechin equivalents. This is followed by the dimers, which contributes about 126 mg/kg as (+)-catechin equivalents (Rebello et al. 2014). Quantity wise Prodelphinidin is the most abundant form of proanthocyanidins polymer (Rebello et al. 2014). This compound exhibits remarkable antioxidant activity and is consequently being used as a therapeutic agent for the treatment of burns. While the most predominant monomer and dimer are B2 Procyanidin (81.95%) and gallocatechin compounds amounting to 158 mg/100 g dry matter (Vu et al. 2018). Remarkable antioxidant activity of these two compounds from banana extract has also been reported (Someya et al. 2002).

As reported by Passo Tsamo et al. (2015b) and Rebello et al. (2014), rutin and its conjugates such as a flavonoid, glycosides, hexoses, and 3-rutinosides are the most dominant flavonols. Due to the anti-inflammatory, antioxidant, and β-amyloid oligomer-reducing activities, Rutin has been considered as an important agent for the treatment of Alzheimer's disease (Xu et al. 2014). Additionally, rutin also exhibits other pharmacological properties such as antimicrobial, antioxidant, and anti-allergic activities (Al-Dhabi et al. 2015). Therefore the use of rutin for the treatment of various deadly and chronic diseases, such as cancer, diabetes, and other cardiovascular diseases has been suggested (Sharma et al. 2013). Furthermore, some studies report that ferulic acid is the predominant hydroxycinnamic acid which is present either in the acid form or as sugar conjugate, or as a conjugate with other compounds (Passo Tsamo et al. 2015a). Ferulic acid has strong antimicrobial, antioxidative, anti-inflammatory, antifungal, and anticancer activities due to which it is widely used as a preserving agent and in skin creams as an antimicrobial agent (Ou and Kwok 2004; Srinivasan et al. 2007). Several studies have also confirmed the presence of catecholamine's (dopamine and L-dopa) in banana peel (González-Montelongo et al. 2010; Kanazawa and Sakakibara 2000). Dopamine is a strong antioxidant which is present in significant quantities in the peel (80–560 mg/100 g) than in the pulp (2.5–10 mg/100 g) (Kanazawa and Sakakibara 2000; Vu et al. 2018). Superior antioxidant activity of dopamine compared to other compounds such as glutathione, luteolin and food additive such as BHT has been reported. Because of this antioxidant activity it has been widely used in medicines for the treatment of Parkinson's disease (Kanazawa and Sakakibara 2000). It is evident from the literature discussed above that the banana and its peel contains many bioactive compounds having valuable bioactivities such as high antioxidant capacity, high antimicrobial activity, anticancer, antiallergic activities etc. This is also evident from their wide spread use as a traditional medicine for the treatment of various illnesses, like burns, cough, anaemia, ulcers, snake bite and diabetes (Shadma et al. 1970). Banana peels also have high nutritional value, indigestible fibre, and minerals. And owing to this nutritional value it is used as a livestock feed, fertilizer, biosubstrate, and other food products. It has been also used as a natural preserving agent to improve the quality and shelf life of the food. The banana peels also have great potential as drugs and in the pharmaceutical industry. The suggested use of banana peels are not only limited to food and pharmaceuticals, and various other

uses of banana peels are suggested such as use as an adsorbent. As many studies have demonstrated excellent absorbing properties of the banana peels. It has been demonstrated that banana peels can affectively adsorb metals such as lead, copper, zinc, cadmium, and chromium (Šabanović et al. 2015; Unctad et al. 2013).

Mango By-Products

Mango is one of the most popular tropical fruit belongs to the family of *Anarcadiaceae*, and have more than 1000 cultivars (Kobayashi et al. 2013). Mango is the third most widely produced tropical fruit after banana worldwide. India and Pakistan are the predominant producers of mango in South Asia. Other countries that produce mango include Egypt, Brazil, Indonesia, Nigeria, and Mexico (Diarra 2014). Because of its sweet taste and flavour mango is generally considered as the King of the fruits and is consumed throughout the world (Kittiphoom 2012). Mangoes can be consumed directly or indirectly in the form of other products such as juices, puree, squash, pickles, leather, jam, and chutney etc. this increasing number of commercial products containing mango has resulted in an increased demand of mango worldwide. This increased industrial utilization of mango and its consumption by the house hold generates large amount of waste in the form of peel and seed (kernel) (Asif et al. 2016). The proportions of pulp, peel, seed, and kernel varies with the cultivars of mango. Mango may contain 20–65% pulp, 15–20% peel, and nearly 20–60% seed (Maisuthisakul and Gordon 2009; Masibo and He 2008). Since only the pulp is consumed, peel and seeds are disposed of as waste. These wastes contain various bioactive compounds such as polyphenols: flavononols, gallotannins, xanthones, and benzophenone derivatives and have been widely used as herbal medicines (Abdel-Aty et al. 2018; Masibo and He 2008). A number of studies have highlighted the presence of various valuable and essential bioactive compounds in mango wastes underlining its nutritional and economic importance. Compounds present in these by-products have many interesting bioactivities such as antimicrobial, antidiabetic, antianalgesic, antioxidative and anticancer activities. Owing to these bioactivities these compounds widely used in pharmaceutical industries (Abdullah et al. 2014; Berardini et al. 2005; Saleh et al. 2014). It has also been reported that the extract of mango kernel is more effective against Gram-positive bacteria than Gram-negative bacteria (Kabuki et al. 2000). Mango seed kernel contains 20.2 mg vanillin, 20.7 mg tannin, 12.6 mg coumarin, 6.0 mg gallic acid, 7.7 mg caffecic acid, 10.4 mg ferulic acid, 4.2 mg mangiferin, 11.2 mg cinnamic acid, and 7.1 mg unknown compounds per 100 g of dry seed weight (Masibo and He 2008; Soong and Barlow 2004). While mango peels contain carotenoids, dietary fibres, and polyphenols like Quercitin, syringic acid, mangiferin pentoside, and ellagic acid (Ajila et al. 2007). Lakshminarayana et al. (1970) reported that at all stages during the development of mango fruit, the number of total phenols was found to be greater in the peel than in the flesh. It has also been reported that approximately, 4000 mg Gallic acid equivalent/kg (dry matter) of total polyphenol is present in

mango peels. While raw peels of mango contain lesser total polyphenols than ripe peels (Ajila et al. 2007; Berardini et al. 2005). The major compounds and the classes of bioactive compounds present in mango waste are given in (Table 2).

Grape By-Products

Grapes are consumed as fruits as well as in the wine industry worldwide. Consumption of grapes result in the production of a number of waste products including seeds skins and stems. These waste products emanate from both industrial production of grapes-based products as well as from households. During the production of white wine, approximately 20 kg pomace is generated as waste per 100 L. Just like other fruits the by-products of grapes also contain a number of nutrients and bioactive compounds such as well-known polyphenols of plant origin like phenolic acids, flavonoids, stilbenes, and tannins. Therefore, instead of disposing of these by-products as waste, they can be used in various industries which will also help to alleviate the environmental burden. Since these waste products contain mainly nutrients and bioactive compounds they are most suited for their use in food and pharmaceutical industries. One of the category of the compounds predominantly found in grapes are polyphenols with well-known antioxidant activity (de Sales et al. 2018). The pomace that contains seeds are also rich in Non anthocyanin phenolic compounds like Gallic acid, Catechin, Epicatechin, β-type (epi) catechin dimer. Gallic acid is especially present in large amount in seeds of grapes. While the skin of the grapes are reported to have high levels of anthocyanins such as Malvidin-rutinoside, Petunidin-rutinoside, Malvidin-hexoside, and Delphinidin-rutinoside. These compounds in pomace also exhibit

Table 2 Essential bioactive compounds and its major classes present in waste parts of mango fruit (Ajila et al. 2007; Asif et al. 2016; Koubala et al. 2012; Soong and Barlow 2006)

	Compounds in mango by-products	
Major class of bioactive compounds	Mango peel	Mango seed (Kernel)
Flavonoids	Proanthocyanidins	Proanthocyanidins
	Epicatechin	Isoquercetin
	Quercetin	Quercetin
	Isoquercetin	Fisetin
	Astragalin	
Xanthonoid	Mangiferin	Mangiferin
Catechin	Epicatechin	Epicatechin
	Epigallocatechin	Epigallocatechin
	Epicatechingallate	Epicatechingallate
Phenolic acid	Gallic acid propyl ester	Gallic acid propyl ester
	3,4-ihydroxybenzoic acid	Gallic acid
	Dihydroxybenzoic acid	Gallic acid methyl ester
Hydrolyzable tannins	–	Gallotannins
Phenolic acid derivative	Ellagic acid	–

interesting bioactivities such as antibacterial and antioxidant activities. The by-products from the grapes are also used in the food industry, as natural antioxidants, and as colour additives (Peixoto et al. 2018). de Sales et al. (2018) studied the effect of grape pomace extracted from white wine production on cellular metabolism. Grape pomace exhibited antioxidant activity increased mitochondrial respiration and decreases glycolytic metabolism. Further, it was also suggested that it can also be used for the pharmaceutical and nutraceutical purposes. Due to these bioactivities of therapeutic value, the grape pomace can be used in pharmaceutical and food industry.

Broccoli By-Products

Broccoli is a nutritious vegetable which is becoming increasingly popular due to its nutritional and therapeutic value (Herr and Buchler 2010). A comparison between broccoli floret and broccoli stalk shows that bioactive compounds in broccoli are predominantly found in the florets part, which constitutes about 25% of the total plant mass. Unlike florets and leaves, stalks have high starch content and are also rich in fibre. Other studies suggest that broccoli sprouts and florets are a good source of flavonoids and phenolic acids. Broccoli sprouts have a greater amount of phenolic acids as compared to broccoli florets. These phenolic acids include benzoic, vanillic, chlorogenic, ferulic, gallic, sinapic, and protocatechuic acid. While the phenolic acids in the florets includes sinapic, ferulic acids derivatives, chlorogenic, and neochlorogenic acids (Fernández-León et al. 2012; Gawlik-Dziki et al. 2012; Nakagawa et al. 2006; Pajak et al. 2014). Although there are different reports on the flavonoids present in broccoli, but it is suggested that kaempferol is the major flavonoids in broccoli sprouts (Gawlik-Dziki et al. 2012; Pajak et al. 2014). Other authors have reported that the broccoli also contains quercetin, apigenin, luteolin, with kaempferol (Fernández-León et al. 2012). Broccoli sprouts are also a good source of sulforaphane than florets, which has been confirmed by several other studies (Nakagawa et al. 2006). The sprouts of broccoli have been shown to exhibit significant anticancer properties in studies based on cell lines. Due to this fact broccoli sprouts based diet is recommended for the treatment of large intestinal tumor (Pasko et al. 2018). The major compounds and the classes of bioactive compounds present in broccoli sprouts and florets are given in Table 3.

Techniques for the Extraction of Compounds from the By-Products of Fruits and Vegetables

A number of different methods are being used conventionally to extract bioactive metabolites from fruits and vegetables waste emanating from agriculture, and industrial processing of these products for further utilization. These include thermal and non-thermal techniques such as pulse-electric fields (PEFs), high hydrostatic

Table 3 Bioactive compounds in broccoli sprouts and florets (Fernández-León et al. 2012; Gawlik-Dziki et al. 2012; Pajak et al. 2014; Pasko et al. 2018)

Major class of bioactive compounds	Compounds in broccoli sprouts and florets	
	Broccoli sprouts	Broccoli florets
Phenolic acids	Chlorogenic acid	Caffeic acid
	p-Coumaric acid	Iso-chlorogenic acid
	Ferulic acid	Sinapic acid
	Gentisic acid	–
	Sinapic acid	–
Flavonoids	Robinin	–
Isothiocyanate	Sulforaphane	Sulforaphane
Fatty acids (Saturated)	Caproic acid	Caproic acid
	Myristic acid	Capric acid
	Lignoceric acid	Lignoceric acid
	Stearic acid	Stearic acid
	Palmitic acid	Palmitic acid
Fatty acids (Unsaturated)	Oleic acid	Oleic acid
	Linoleic acid	Linoleic acid
	Eicosenoic acid	Eicosenoic acid
	Erucic acid	Erucic acid
	α-linolenic acid	–
	Docosadienoic acid	–

weight, ultrasound, microwave, radio-frequency etc. Extraction is a basic method which is used for the isolation of phytochemicals from crude materials. A number of modern and conventional approaches are used for the purification of specific compounds from the mixture of compounds in these extracts. These techniques employ fluids at high pressures (sub-critical and super-critical states), hydrostatic pressures, electric fields, layer filtration, and ultrasounds. These techniques are referred to as microwave-assisted extractions (MAE), pressurized fluid extractions, ultrasound-assisted extractions (UAE), supercritical fluid extraction (SFE) and chemical-assisted extractions (Wijngaard et al. 2012). The approach and the technique used for the extraction of a specific phytochemical from a source is chosen based on the nature of the target compound and the properties of the source. Other factors that influence the choice of method to be used are yield, economic feasibility, and simplicity of execution etc. However, the yield remains one of the most important criteria.

Ultrasound-Assisted Extraction (UAE)

UAE is an inexpensive, speedy and efficient method for the extraction of bioactive compounds from fruits and vegetables (Da Porto et al. 2013). It uses the effective properties of acoustic-cavitations created in dissolvent under the transitory

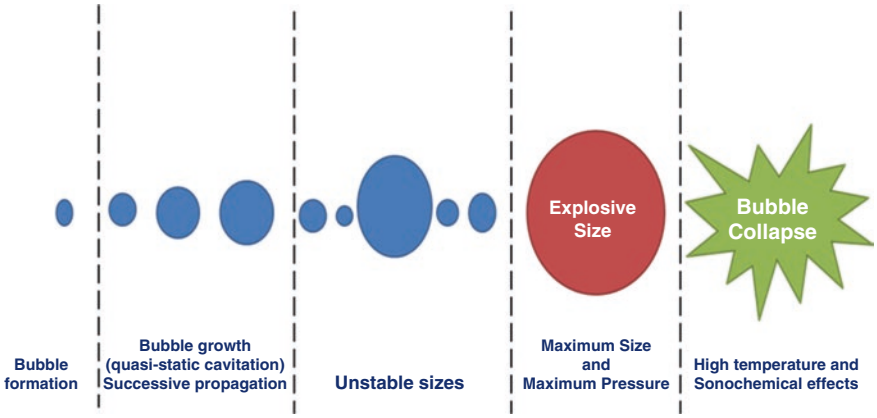

Fig. 3 Principal of UAE, bubble cavitation: formation, growth, and collapse

ultrasound-waves. Ultrasound is a specialized sound wave that is not heard by humans with a frequency ranging between 20 kHz–100 MHz. Similar to the entire wave forms; it goes through a medium in crest and amplitudes with expansion and compaction. The compaction pressure elevates the temperature, which results in the collapsing of the air pockets of maximum size (Fig. 3). Smaller air pockets either serve as new cavitation cores or are simply dissolved. Therefore the resultant 'waves' passes through the solvent, enhancing the mixing and resulting in the disruption of the cellular envelops. Ultrasound also accelerates the penetration of the solvent into the sample matrix, consequently increasing the contact surface area between the solid and liquid phase. This enhances the extraction of dissolvable solids from the material into the solvent (Rostagno et al. 2003; Wang et al. 2008). The extraction efficiency of UAE depends on various parameters such as solid–solvent ratio, time, temperature and power. The extraction of phenolic compounds from orange peel is three times faster using the UAE as compared to other conventional methods (Khan 2010). In addition, it has been found that ultrasound assisted extraction of flavonoids from hawthorn seeds is faster and gives greater yield as compared to flux extraction method (Pan et al. 2012).

Microwave Assisted Extraction (MAE)

Another method for the extraction of different bioactive compounds employs microwaves and is called microwave assisted extraction (MAE). The microwave is an electromagnetic wave that possess both magnetic and electric field oscillating perpendicular to each other. The frequency of microwaves ranges between 300 MHz and 300 GHz. During the extraction, heat is generated due to the dipolar rotation, and ionic induction of electric field. Ionic conduction generates heat due to the resistance of flow of ions in the medium. While due to the dipole rotation ions

continue to change their direction with the field charge resulting in the collision between molecules. And this collision between molecules generates heat (Bouras et al. 2015). In MAE process, increased temperature and pressure loosens the compound from the active site of the sample matrix, after that solvent diffuses into the sample matrix and then finally, the compounds are released from the sample matrix to solvent (Ani et al. 2012). This extraction technique is widely used for the extraction of phenolic compounds and other antioxidants from different fruits and vegetables, such as tomatoes, grapes, blackberries etc. (Li et al. 2012; Liazid et al. 2011; Wen et al. 2015). Several studies confirmed that MAE is a rapid, efficient and green extraction technique that requires a lesser solvent, reduces extraction time, provides a better quality of extract and high extraction efficiency as compared to other conventional methods (González de Peredo et al. 2018). The efficiency of the MAE process depends on a number of variables such as solvent, solid/solvent ratio, extraction time, particle size, extraction temperature, microwave power and time (Routray and Orsat 2012).

Supercritical Fluid Extraction (SFE)

SFE is a green technology and has a wide range of application in the extraction of compounds from the by-products of fruit and vegetable. This technology mainly depends on the supercritical state of the fluid. Supercritical state is a condition which is achieved when a fluid is exposed to high pressure and temperature which is beyond its critical point. The value of pressure and temperature above which liquid and gas phases of a substance have the same density is known as a critical point. Therefore, at this supercritical point, a fluid loses typical properties associated with its liquid or gaseous phase. As the process is irreversible the state of fluid cannot be reverted back to liquid or gas by modifying pressure and temperature. Liquid and Gas like properties of supercritical fluid such as density, solvation power, diffusion, viscosity, and surface tension, make it suitable for extracting compounds rapidly with high yield (Azmir et al. 2013). The critical temperature and pressure of the Carbon dioxide are 31.2 °C and 72.9 atm, respectively. It is considered as an ideal solvent for SFE method because it allows the extraction at low temperature and pressure and therefore is suitable for heat sensitive compounds. The only limitation with CO_2 is that it is a nonpolar substance; it can only be used for the extraction of nonpolar compounds and other limited components such as lipids and fats. For the extraction of polar compounds, it is necessary to add some co-solvents such as dichloromethane, methanol, hexane, and isopropanol along with the CO_2 to increase the polarity of the solvent mixture (Pham 2017). The extraction efficiency of SFE is mainly affected by solvent and co-solvent flow rate, extraction time, temperature, pressure, bed diameter, bed height, size of the sample, density, and porosity (Azmir et al. 2013; Mousavi et al. 2018).

Pressurized Liquid Extraction (PLE)

PLE is a well-known extraction method for the extraction of bioactive compounds from plant products. This method has some other names such as high pressure solvent extraction, enhanced solvent extraction, accelerated solvent extraction and pressurized fluid extraction. In PLE process the solvent is accelerated at high pressure due to which the temperature of the solvent raises above its atmospheric boiling point while the solvent remains in a liquid state. The increased temperature of the solvent speed up the extraction process by increasing the solubility and mass transfer properties, while the viscosity and surface tension of the solvent also decreases due to which it penetrates into the sample matrix easily and reduces the interaction between bioactive compounds and matrix. At the same time it also increases the solubility of compounds into the solvent (Lozano-Sanchez et al. 2014). Water and ethanol are normally used as solvents even they are also recommended as GRAS (generally recognize as safe) solvents. PLE has many more advantages over other extraction methods such as it improves the extraction yield, decreases extraction time and solvent consumption. It has been reported that PLE gives higher yield of carotenoid (approximately two times) from pressed palm fibre with ethanol as solvent as compared to solvent extraction method at the optimal condition of temperature 55 °C, pressure 4 MPa and flow rate 2.4 g/min (Cárdenas et al. 2015) (Table 4).

Table 4 Preliminary extraction of various bioactive compounds from the waste of fruits and vegetables using different extraction methods

Extraction method	By-product of fruit and vegetable	Bioactive compounds	References
MAE	Grape seeds	Phenolic compounds	Hong et al. (2001), Li et al. (2011)
MAE	Grape seeds (*Vitis vinifera*)	Phenolic antioxidants	Krishnaswamy et al. (2013)
MAE	Grape skins	Anthocyanins	Liazid et al. (2010)
MAE	Apple pomace	Polyphenols (chlorogenic acid, caffeic acid, syrigin, coumaric acid, phlorizin and quercetin)	Bai et al. (2010)
MAE	Apple pomace	Pectin	Wang et al. (2007)
MAE	Sea buckthorn Berries Pomace	Phenolic compounds	Perino et al. (2011)
MAE	Orange peels	Pectin	Prakash Maran et al. (2013)
MAE	Citrus mandarin peels (Kinnow fruit)	Gallic acid, vanillic acid, *p*-coumaric acid, and ferulic acid	Hayat et al. (2009)
UAE	Apple pomace	Flavan-3-ols, procyanidins, dihydrochalcones phenolic acids, flavonols	Virot et al. (2010)
UAE	Citrus peels	All-trans-β-carotene	Sun et al. (2011)
UAE	Grape by-products	Anthocyanin	Corrales et al. (2008)

(continued)

Table 4 (continued)

Extraction method	By-product of fruit and vegetable	Bioactive compounds	References
UAE	Orange peel (*Citrus sinensis L.*)	Flavanone glycosides	Khan et al. (2010)
UAE	Blueberry (*Vaccinium ashei*) wine pomace	Phenolic compounds and anthocyanins	He et al. (2016)
UAE	Winter melon seed (*Benincasa hispida*)	Antioxidants	Bimakr et al. (2013)
UAE	Coconut shell powder	Phenolic compounds	Rodrigues et al. (2008)
SFE	Grape seed (*Vitis vinifera L.*)	Linoleic, stearic and oleic acids	Prado et al. (2012)
SFE	Grape bagasse (*Vitis vinifera*)	Syringic, vanillic, gallic, *p*-hydroxybenzoic, and quercetin	Farías-Campomanes et al. (2013)
SFE	Orange pomace	Flavonoids	Benelli et al. (2010)
SFE	Apple peels	Glycosides derivatives, quercetin glycosides	Díaz-Reinoso et al. (2006)
SFE	Apple and peach pomace	Phenolic compounds	Hasbay et al. (2007)
SFE	Apricot pomace and bagasse	β-carotene	Khosravi (2010), Sovova and Stateva (2011)
SFE	*Citrus depressa* Hayata peels	Flavones (Nobiletin and tangeretin)	Lee et al. (2010)
SFE	Cherry pomace	Phenolic compounds	Adil et al. (2008)
EAE	Tomato processing waste	Lycopene	Zuorro et al. (2011)
PLE	Pressed palm fibre	Carotenoids	Cardenas-Toro (2015)
PLE	Sea buckthorn (*Hippophaë rhamnoides* L.) seed	Antimicrobial and antioxidant components	Michel et al. (2012)

MAE microwave assisted extraction, *UAE* ultrasound assisted extraction, *SFE* supercritical fluid extraction, *EAE* enzyme assisted extraction, *PLE* pressurised liquid extraction

Utilization of Bioactive Compounds Extracted from By-Products of Fruits and Vegetables

Anti-microbial compounds of plant origin are an attractive alternative for generally used antibiotics and antimicrobials. Phytochemicals from various plants have been conventionally used as traditional medicine to treat various microbial and non-microbial diseases. One of the most popularly used antimicrobial agents of plant

origin are essential oils (EOs). These oils are a mixture of aromatic compounds with the peculiar smell and are volatile in nature. The citrus fruits based industries also generate a high quantity of essential oil containing by products as waste. Oils acquired from the skin of fruits and vegetables have been utilized for various applications. Concentrates of fruits or essential oils are also used in Mozzarella cheese as a preservative and or antimicrobial agent. The use of such concentrates and bioactive agents has increased rapidly in recent years (Conte et al. 2007). Interestingly these compounds are shown to control undesirable microflora without affecting the indigenous micro-biota.

Mango Peel and Kernel

Peel and Kernel are the by-products of mango that are produced during the processing. Protein, fat, and starch are the major components of mango seed although mango seed kernels have low protein content. Mango seed kernel is a good source of polyphenols, phytosterols, sitosterol, and tocopherols. Due to the presence of a significant amount of fat, protein and some natural antioxidants it can be used as a valuable ingredient in functional food and is also used in cosmetics (Kittiphoom 2012). Many researchers have concluded that the oil extracted from the mango seed kernel is safe, nutritious and nontoxic; and can be used as an alternative for edible oils. Additionally, mango peels can also be used as a livestock feed and as an adsorbent. It has been demonstrated that peels can be used for the removal of lead from industrial effluent (Araya 2015; Kanjilal et al. 2014).

Banana Peel

Bananas are produced in large quantities annually. The main waste product emanating from banana is its peel which constitutes about 35% of the total fruit weight. The peel mostly discarded as waste also contains compounds with potential therapeutic values. Studies have shown that these compounds from banana peel have the potential to cure ailments like stomach ulcers, depression, anemia and blood pressure (Pereira and Maraschin 2015). Other than being used as traditional medicine, the banana peel is also used a livestock feed, due to its rich nutrient content. The nutrient content of the peels include carbohydrates, protein, and lipids and may contain even essential minerals like potassium (Anhwange 2008). Banana peel is also used as organic fertilizer and various methods are used to make fertilizer from banana peels (Kalemelawa et al. 2012). The peel is also used for the production of wine and in the cultivation of edible mushrooms (Oberoi et al. 2011). Banana peels are also used as excellent absorbent for several heavy metals like lead copper and zinc (Castro et al. 2011).

Pomegranate Peel

Pomegranate peel extract (PE) demonstrate remarkable cancer prevention activity, while the seed extracts from pomegranate did not show any such activity, presumably to the chemical composition of the two tissues. Faizan and Kumar (2018) found that the amount of total phenolic content in the pomegranate peel was 140.61 mg GAE/mL when extracted with ethanol. It was also observed in the study that these phenolic compounds exhibited antioxidant and antimicrobial activities. For example, the notable antimicrobial activity of the peel against *Bacillus cereus* and *Staphylococcus aureus* was observed. Furthermore, the use of pomegranate peel concentrate in chicken and meat items improved their shelf life by 2–3 weeks during the cold-storage. Pomegranate peel extracts were also effective in controlling airborne oxidative-deterioration in poultry products (Kanatt et al. 2010). In addition to peels, other wastes from the pomegranate such as seeds etc. may also serve as a good source of bioactive compounds and may have a potential role in the pharmaceutical industry (Kaderides et al. 2015).

Orange Pomace

Orange pomace is rich in fibre (40.46% of orange fruit) but other than fibre it also contains other organic substances and minerals but is low in its fat content (2.15% of orange fruit). The orange pomace can be used as a substance with enhanced water/oil adsorption and binding activity as the fibres from orange pomace have high water hydration limit (4.40 mL/g).

Aloe Vera

The use of *Aloe Vera* as medicinal plant is gaining popularity and currently is being used in various therapeutics (Sahu et al. 2013). Although, *Aloe Vera* is not quite palatable due to its unpleasant taste but it has various health benefits as it contains more than 200 bioactive chemicals. Health benefits of *Aloe Vera* include its application in lung cancer, wound healing, minimizing frost bite damage, treating burns, reducing low density lipoprotein (LDL), protection against skin damage from X-rays, fighting acquired immune deficiency syndrome (AIDS), intestinal problems, increasing high density lipoprotein (HDL), and reducing blood sugar in diabetics (Ahlawat and Khatkar 2011; Sahu et al. 2013; Surjushe et al. 2008). Gel extracted from the leaves of the plant shows significant antioxidant activity which is at par with the commercially used antioxidant agents such as butylated hydroxyltoluene (BHT). The antioxidant property of the plant is attributed to the presence of high concentrations of glucosides and polysaccharides. Therefore, *Aloe Vera* is of great use in antiaging creams, other pharmaceuticals and in the food industry (Gupta and Malhotra 2012).

Pineapple

Pineapple is one of the most widely occurring fruits of the family Bromeliaceae and is found around the world. It is the third most consumed fruit worldwide after orange and squashed apple (Upadhyay et al. 2010). Pineapple is also a good source of bioactive compounds and these may be obtained also from the waste of pineapple such as lingering mash, strips, stem, and leaves (Kodagoda and Marapana 2017). Processing and consumption of pineapple yield a significant amount of waste which may result in environmental concerns if not utilized or disposed of properly. Sugars are excessively found in pineapple strip that can be utilized as supplements in aging procedures. The strip can be utilized for the production of methane, ethanol, and hydrogen. Bromelain is also an industrially important enzyme that can be obtained from pineapple stem. Bromelain is known for its anti-inflammatory activity and is used for treating the swelling especially of nose post-surgery (Tochi et al. 2008). This enzyme has also been used as a meat tenderizer, bread batter improver, tooth brightener, animal feed, and in the material industry (Choonut et al. 2014; Kodagoda and Marapana 2017).

Potato Peels

Potato peels are generated in large quantity, as it is widely used around the world. Potato peels are a rich source of dietary fibres which plays an important role in gut mobility and in well-being of humans (Yang et al. 2017). For the preparation of potato peel fibres, potato peels are washed with water and dried in a boiler at 600 °C for 12 h. After this, they are processed to a molecular size of 500 μm and sieved and are finally refrigerated. The yield of the fibre (Ncobela et al. 2017), from peels is in the range of 61–125 g/kg of crude. Potato pomace is also a good source of fibre (27–35 g/100 g) (Sharoba et al. 2013).

Tomato Peel

Tomato Peel can be utilized for the production of pectin. Grassino et al. (2016) reported that pectin from tomato peel could be used as a corrosion inhibitor. It was also found that the inhibition efficiency for tin is 73%. Also, the processed tomato waste can be utilized in pan bread instead of wheat flour. Ćetković et al. (2012) reported that the by-product of tomato, obtained from the juice processing industries is a good source of phenolic antioxidants and anti-cancer agents. Therefore, it can be used as a cancer preventing agent in many medicines. It was further found that the extract of tomato Saint Pierre (variety of tomato) exhibit maximum activity against cancer cells in the HeLa cell line as compared to other varieties tested.

Tomatoes are the major source of lycopene which has many health benefits such as it decreases the risk of heart disease and cancer. Lycopene extracted from tomato by-product shows anti-oxidant, hypolipidemic, and anti-carcinogenic activities therefore, it can also be used for the manufacturing of meat products (Viuda-Martos et al. 2014).

Commercial Products Available in Market Having Compounds Extracted from By-Products of Fruits and Vegetables

With the increasing demand of the food and the health conscious consumers the food industry is developing new food products containing additional health benefits and are trying to minimise the waste from the industry. This has led to the new concepts like "super food" and "functional foods". Some of the commercial products containing additives from vegetables and fruit by-products for improving properties such as flavours, color, shelf life, and health benefits are shown in Fig. 4. Examples of some of these commercial products are also discussed below.

1. Pineapple waste has been used for the production of lactic and citric acids through submerged and solid-state fermentation.
2. Peels of pineapple are utilized as biogas feeds in biogas plant, due to high protein and carbohydrate contents.
3. The dietary fibres (DFs) extracted from various by-products have been utilized as a wheat-flour alternative in cake and pastry preparations. The peels of the potato are rich in dietary fibre, which is utilized in bread making industry as a good source of dietary fibre.
4. Recently it was examined that the extract of olive oil waste are rich in polyphenols, which acts as a characteristic antioxidant in sheep meat patties. Outcomes were encouraging, demonstrating that the polyphenolic concentrates could enhance the item usability, time-span by the prevention of oxidative-decays and discolourations.
5. Processed beef-sausages with the addition of grape-pomace (1% w/w) had a diminished rate of lipid-oxidation and a characteristic flavour (including colour and tastes) (Riazi et al. 2016).
6. The "sugar syrup" extracted with solvent from citrus peel is utilized as a natural food-grade sweetening agent (AU1983/0011308D).
7. Tomato waste extracts such as lycopene are utilized as nourishment, cancer preventing agent and supplement (PCT/EP2007/061923).
8. Extracts of bioactive silver-skin from the waste of coffee silver-skins have potential utilization in health, food and cosmetic industries (WO2013/004873) (Socaci et al. 2017).

Fig. 4 Web-grab of some commercial products containing bioactive compounds from vegetable and fruit by-products available in the market

9. Waste from fruits such as apple, orange, and pear are utilized in cake making due to the presence of soluble dietary fibre fraction and pectin substances (Sharoba et al. 2013).
10. The compound proanthocyanidines extracted from the seeds of cranberry and grapes are widely used as a coloring additive in soy sauce (JP1998/0075070) (Socaci et al. 2017).

11. The polyphenols extracted from the seeds of grape pomace are used in food supplements (WO/1999/030724) (Galanakis 2015).
12. The compound hydroxytyrosol extracted from olive leaves is used as a natural antioxidant in many food products (EP 1582512 A1) (Socaci et al. 2017).

Conclusion

The present chapter discusses about the valuable bioactive compounds containing by-products that can be obtained from various wastes emanating from the consumption of fruits and vegetable in industries and households. These compounds exhibit bioactivities ranging from antimicrobial, antioxidant, to anti-cancer activities and are obtained from the by-products such as pomace, shells, peels, seeds, and skins. These compounds include natural and essential oils, organic-acids, dietary fibres, flavors, catalysts and phenolics etc. Citrus fruits for example are rich source of essential oils and yields 0.5–3.0 kg oil/tonnes of organic product. Being volatile and having antimicrobial activity these oils can be used as aroma, in candy parlours, in pharmaceuticals, and can also be used as food additive to enhance the shelf life of food. Seeds, shells, pomace, and peels are rich source of polyphenols and interestingly contain twice the amount found in edible tissues. The fat in mango seed is a promising source of edible oil and its unsaturated fat and triglycerides are similar to those found in cocoa spread. Food waste like tomato peels, pomace of carrots can also be used for the extraction of pigments such as carotenoids and anthocyanin etc. Vegetable and fruit waste are also good source of dietary fibers like starch-free polysaccharides, cellulose, and pectin. Adding these components from the waste in the food helps to increase food bulks. Consumption of such foods consequently helps in prevention of constipation, and the exclusion of toxic elements.

Despite the fact that these by-products contain a number of commercially bioactive compounds and other valuable components, large quantities of such by-products are being still disposed as waste. This may be due to the lack of effective methods to efficiently and economically obtain these compounds from these wastes. Another factor that may also result in this wastage is the logistic to collect these wastes. But the waste that is generated by these fruits and vegetable based industries can be effectively reused for the manufacturing of other human health and well-being products. A number of researches are working around the globe to develop economic and efficient methods for the same. Customized methods can be developed for obtaining different products from different wastes for different purposes. A lot of other by-products should be identified as the potential source of these compounds and the information should also be shared with the industries so that these waste products are utilized instead of being disposed of as waste. However, a lot still needs to be done to harness the true potential of these by-products.

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Phytochemicals of Whole Grains and Effects on Health

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Abstract Grains are important raw materials for staple food products in the diet. Since they are not only a good source of carbohydrate content, provides for daily energy intake, but also protein, vitamins B-complex source for an adequate and balanced diet. Moreover, in recent times, it was elucidated that whole grains involve several bioactive compounds namely phytochemicals. Phytochemicals are non-nutritive dietary bioactive compounds and secondary metabolites generated by plants to protect themselves against environmental stress or threats. Whole grain phytochemicals comprise of dietary fiber as β-glucan, arabinoxylan, inulin, resistant starch; phenolic compounds as phenolic acids, anthocyanins, tocols (tocotrienols and tocopherols), lignans, alkylresorcinols, carotenoids (lutein, zeaxanthin, etc.) and other phytochemicals as phytic acid, phytosterols, γ-oryzanol, avenanthramides, benzoxazinoids. Phytochemicals could improve health and/or hinder some chronic diseases by means of whose antioxidant, anticarcinogenic, antimicrobial, antimutagenic, and anti-inflammatory activities. Epidemiological studies support that consumption of whole grains and food products are related to decreasing the risk of coronary heart disease, type 2 diabetes, obesity, oxidative stress, and some cancer types. The phytochemicals are mainly present in outer layers of grains as germ and bran parts. For this reason, the content of whole grains phytochemical is higher than refined ones owing to the milling process. In some cases, the processing negatively affects the bioactive components but there are contradictory remarks and studies about the stability of phytochemicals during processing. This chapter will briefly discuss not only phytochemicals of whole grains and effects on health but also the effect of processing on whole grain phytochemicals.

Keywords Phytochemicals · Grains · Health · Processing · Bioactive compounds

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Introduction

Cereals are edible seed and taxonomically belongs to *Poaceae* namely *Gramineae*, in other words, grass family (Jones and Engleson 2010; Gani et al. 2012). Dates from commencing of civilization, cereals are staple foods not only for human consumption but also livestock feed (Poutanen 2012; Gani et al. 2012). Since cereals are one of the main raw food material and fundamental energy sources which supply almost half of the energy for humans throughout the years in the world. Cereal-based foods could supply mostly the intake of the carbohydrate and dietary fiber (Poutanen 2012).

The main cereal grains are composed of wheat, rice, maize (corn), rye, barley, oats, triticale, sorghum, and millet (Slavin 2004). Among them, the most consumed ones are wheat, rice, and maize, despite oats, rye, barley, millet, and sorghum are more extensive in some countries based on cultural, climate (Seal 2006) and regional differences (Schaffer-Lequart et al. 2017). In this regard, the most important cereals for human nutrition are wheat in Europe, rice in Asia which corresponds to nearly half of the cereal consumption annually, maize in Central America and South America, millets and also sorghum in Africa (Poutanen 2012). According to recent data of the Food Agricultural Organisation, the ultimate forecast for production of cereal around the world in 2018 stands at 2595 million tonnes (Anonymous 2018a).

Whole grains comprised of three primary parts as the bran, endosperm, and germ (Jacobs and Gallagher 2004; Seal 2006) from outside to in and the relative ratios of these parts vary from one species to other species (Seal 2006). In this regard, all cereal grains contain a preservative hull and also the endosperm, bran, germ parts underneath of it. The germ part involves plant embryo and contributes lesser to the dry weight in many grains as 4–5% in wheat and barley typically, whereas the germ part in maize comprises higher ratios of the total grain quantitatively than other grains as wheat, oats, and barley (Slavin 2004). The germ fraction supplies critical nutrients which are needed to develop new plant and, rich in vitamin E, antioxidants, other phytochemicals and lipid-soluble compounds (Jones and Engleson 2010). The endosperm part is composed of about 75–80% of the kernel weight (Liu 2007), viz. the largest part of the grain (Jacobs and Gallagher 2004) and main energy sources for growing plants (Jones and Engleson 2010), during germination due to its high amount of starch content about 50–75% of the endosperm (Slavin 2004). The second largest part of the grain is the bran part (Jacobs and Gallagher 2004), which surrounds and covers the endosperm and germ fractions and preserves the grain from the weather, insects, molds, and bacteria (Slavin 2004). The outer bran layer is comprised of non-digestible, insoluble, inadequate fermentable carbohydrates like cellulose, hemicellulose, arabinoxylan and also other phytonutrients like polyphenols whereas germ and starch endosperm involve soluble fiber, resistant starches, fermentable oligosaccharides, lignin, polyphenols, oils, vitamins and minerals (Slavin et al. 2013).

Earlier times, the entire grains in other words whole grains were used by human (Poutanen 2012) and were the part of diet nearly for 10,000 years ago (Slavin 2004).

During those dates, people utilized gristmills which could not able to separate the bran and germ fractions from the endosperm completely (Slavin 2004). The endosperm was the most important fraction in terms of technological and scientific attention for food processing until the end of the past century (Kong and Singh 2008; Poutanen 2012). In the 1830s, Sylvester Graham was suggesting whole grain food beforehand the common availability of refined flour (Jacobs and Gallagher 2004). However, subsequent to using roller mill which separates more effectively bran and germ parts from the endosperm than gristmills by 1870s, it created a great demand for refined grain products (Slavin 2004), particularly in Western industrialized countries (Seal 2006). The utilizing of the rolling miller was a not only effective, profitable and simple way to supply refined grains (Jacobs and Gallagher 2004), but also they were thought higher characterization about nutritional, sensorial and also hygienical when comparing unprocessed grains (Vitaglione et al. 2008). Thus, most of the population has intake generally refined grain products for the last 100 years (Slavin 2004).

Health sides of whole grains are known for a long while. Dates back to fourth century BC, Hippocrates discerned the health benefits of whole grain bread. In the early 1800s to mid-1900s, scientists suggested whole grains could hinder constipation (Slavin 2004). In the early 1900s, there was claims and discussions about whether refining was proper. Refined grains made a contribution to vitamin deficiency diseases as pellagra and beri-beri. In 1937, discoveries of thiamin and niacin were the lacking factors cause these ailments was surprising. The restorative power of these substances causes a belief that nutritional value could be restored by enriching of refined grain products, what could be called as the "Wonder Bread" phenomenon. Walker, Burkitt, Cleave, and Trowell countered to this view by developing the notion that whole foods were healthful, yet they interpreted their ideas into statements about dietary fiber (Jacobs and Gallagher 2004).

Then, Trowell first proposed the 'fiber hypothesis', published in 1972, suggested that whole grain enable dietary fiber including derived from whole grains with other components which have affirmative effects on human health and therewith intake of whole grains augmented slightly. After that, this hypothesis has become 'high fiber food' hypothesis with the result of that 'fiber' has become the focus of public health suggestions (Seal 2006). Nowadays, there is a growing interest to outer layers of grains. Therefore, the importance of the whole grains was getting well understood on the health due to whose phytochemicals, vitamins and minerals content (Kong and Singh 2008; Poutanen 2012).

The progression of milling technologies could control extractions rates. The average extraction rate is nearly 75% in wheat and the rest of it namely, the bran fraction is separated and generally utilized for animal feed. The extraction rate also defines the composition of flour with regard to nutritional value (Hemery et al. 2007; Poutanen 2012). The relation between consumption of cereal based food and health outcomes is depends on various factors such as the variety of cereal, the form of the cereal such as whole grain or refined, the fiber and polyphenol content, micronutrient composition, and glycemic index. Epidemiological studies pointed consistently out that there is an association between cereal consumption, particularly

whole grain and preclusion of many epidemics of non-communicable diseases, but the mechanisms involved are still not well elucidated (Gibson et al. 2013).

Many different terms and explanations were utilized to define whole grains in the world, and these disparities made it difficult to comprehend of whole grains (Slavin 2004). "Whole grain" is an abbreviation of "whole cereal grain" for an American term (Jacobs and Gallagher 2004). The European countries were generally used 'wholemeal' term to describe a finely ground whole grain flour or whole grain bread. On the other hand, 'whole grain' term was utilized and described not only bread, cereal products, and also both finely and coarsely ground flour in the USA. In this regard, several definitions of whole-grain have been defined to provide a common comprehension of whole grains (Slavin 2004).

First of all, AACC (American Association of Cereal Chemists) members defined and agreed on whole grain term in 2000. According to this term, 'whole grains should include intact ground, cracked or flaked caryopsis (seed or kernel), whose principal anatomical components endosperm, germ, and bran are present in the same relative proportions as they exist in the intact caryopsis' (AACC 2000; Slavin et al. 2013). In this regard, whole grains involve all cereals and pseudocereals (Anonymous 2018b). In 2004 this description was adopted by the 'Whole Grains Council' (WGC) as whole grains or whole grains foods are made from kernel, in other words entire grain seeds, that comprise whose all main parts as germ, endosperm, and bran from the inside out, and nutrition values in the same ratios even if they expose to some processes like cracking, crushing, cooking, and/or extruding and rolling. In respect to this, they claimed that whole grains are composed of wheat and whose varieties such as spelt, einkorn, emmer, farro, Khorasan wheat, Durum wheat and also whose forms as wheatberries and bulgur; rye, oats (involving oatmeals), barley, maize (involving popcorn and whole cornmeal), rice (brown and coloured rice), millet, sorghum stated in other words milo, triticale, teff; pseudocereals (amaranth, buckwheat, quinoa) and some *Gramineae* members as Job's tears, canary seed, fonio and montina (Anonymous 2004). In 2006, the AACC International Whole grains Task Force in a letter to FDA (US Food and Drug Administration) confirmed that whole grains are referred to not only whole grain cereals but also pseudocereals whose overall macronutrient composition is analogue with cereals (Jones and Engleson 2010). In 2009, the FDA identified whole grains ecologically with AACC but only endosperm part explained as most of the inner part of the kernel. In terms of FDA, whole grains include all grains and pseudocereals in a similar vein with AACC (Anonymous 2018b). Pursuant to term, whole grains may embody following cereals like wheat, rye, oats, barley, maize, rice, millet, sorghum, triticale, teff, bulgur and some pseudocereals such as buckwheat, amaranth, and quinoa. Nevertheless, in an agreement with AACC, WGC and FDA, legumes as soybean, chickpea; oilseeds as *sunflower seed*, *flax and chia* (Anonymous 2004). For regulation of the European Parliament and of the Council, whole grains describes as irrespective of characteristics produced at each stage of milling and end of the part has been only removed (Anonymous 2018b). Also, there is not a common idea about the extent of whole grains between other issuing bodies. According to Sweden health claim code of practice, whole grains comprise of only some cereals like wheat, rye,

oats, and barley. Danish Task Force added that different milling fractions of these grains are allowable on the condition that relative ratios of germ, endosperm, and bran must be the same as in the intact germ. In addition, pseudocereals, fresh maize flour, and popcorn are not accepted as whole grains. Health authorities of three Scandinavian countries (Sweden, Norway, and Denmark) approved some rules about the declaration of healthy foods as an intitle of Scandinavian Keyhole. Taking into consideration that they accepted the same cereals as whole grains except triticale. Furthermore, within this context, they declared the parts of whole grains which are exposed to dehulling, grounding, cracking, flaking as processed fractions should adding back to final product even if they are separated (Frølich and Åman 2010). Even though EFSA (European Food Safety Authority) elucidated health claims related to whole grains in 2010 (EFSA 2010). EU countries organized a consortium as 'HEALTHGRAIN' and come to an agreement with definition and content of whole grains, a nutritional recommendation as a guidance and labeling objectives in 2010 (Van der Kamp et al. 2014). In contrary to the updated AACCI definition, minimal losses during the processing of whole grain-based foods are allowed vis à vis this definition (Seal and Brownlee 2015). In this regard, during processing, only fewer than 2% of the grain and 10% of the bran losses are permittable for consistent quality (Anonymous 2018b).

Ever since the early 1980s, epidemiological studies continuously showed that people who eat more whole grains reduced the risk of certain chronic diseases (Schaffer-Lequart et al. 2017). Therefore, intake of whole grains and/or whole grain-based food products in the diet regularly is related with enhancing health and playing a role with preventing the risk of some health diseases such as cardiovascular disease, hypertension, obesity, type 2 diabetes, metabolic syndrome and some types of cancer (Borneo and León 2012). This notion is still remarkable, even after adjustment for dietary fiber (Ye et al. 2012; Schaffer-Lequart et al. 2017). Moreover, recent studies showed that intake of plant-based bioactive compounds as a complex mixture of whole grains, fruits, and vegetables could be more beneficial and healthful than individuals isolated compound (Liu 2007; Slavin 2013) by showing synergistic effects (Gani et al. 2012). Thus, it is recommended that people should consume a wide variety of plant-based foods daily in order to get the highest health benefits (Liu 2013). Therefore, several states, non-profit organizations, trade, and industrial communities non-profit health, and industrial and trade groups have supported the consumption of whole-grains more than the last 35 years (Slavin 2004). Nevertheless, there has been no worldwide standard of what involves a "whole-grain food in spite of defining the "whole grain" term properly. Thus, it is creating challenges for not only researchers, the food industry, regulatory authorities, but also consumers all over the world (Ferruzzi et al. 2014). To develop a definition for whole-grain foods that could be accepted worldwide and applied to dietary recommendations and planning was issued by the U.S. Dietary Guidelines Technical Advisory Committee (DGTAC) as part of the 2010 Dietary Guidelines for Americans (DGA)" (Ferruzzi et al. 2014). In 2010, the Dietary Guidelines Advisory Committee (DGAC) calls for the development of criteria for labeling whole grain foods to reduce confusion both for researchers and consumers. To ensure that products labeled with a whole grain

health claim will provide substantive amounts of whole grains, it must be the first ingredient listed on the ingredient label, meaning it makes up 51% of the product by weight. Also, most of the whole grains could be processed as grounding, cooking, parboiling, extruding, pearling, rolling, and milling and terms are not put in order, therefore, it could be ambiguous. Due to most whole grains are processed, the FDA acts in concert with the AACC and established a labeling definition. To be considered a "whole grain," each of the principal components of the grain as endosperm, germ, and bran must be present in the same relative proportions as they exist naturally in the seed (Harris and Kris-Etherton 2010). So the ratios of whole grains in whole grain foods for the health claims are different between some countries (Van der Kamp et al. 2014). Therefore, many countries try to find a common way to describe whole grain food products (WGFP) to be labeled (Slavin et al. 2013). In the USA and the UK, WGFP must contain equal or greater than 51% whole grain ingredients on a wet weight basis. In Denmark and Sweden, the minimum level of whole grains is 50% whole grain in dry weight basis. Moreover, this level is higher than others in Germany and their whole grain wheat and rye bread includes 90% whole grain (Van der Kamp et al. 2014), whereas pasta 100%. Recently, AACCI approved that WGFP must contain equal or more than 8 g whole grain for per 30 g end product (Slavin et al. 2013).

Whole Grain Phytochemicals

"Phytochemical" term is derived from the league between the words "chemical" and *phyto*, which means plant in Greek (Liu 2013), and named as also plant bioactive substances (Gani et al. 2012). They are plant-based non-nutrient bioactive compounds that have health-enhancing benefits to decrease the risk of major chronic disease beyond whose nutritional values (Slavin et al. 2013; Liu 2013; Rebello et al. 2014; Abuajah et al. 2015; Zhu and Sang 2017). Upwards of 5000 phytochemicals have been isolated and defined in plants like grains, fruits, and vegetables however it is predicted that wide range and a large quantity of them are still remains unknown (Liu 2013). According to another study, this value is over 900 (Abuajah et al. 2015).

As seen in Fig. 1, phytochemicals are generally divided into terpenes, polyphenols, carotenoids, glucosinolates, dietary fibers, lectins and other phytochemicals such as capsaicinoid, betalains, chlorophyll, allium compounds, polyacetylene, and alkaloids.

Health beneficial whole grain phytochemicals are mostly present in germ and bran parts (Liu 2007; Slavin et al. 2013) and distributed as bound, free and soluble-conjugated forms (Liu 2007).

Many studies stated that the combination of biologically active compounds in whole grains which are generally located in germ and bran fractions could be more effective than individual isolated compounds (Gani et al. 2012). In many epidemiological researches, analysis of data attained that intake of whole grains and its compounds, namely bran, germ, and endosperm, demonstrated an independent relation

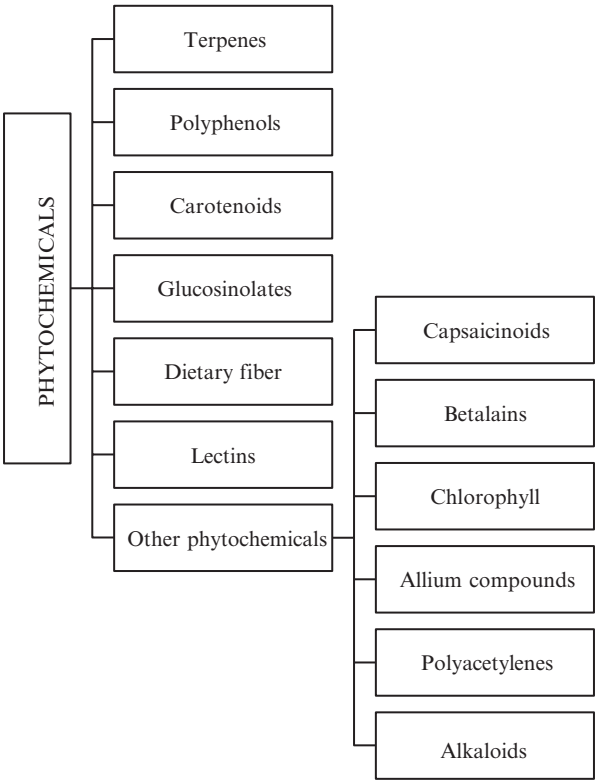

Fig. 1 The general classification of phytochemicals

only for the wheat bran (Stevenson et al. 2012). Thus, the bran is a key factor in identifying the health benefits of whole grain (Călinoiu and Vodnar 2018).

The phytochemicals present in fruit and vegetables are analogue to whole grains (Craig 1997; Craig and Beck 1999). Phytochemicals of whole grains are generally subdivided as dietary fiber, phenolic compounds, carotenoids and other phytochemicals such as phytic acid and phytosterols as seen in Fig. 2.

Dietary Fiber

The carbohydrates which are found in whole grains could be separated into two different groups, based on the ability of digestion in the human digestive system. Principally, the small bowel enzymes are not capable of the digest any complex carbohydrates present in whole grains except starch. Starch could only digestive by amylases that present in the human digestive system. In fact, non-digestibility is based upon the definition of dietary fiber. Non-digestible complex polysaccharides

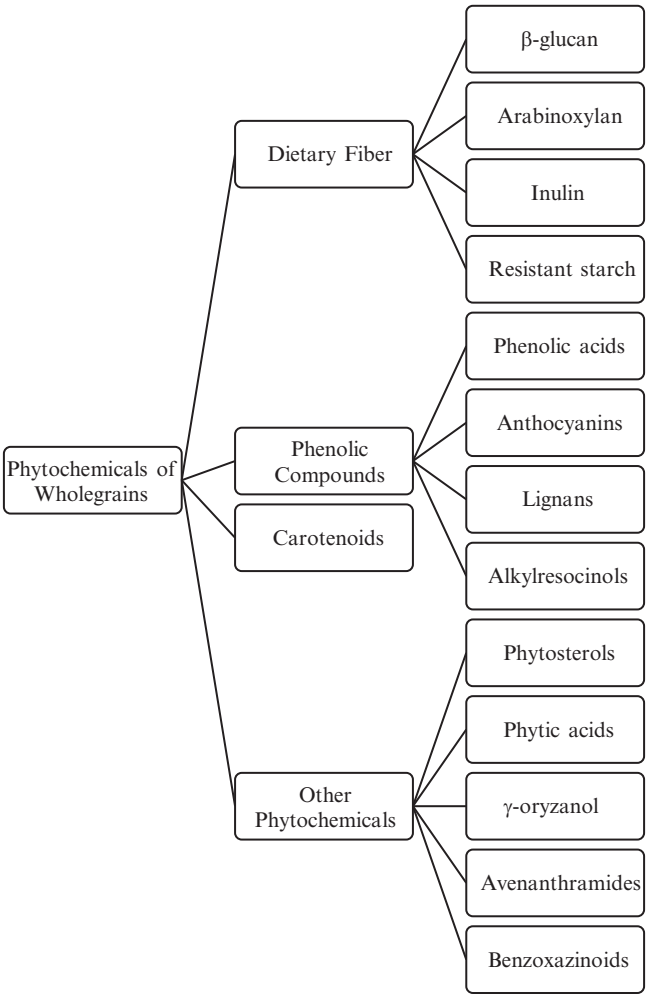

Fig. 2 The general classification of whole grain phytochemicals

are constituted non-starch polysaccharides (NSPs) and oligosaccharides (OS). Also, non-starch polysaccharides of whole grains are subdivided into two main groups due to whose solubility in water as soluble and insoluble non-starch polysaccharides, or in other names "soluble fiber" and "insoluble fiber" (Borneo and León 2012).

NSPs involve all the plant polysaccharides except that starch which is the key compounds of the cell walls of grains. The main NSPs are cellulose, β-glucans, pentosans, pectins, heteroxylans, and xyloglucan that could not be hydrolyzed by the human endogenous enzymes. NSPs together form a major part of the dietary fiber of grains, dietary fibers is chemically described as non-starch polysaccharides by a majority. The physiochemical features of NSPs such as water dispersibility and

water holding capacity, viscosity and fermentability into short chain fatty acids (SCFAs) correspond to dietary fiber (Kumar et al. 2012).

Dietary fibers (DF) are the edible fragments of plants or similar carbohydrates that are resistive to digestion by human digestion enzymes, absorbed in the small intestine and partially or completely fermented in the large bowel (Okarter and Liu 2010; Bartłomiej et al. 2012). According to the dietary fiber definition of AACCI, DF includes "cell wall polysaccharides (polysaccharides, oligosaccharides), lignin and associated substances resistant to hydrolysis by the digestive enzymes of humans". In this definition, "associated compounds" are subsumed for the first time in an official definition of dietary fiber (Vitaglione et al. 2008; Borneo and León 2012). Definition of dietary fiber was updated in 2008 by the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU). In their definition, the dietary fiber involves also polysaccharides composing of ten or more monomers but it could be reduced to three depending on local regulations. Dietary fiber composes of three main categories of polysaccharides as the first one is polymers which are intake in the natural form with foods, the second one is polymers extracted from raw foods by physical, chemical and enzymatic methods and the last one is synthetic polysaccharides (Cummings et al. 2009; Bartłomiej et al. 2012). Dietary fibre could be subdivided into two main categories due to whose water solubility as water soluble and water insoluble (Charalampopoulos et al. 2002). The water-soluble fibres are mainly composed of β-glucans, arabinoxylan, gums, pectin and mucilage whereas the water-insoluble fibres are comprised of lignin, cellulose, and hemicellulose (Charalampopoulos et al. 2002; Abuajah et al. 2015). Among the cereals, rice involves almost no soluble fibre and wheat has a low level in soluble fibre than most grains (Slavin 2004). Each category ensures different therapeutic effects. In this regard, soluble fibre decelerates intestinal transit, retards gastric emptying, and decreases glucose and sterol absorption by forming a viscous solution. Also, soluble fibre reduces serum cholesterol, insulin and postprandial blood glucose contents (Charalampopoulos et al. 2002) and improves glucose response, whereas insoluble fibre is related to enhance laxation (Slavin 2004).

Whole grains are good reserves of dietary fibers (Liu 2007). Rye, oats, and barley involve approximately one-third soluble fibre and residual part is insoluble fibre when comparing the content of dietary fibre in whole grains (Slavin 2004). The fiber content of the whole wheat grains ranges between 11.6% and 12.7% in dry weight based and most of them are located in the outer layers of the grain as pericarp and seed coat which is usually known as bran (Stevenson et al. 2012). According to another study, dietary fiber contents in wheat grains range from 11.6 to 17.0 g/100 g and lower than wheat bran (36.5–52.4 g/100 g) based on dry matter (Zhu and Sang 2017). Also, the content of soluble fiber in wheat is considerably fewer than in other cereal grains such as barley and oats, 3–11% and 3–7%, respectively (Stevenson et al. 2012). The content of soluble fibre in barley, rye, and oats is much higher than wheat (Seal 2006). Additionally, the refining process results in removing proportionally more of the insoluble fibre than soluble fibre in grains (Slavin 2004).

Dietary fibers are important compounds for a healthy diet. Consumption of whole grain-based dietary fibers are related with lowering the risk of chronic

diseases and has a protective role on coronary heart disease some type of cancers, diabetes, insulin resistance, weight gain, and metabolic syndromes. All these effects are associated with several physiological mechanisms that contain eliminating or binding cholesterol and bile acids, alleviation of hormonal activity, induction of the immune system, enabling toxicant transit through the digestive system, generation of SCFAs in the colon, reducing glycemic index and calorific value of foods, developing insulin response, providing scavenging free radicals and bulk in foods (Liu 2007).

Whole grains are reserves of fermentable carbohydrates as dietary fibre, resistant starch, and oligosaccharides. Indigested carbohydrate which arrives the colon is fermented to SCFAs and gases by the intestinal microflora. SCFAs involve butyrate, acetate, and propionate. Among them butyrate is mainly favored by the colonic mucosa cells (Slavin 2004) because of being fuel for them and also contributes to faecal pH, affecting colonic function (Stevenson et al. 2012). Production of SCFAs results in physiological alters to the colonic contents influencing water retention capacity, bulking and viscosity (Stevenson et al. 2012) therefore, reducing serum cholesterol and cancer risk (Slavin 2004). Cereal-based foods are also a significant source of dietary fiber. For instance, a slice of 40 g of white bread could supply approximately 1 g of dietary fiber, whereas a similar slice of whole grain bread could supply about 3–4.5 g of dietary fiber. In this regard, the different choice of bread type has an important effect on obtaining dietary fiber. Also, by taking into consideration of these data, consumption of six portions of whole meal bread could provide close to the suggested intake of dietary fiber of 25–35 g/day (Poutanen 2012).

β-Glucan

(1→3,1→4)-β-D-Glucans, which are generally known as β-glucans, consist of D-glucopyranose residues (Bartłomiej et al. 2012). β-glucan is a complex carbohydrate that consists of linear polymers of glucose molecules linked each other by account for 70% of β-(1–4) and 30% of β-(1–3) glycosidic bonds (Liu 2007; Okarter and Liu 2010; Borneo and León 2012). Due to these bonds, β-glucan is more soluble, viscous and flexible than cellulose (Liu 2007; Gani et al. 2012). Put it differently, β-glucans are polysaccharides which are comprised of glucopyranosyl units (Zekovic et al. 2005; Okarter and Liu 2010).

In cereals, β-glucan is one of the non-starch polysaccharides which is the component of cell walls (Ahmad et al. 2012) and content is affected by mainly genetic as their genotypes or waxy, non-waxy varieties; and also environmental factors like heat stress, available water content during maturation, moisture content, fertilizer implantation (Brennan and Cleary 2005), the existence of hull fraction and amylose content (Fardet 2010).

β-glucan content of whole grain wheat is generally lower than 1% on a dry weight based, and so whole grain wheat is not recognized as a source of β-glucan (Charalampopoulos et al. 2002). Among the cereals, particularly barley and oats are

dietary fiber source (Ahmad et al. 2012). Therefore it could be assumed as a marker for whole barley or whole oats because this compound is the characteristic phytochemical for them (Okarter and Liu 2010). Barley and oat β-glucans present in the walls of the endosperm cells which environ starch, protein matrix and lipid sources of the grain (Gangopadhyay et al. 2015). The ratio of dry matter based total β-glucan content is 3.0–27.17% (w/w) in whole grain barley. The water-soluble β-glucan in whole grain barley is 0.5–8.3%, whereas higher in whole grain oats 3.9–7.5%, and also the water-insoluble β-glucan content is 1.2–21.7% (w/w) in barley, while 5.2–10.8% in dehulled oats (Fardet 2010). Moreover, it is indicated that β-glucan is mainly located in the aleurone layer in oats whereas becoming intense in the endosperm fraction in barley. Besides, fewer amounts of the β-glucan present in rye (1–2%) wheat less than 1%, and also only trace quantity of β-glucan are stated in maize, rice, sorghum and other cereals (Liu 2007; Gani et al. 2012). Beforehand, β-glucan is only known as a component of the cell wall, but today β-glucan is linked with beneficial effects on human health.

β-glucan is the primary bioactive component an which is liable for reducing the serum and plasma cholesterol, particularly from oat bran (Liu 2007; Gani et al. 2012). Relevant researches are encouraged that intake of β-glucan during 1 month could reduce total cholesterol level account for 10% and LDL cholesterol for 8% (Liu 2007).

In generally, researches indicate that β-glucan could also reduce or attenuate postprandial glycemic and insulin responses (Wood 2007; Gani et al. 2012; Sibakov et al. 2013). In this regard, consumption of β-glucan could be related with lowering risk of insulin sensitivity, type 2 diabetes by, controlling blood sugar in diabetes (Gani et al. 2012), and also coronary heart disease and metabolic syndrome (Wood 2007). These are attributed to whose feature of high viscosity as a soluble fiber to bind cholesterol and facilitate their elimination from the body (Liu 2007), and retarding gastric emptying that permitted dietary sugar to absorb stepwise, as well as by probably augmenting insulin sensitivity, respectively (Liu 2007; Gani et al. 2012). Also, it is predicted that a reduction in cholesterol content in blood serum by 1% eventuate in decreasing the coronary heart disease risk by 2% (Bartłomiej et al. 2012).

Other researches also identified that β-glucan, especially obtain from oats plays a role in the management of body-weight and reducing blood pressure (Gani et al. 2012). Like arabinoxylans, β-glucans could be fermented by bacteria localize the large bowel which could enhance the SCFAs formation (Bartlomiej et al. 2012). It was elucidated that oat-based foods are proper for the lactic acid bacteria which are capable of producing exopolysaccharides (EXP). This attributes to behaving like dietary fiber because of whose capacity of withstanding to degradation by gastrointestinal enzymes (Lambo et al. 2005). Moreover, β-glucans have a high water-binding capacity (WBC) thereby contributes to removing detrimental carcinogenic substances from colons faster, thereby scanting the colorectal cancer risk (Bartłomiej et al. 2012).

Therefore, the Food and Drug Administration (FDA) declared that consumption of oat and its products should be used to decrease hazard of heart disease and

advanced that 3 g of β-glucan from oats could reduce total cholesterol. After that FDA permitted to utilize β-glucan in food products in case of made taking part in label compulsory (Liu 2007; Okarter and Liu 2010; Ahmad et al. 2012).

Arabinoxylan

Arabinoxylans (AX) are polymers of pentoses and herewith they are titled with 'pentosans' term (Bartłomiej et al. 2012). AX is hemicellulose which is one of the most plenteous cell wall polysaccharides of cereal grains after cellulose (Ragaee et al. 2013). Also, total arabinoxylans and water extractable arabinoxylans are most abundant in the bran fraction of whole grains (Bartłomiej et al. 2012). Arabinoxylans (AX) was for the first time defined in wheat flour in 1927 (Bartłomiej et al. 2012). Arabinoxylan is the main component of wheat, rye and rice bran fiber complex (Sibakov et al. 2013). Quantification of AX in wheat, rye, barley and oat bran range between 9.0–18%, 12.1–14.8%, 4.8–9.8% and 4.0–13.0% in dry based, respectively (Bartłomiej et al. 2012). According to another research, AX content is higher in rye correspond to 9.1% and following with wheat (7.3%) (Ragaee et al. 2013), barley, oats, rice and sorghum (Cui et al. 2013). When comparing with the flour forms the whole rye grains flour has the highest AX content varied from 3.1% to 4.3% in dry weight based following with whole wheat grain flour, barley grain flour and oats grain flour account for 1.7–2.0%, 1.4–2.25% and 0.35–1.25% in dry matter based, respectively (Bartłomiej et al. 2012). Arabinoxylans have the health improving effects and also antioxidant capacity based upon the presence of phenolic groups of ferulic acid that is mainly comprised of AX. Ferulic acid could be the ester form and either bond. Therefore it could participate in cross-links between polysaccharides or between polysaccharides and lignins that cause AX more resistant to digestion. The cross-linking arises from dimerization of ferulic acid residues caused by photochemical reactions or reactions between free radicals (Bartłomiej et al. 2012).

Inulin

Inulin is a polymer of fructose units whose degree of polymerization ranged between 2 and 60 (Ragaee et al. 2013), and the link between fructose units in inulin is a β-(2–1) glycosidic bonds (Liu 2007).

Inulin non-digestible dietary fiber and so has prebiotic properties which enable to grow benefits probiotics also known as microorganisms are good for the gastrointestinal system (Liu 2007; Borneo and León 2012). Because inulin puts up resistance to hydrolyzation by human digestive enzymes due to its glucosidic bridge (Ragaee et al. 2013). Therefore, inulin is a source for probiotics as lactobacilli, and bifidobacteria in the intestine because induce the growth and enhance the balance of these bacteria in the bowel. Among them, bifidobacteria could hinder the growth of detrimental bacteria, to promote the immune system, and to the synthesis of vitamins

B complex and enable the absorption of minerals. When bifidobacteria digest inulin, short chain fatty acids, like propionic acid, acetic acid, and butyric acids emergence. Acetic acids and propionic acids contribute as an energy source for the liver, whereas butyric acids have cancer prohibiting properties in the bowel. Beside, inulins could enable the absorption of some minerals such as magnesium, calcium, and iron in the colon because of the formation of SCFAs. Among them, calcium and magnesium are significant regulators for cellular activity. In high concentrations calcium could assist the formation of insoluble bile or salts of fatty acids and so could decrease the harmful effects of fatty acids or bile on colon cells (Liu 2007).

Resistant Starch

Starch is a polymer of glucose units linked by α-(1–4) bonds and composes of amylose and amylopectin fractions. Starch could be degraded by α-amylases yet, some of them could not easily digest and put up "resistant" to amylolysis (Borneo and León 2012). Resistant starch (RS) is resistant to digestion in the upper intestinal and so pass through the large intestine to be fermented by the colonic microflora (Liu 2007). And similar with oligosaccharides, particularly fructooligosaccharides supply fermentable carbohydrates for colonic microflora (Charalampopoulos et al. 2002). By this means, they produce short-chain fatty acids like acetate, butyrate, and propionate to enhance colon health (Liu 2007). Therefore, resistant starch act as prebiotics for reducing the risk of intestine diseases (Charalampopoulos et al. 2002). Besides, they use to reduce the caloric content of foods by not only providing lower calorie intake but also modulating lipid metabolism by promoting lipid oxidation in human subjects (Higgins et al. 2004). Moreover, they moderate blood sugar levels and in this way, they could decrease the risk of diabetes, heart disease, and other chronic health diseases (Liu 2007). Resistant starches subdivided into four types as RS1, RS2, RS3, and RS4. RS1 states for physically inaccessible trapped starch and mainly present in and unprocessed cereal grains, seeds, and legume; RS2 express to ungelatinized starch and natural raw starch; RS3 represents for retrograded starch form and RS4 refers to chemically modified starches (Borneo and León 2012). In recently, RS5 is also included and originates in formation of amylose-lipid complexes (Raigond et al. 2015).

Phenolic Compounds

Phenolic compounds have chemically one or more aromatic rings with one or more hydroxyl groups. They are the outputs of secondary metabolism and have crucial roles as in the growing up and reproduction of plants, behaving as defensive mechanisms against parasites, pathogens, predators, UV radiation and also making a contribution to the color of plants. Together with their functions in plants, in human diet

obtain health benefits related to protection and/or decrease in risk of chronic diseases (Liu 2007, 2013; Gangopadhyay et al. 2015).

Phenolic compounds are mainly classified into phenolic acid flavonoids stilbenes, coumarins, tannins, and anthocyanins. Phenolic acids and flavonoids are the most common phenolic composites which are present in whole grain cereals (Liu 2007; Gani et al. 2012; Gangopadhyay et al. 2015).

The content of phenolic compounds in whole grains vary by depending on grain type, genotype/varieties, part of the grain sampled, handling, and processing types of grains (Liu 2007; Gangopadhyay et al. 2015). Bran fraction, phenolic compound content is approximately 15–18-fold higher than the endosperm fraction that composes of 17% from the content of total phenolics. Thus, total phenolic content and implicitly the total antioxidant activity slightly reduce from the aleurone layer to the internal parts of the grain (Călinoiu and Vodnar 2018).

Flavonoids

Flavonoids are constituted C6–C3–C6 skeleton that comprises of aromatic rings. They are a large group of polyphenolic compounds (Gangopadhyay et al. 2015) and classified into five main subgroups as anthocyanins, flavones, flavanones, flavonols, and isoflavonoids (Gani et al. 2012). Until today, more than 4000 flavonoids determined in nature (Liu 2013) and notified that they have antioxidant, anticancer, anti-inflammatory, anticarcinogenic, antiallergic properties and preservative feature of the gastrointestinal system (Gani et al. 2012). Flavonoids correspond with nearly two-third of the phenolics in our diet and the remaining one third are from phenolic acids (Liu 2013).

Nevertheless, cereals contain a few amounts of flavonoids (Gangopadhyay et al. 2015) except barley that includes measurable contents of catechin and some procyanidins (Gani et al. 2012). Up to the present, there are a few researches about flavonoid contents of whole wheat grains. Latest studies stated that flavonoids are mainly *C*-glycoside form as particularly apigenin-*C*-diglycosides (Zhu and Sang 2017). Flavonoids are present in the pericarp fractions of all cereals. It was reported that among cereals, sorghum has the broadest varieties of flavonoids (Gani et al. 2012). Flavonoid contents in different wheat varieties are varying between 740 and 940 μmol of catechin equiv/100 g, whereas 60 and 80 μmol of catechin equiv/100 g in endosperm fraction (Zhu and Sang 2017).

Phenolic Acids

Phenolic acids are classified into two main groups as hydroxycinnamic acid and hydroxybenzoic acids and whose derivatives (Liu 2013), based on C3–C6 and C1–C6 skeletons, respectively (Călinoiu and Vodnar 2018). Hydroxybenzoic acid derivatives contain protocatechuic, *p*-hydroxybenzoic, vanillic acid, gallic acid, syringic acid, besides the hydroxycinnamic acid derivatives involve in ferulic acid,

p-coumaric acid, caffeic acid, and sinapic acid (Liu 2007, 2013; Călinoiu and Vodnar 2018).

The phenolic acids in cereals could be in both bound and free form. Free phenolic acids are mainly present in the outer layer of the pericarp whereas bound phenolic acids are esterified to cell walls and also acidic or basic hydrolysis is needed to set free of these bound compounds from the cell matrix (Gani et al. 2012; Gangopadhyay et al. 2015). Hydroxybenzoic acid derivatives are generally found in the bound form and they are typically compounds of complex structures like lignins and hydrolyzable tannins. They could also present as derivatives of organic acids and sugars in plant-based foods. Hydroxycinnamic acid derivatives are also found in the bound form and linked to cell wall structural compounds like cellulose, lignin, and proteins through ester bonds (Liu 2007).

The widest variety of phenolic acids found in millet and sorghum (Gani et al. 2012). One of the main phenolic compounds of whole grain cereals is phenolic acids and predominantly ferulic acid (trans-4-hydroxy-3-methoxycinnamic acid) which is hydroxycinnamic acid derivatives and mainly present in aleurone, pericarp, and embryo cell wall in whole grains (Ragaee et al. 2013). Ferulic acids are mainly present in the leaves and seeds of plants, generally covalently combined with plant cell wall polysaccharides as unsoluble carbohydrate biopolymers, lignin, fibers, glycoproteins and polyamines (Liu 2013). In whole grain cereals, ferulic acid could be present in free, soluble (conjuncted) on insoluble (bound) forms (Borneo and León 2012; Călinoiu and Vodnar 2018), and generally bound ferulic acids are the dominant form in grains (Liu 2013) account for more than 93% of the total ferulic acid content (Okarter and Liu 2010). The ferulic acid concentration is highest in whole grain corn when comparing with other grains and following by wheat, oat and rice and correspond to (Ragaee et al. 2013), 906 μmol ferulic acid/100 g, 333 μmol ferulic acid/100 g, 185 μmol ferulic acid/100 g, and 154 μmol ferulic acid/100 g, respectively (Okarter and Liu 2010). After the ferulic acid, the most common phenolic acids are vanillic acid, syringic acid and *p*-coumaric acid in wheat bran (Ragaee et al. 2013). The content of total vanillic acid is ranged between 8.4 and 12.7 μg/g, total syringic acid is ranged from 8.9 to 17.8 μg/g. and the total *p*-coumaric acid is ranged from between 10.4 and 14.1 μg/g (Moore et al. 2005; Okarter and Liu 2010). The almost total content of ferulic acid (98%) is localized the aleurone and the pericarp fractions of wheat grain (Călinoiu and Vodnar 2018). The concentration of ferulic acid ranges from 535 to 783 mg/kg in mature grains. Ferulic acid could be present in free and also esterified forms as ferulic acid dehydrodimers (DiFA) whose relative concentration is 808 mg/kg (Shahidi and Naczk 2003).

The antioxidant activity of wheat grain fractions is adversely relevant to the aleurone content owing to its high concentration in hydroxycinnamic acids, the principal one being the ferulic acid. The bioavailability of the ferulic acid is restricted because of whose powerful boundary with indigestible cell wall material in grains (Călinoiu and Vodnar 2018). Stages of food processing, like fermentation and thermal processing such as pasteurization, and freezing unleash free and

soluble-conjugated ferulic acids from bound phenolic acids (Dewanto et al. 2002; Liu 2013). In a study, subject to wheat whole grain to alkaline hydrolysis showed that germination could increase phenolic acid content. According to results ethanol soluble ferulic acid, *p*-coumaric acid and syringic acid composition are 3 mg/kg, 1 mg/kg, 2 mg/kg respectively in ungerminated grains whereas 26 mg/kg, 8 mg/kg, 8 mg/kg in a consequence of 48 h germinated grains. Moreover, it was added that the highest phenolic acid content is reached after nine days of germination process (Shahidi and Naczk 2003).

Phenolic acids are known as their antioxidant activity and that is an association with health benefits (Borneo and León 2012).

Anthocyanins

Another study pointed out that cyanidin-3-galactoside and peonidin-3-glucoside are also determined in purple and blue wheat as anthocyanins. Average total anthocyanins content is approximately 100 mg/kg in purple whole wheat, 150 mg/kg in blue whole wheat whereas 5 mg/kg in red whole wheat (Zhu and Sang 2017).

In colored maize, the most common anthocyanins are cyanidin, pelargonidin, and peonidin, which are mainly present in the pericarp and aleurone layer of the endosperm (Suri and Tanumihardjo 2016).

Tocols

Tocopherols and tocotrienols, which are widely named as tocols are chemical components also known as vitamin E (Bartłomiej et al. 2012). They are lipid-soluble compounds that involve a phenolic-chromanol ring bond to an isoprenoid side chain that could be saturated viz. tocopherols or unsaturated viz. tocotrienols (Abuajah et al. 2015). Also, tocols are a group which composes of whose four forms of tocopherols as α-tocopherols (αTP); and β-tocopherols (βTP), γ-tocopherols (γTP), δ-tocopherols (δ TP) and four forms of tocotrienol as α-tocotrienols (αTT), β-tocotrienols (βTT), γ-tocotrienols (γTT), δ-tocotrienols (δTT) which all have vitamin E and antioxidant activity (Ragaee et al. 2013).

It is revealed in many reports that tocols have vitamin E activity which depends on physiological factors and chemical structure. In this regard isomers of tocols have different vitamin E activities as follows: α-tocopherols > β-tocopherols > α-to cotrienol > γ-tocopherol > β-tocotrienol > δ-tocopherol or no activity for γ-tocotrienol and δ-tocotrienol. Among them, the α-TP has the highest vitamin E activity whereas, α-TT exhibits the highest antioxidant activity (Gani et al. 2012; Gangopadhyay et al. 2015). In accordance with dietary guidelines, the estimated average requirement of vitamin E is 12 mg and the suggested dietary allowance is 15 mg of 2R-α-tocopherol in a day (Gani et al. 2012).

Tocols are mainly found in plant-based foods. The major reserve of tocols are vegetable oils, yet great amounts of these compounds are also found in most cereal

grains (Gani et al. 2012; Gangopadhyay et al. 2015). The distribution of tocol isomers is irregular in the kernel. Nearly almost tocotrienols are present in the bran whereas particularly α-tocopherol and γ-tocopherol are located in germ fraction (Gangopadhyay et al. 2015). Therefore, refined cereals have less vitamin E content due to the refining process (Fardet et al. 2008). According to a study, whole grain maize and rye have higher tocol contents than other cereal grains (Fardet et al. 2008). The contents and profiles of tocols are different in grains. Another study revealed that wheat and rye, which contained 27.81 μg and 27.78 μg in dry weight based and have higher amounts than barley (18.73 μg) and oats (11.59 μg), respectively. Also rye, wheat, and barley were the rich in α-tocopherol, β-tocotrienol and α-tocotrienols, respectively (Bartłomiej et al. 2012).

Tocols act as a radical scavenger, therefore, it is known as its antioxidant activity and hindering polyunsaturated oxidation in particularly cell membranes (Fardet et al. 2008). Aside from antioxidant activities, whole grain tocols could have many human health benefits involves modulating some degenerative diseases like cardiovascular diseases (CVD), cancer, and also decreasing blood cholesterol levels by prohibiting biosynthesis of cholesterol and lipid peroxidation, and act as a potential anti-inflammatory agent (Gani et al. 2012). They have been found stable in unprocessed groats for a period of 7 months at room temperature. Nevertheless, they undergo degradation in all the processed products (Gangopadhyay et al. 2015).

Lignans

Lignans are one of the polyphenolic bioactive compounds which are composed of a group of dietary phytoestrogen components consist of two coupled C6–C3 units (Liu 2007). Dietary lignans are comprised of seven different phytoestrogen compounds as lariciresinol, matairesinol, 7-hydroxymatairesinol, medioresinol, pinoresinol, secoisolariciresinol, syringaresinol that are mainly located in outer layers of grains. Some of these plant-based dietary lignans as matairesinol and SDG could be metabolized in the gastrointestinal system and converted into mammalian lignans as enterolactone (EL) and enterodiol (ED), respectively (Ragaee et al. 2013; Thompson et al. 2001). Lignans have a diphenolic structure which is analogue to that estrogenic component (Slavin et al. 1999).

Lignans are dietary phytoestrogens which are found in a widespread variety of plant-based foods involving flaxseeds, fruits, vegetables, legumes and also whole grains as corn, oats, wheat, and rye (Gani et al. 2012). Among them, flaxseeds are the highest content of dietary source in plant lignans (Liu 2007). The concentration of whole grain lignans from high to low as rye, oats, and wheat which are in between 2500 and 6700 μg/100 g, 820 and 2550 μg/100 g, 340 and 2270 μg/100 g, respectively (Ragaee et al. 2013). Another study mentioned that wheat and rye bran fractions have a higher concentration of lignans as 92.24 μg/g than other grains. Another research revealed that different cultivars of the wheat concentration of total lignans varied from 2.60 to 5.00 μg/g in dry seed weight based (Zhu and Sang 2017). In vitro studies showed that secoisolariciresinol diglycoside (SDG) could reduce early

biomarkers of colon cancer, also the new precursors of mammalian lignans as ED and EL could diminish spreading colon tumor cells in conjunctioSCFAs (Thompson et al. 2001). The ED and En with Ca^{2+} and L are abundant in whole grain rye. In a study, dry matter based SDG level in wheat pasta samples is ranging between 34.20 and 58.81 μg/g (Zhu and Sang 2017).

Lignans are phytoestrogens and lately related to reducing the prevalence of hormone-dependent health problems (Heinonen et al. 2001). Because, lignans are hormonally active compounds in grains and so, they could protect against hormonally mediated diseases (Slavin et al. 1999). ED and EL which have high antioxidant activity and less estrogenic activity make them very beneficial in promoting health benefits and combating various chronic diseases (Liu 2007; Gani et al. 2012). The mammalian lignans as ED and EL which are the metabolites of lignans may prevent against heart disease and hormone-related breast cancer and prostate cancers. Also, they could inhibit cell growth of colon cancer (Liu 2007; Gani et al. 2012).

Alkylresorcinols

5-n-Alkylresorcinols, namely alkylresorcinols are synthesized by higher plants of families as *Gramineae*, *Myristicaceae*, *Proteaceae* and *Anacardiaceae* (Bartłomiej et al. 2012). Alkylresorcinols are plant-based phenolic lipids (Fardet 2010) and amphiphilic compounds (Ross and Kamal-Eldin 2001). Amphiphilic characterization is related to being a 1,3-dihydroxybenzene derivative with an alkyl chain at position 5 of the benzene ring (Fardet 2010; Gani et al. 2012). They are mostly found in aleurone fractions of whole grain cereals (Ross and Kamal-Eldin 2001) and especially dominated in rye between other whole grains. The content of alkylresorcinols could almost two times more in rye than wheat (Fardet 2010; Gani et al. 2012).

The content of alkylresorcinols in dry weight based is higher in the rye and mainly located in its bran fraction as 734 μg/g among other cereals and keep up with wheat account for 583 μg/g and barley as 45 μg/g, respectively (Ragaee et al. 2013). The content of alkylresorcinols in whole grain wheat especially bran fraction is 2110 mg/kg and higher by a long way than wheat flour (380 mg/kg) (Shahidi and Naczk 2003). On the other hand, the total amounts of whole grain wheat breads are ranged between 422 and 609 μg/g in the dry matter based (Zhu and Sang 2017). According to other research, the alkylresorcinols content of rye grains vary between 360 and 3200 μg/g in dry weight based, whereas 317–1430 μg/g and 41–210 μg/g in wheat and barley, respectively (Bartłomiej et al. 2012).

Alkylresorcinols have antifungal and antibacterial features and also antioxidant activity *in vitro* (Ragaee et al. 2013). Their antibacterial and antifungal activities are attributed to whose hydrophobic alkyl chain that enables to react with proteins involving enzymes and therefore hinders their catalytic activities (Bartłomiej et al. 2012). Alkylresorcinols are biologically active antioxidants and whose absorption levels are high be a counterbalance up to 80%. Also, their potential antioxidant activities are proved in in-vivo studies that are protective to lipid oxidation.

Alkylresorcinols are provided to antioxidant activity that is subject to chain lengths such as incorporation with cell membranes and whose amphiphilic characters. However, it is remarked that their radical scavenging activities are less efficient than vitamin E and also sensitive to some production processes as fermentation, baking, and extrusion (Fardet et al. 2008). In vitro studies stated that alkylresorcinols have positive effects on reducing cholesterol level and anticancer cytotoxic effects (Fardet 2010). Their antioxidant activity is associated with the anticancer effect, but it is not certain in vivo studies (Ross and Kamal-Eldin 2001). For instance, in vivo studies on mice with implemented cells of prostate cancer, alkylresorcinols demonstrated no effect on cancer cells (Bartłomiej et al. 2012).

Carotenoids

Carotenoids are natural pigments of fruits, vegetables, whole grains and responsible for yellow, red and orange colors which are synthesized by microorganisms and plants (Borneo and León 2012). They are lipid-soluble compounds (Abuajah et al. 2015) and including at least 40 carbon skeleton (Okarter and Liu 2010) could be present esterified to fatty acids or unesterified in plant tissues (Abuajah et al. 2015). In nature, it is defined more than 600 different carotenoids (Gani et al. 2012) and generally present in the all-trans form (Okarter and Liu 2010), yet, among them, only 40 are present in the human diet (Borneo and León 2012). Carotenoids are subdivided into two main groups as carotenes as hydrocarbons and whose oxygenated derivatives as xanthophylls (Borneo and León 2012). The most prevalent carotenoids which have antioxidant activity and source of yellow pigments are lutein, zeaxanthin, and β-cryptoxanthin in whole grain cereals (Ragaee et al. 2013).

Cereals are a major reserve of carotenoids. On the contrary to other bioactive compounds such as polyphenols, carotenoids are located mainly in endosperm fraction (Fardet et al. 2008). Among whole grain cereals, maize has the highest carotenoids content account for 11 mg/kg in the dry matter based totally, also particularly lutein and zeaxanthin are dominant correspond with 6–18 μg/g; 4–8 μg/g, respectively. In accordance with whole grain soft wheat contain about only 1.5 mg/kg carotenoids while wheat germ has approximately 5.5 mg carotenoids/kg concentration of carotenoids in the dry matter based. Lutein concentration is depend on different wheat varieties such as 0.3–1.4 μg/g; 1.2–5.8 μg/g; 0.8–1.1 μg/g (Fardet et al. 2008). Zeaxanthin contents of 11 wheat varieties from around 8 to 27 mg/g grain, and β-cryptoxanthin contents from around 1.0 to 13.5 mg/g grain (Fardet et al. 2008). The content of major carotenoids range from 26.4 to 143.5 μg/100; 7.0 to 27.1 μg/100 g; 1.1 to 13.3 μg/100 g respectively, in wheat. Among the wheat species concentration of carotenoids particularly lutein is higher *Triticum durum* and *Triticum monococcum* (Einkorn) than *Triticum aestivum* (Ragaee et al. 2013). *Triticum durum*, *Triticum turgidum* have intermediate levels of lutein account for 5.41–5.77 μg/g, whereas one of the most common wheat namely *Triticum aestivum* had the lowest content correspond to 2.01–2.11 μg/g (Abdel-Aal et al. 2002). On the

other hand, corn flours contain reasonable concentrations of the different carotenoids like β-cryptoxanthin (3.7 mg/kg), lutein (11.5 mg/kg), and also zeaxanthin (17.5 mg/kg) (Brenna and Berardo 2004; Ragaee et al. 2013).

Carotenoids have antioxidant and pro-vitamins functions (Gani et al. 2012). Antioxidant activities of carotenoids are attributed to whose several characteristic features as enable to cyclized one or both ends of the structure and could be hydrogenated to different degrees. They also have oxygen involving groups and a long series of alternating single and double bonds. Thus, carotenoids could scavenge free radicals and so being free and remain stable radicals in the process, due to whose capability of localization delocalize the free radical amongst its alternative single and double bonds (Okarter and Liu 2010). Despite the fact that fruits and vegetables known as a main source of carotenoids, there is a growing interest of whole grain carotenoids (Borneo and León 2012).

Other Phytochemicals

Phytic acid or stated in other words inositol hexaphosphate (IP6) is bioactive compounds. Also, its salt form is called phytate (Gani et al. 2012). Phytic acid is majorly present in the bran parts of whole-grains, particularly in the aleurone layer (Fardet et al. 2008; Gani et al. 2012), correspond to 90% and 10% in the embryo (Dost and Tokul 2005; Stevenson et al. 2012) while mainly present germ fraction in corns (Gani et al. 2012). The amount of phytic acid in whole grain wheat, maize, sorghum are 82 mg/100 g, 635 mg/100 g, and 829 mg/100 g respectively (Ragaee et al. 2013). Phytic acid is a principal phosphorus storage components in cereal grains correspond with approximately 1–7% in the dry matter based (Fardet et al. 2008). That contributing more than 70% of the total phosphorus content of the kernel (Fardet et al. 2008; Gani et al. 2012). It is generally known that phytic acid has an antinutritional effect which is related to chelates minerals and trace elements such as Fe, Ca, Zn and/or Mg and so limiting whose bioavailability (Fardet et al. 2008; Gani et al. 2012). Besides this, it is revealed that phytic acid has antioxidant activities not only in vitro by suppressing iron-catalyzed oxidative reactions due to whose ability to chelate free Fe and may be a potent antioxidant but also in vivo by blunting lipid peroxidation (Fardet et al. 2008). Thus, there is a potential health problem about nutritional deficiency particularly iron and zinc deficiency for populations whose diets are based on cereals and legumes (Raboy 2001; Stevenson et al. 2012). On the other hand, it was revealed in recent studies that phytic acid could prevent some health problems such as coronary heart disease, atherosclerosis, formation of kidney stone and some cancer types respectively (Ragaee et al. 2013). Researches are detected that endogenous or purified in other words exogenous phytic acid has a protective effect to colon cancer. This effect is explained by the antioxidant effect of phytic acid which has the capability to bind iron which is a catalyst for lipid peroxidation. Moreover, it was enlightened that purified phytic acid is more effective than endogenous phytic acid about this issue (Thompson et al. 2001). The

phytic acid content is effected during milling. In this regard, refined flour has almost no phytate although wheat involves about 1.13% phytate in dry weight based. In refined flour, phytic acid content varies from 200 to 400 mg/100 g while these values are 600–1000 mg/100 g in whole flour (Febles et al. 2002). Moreover, it is higher in n wheat bran account for 3116–5839 mg/100 g in the dry matter based (Stevenson et al. 2012).

Phytosterols are secondary metabolites (Liu 2007; Ragaee et al. 2013), and collective term to state plant sterols and stanols that have an analogue structure with cholesterol. Plant-based sterols involve stigmasterol, sitosterol, campesterol in other respects stanols include as stigmastanol from only in the side chain groups (Liu 2007; Gani et al. 2012). In whole grains, plant sterols could be found in different forms as free sterols, steryl esters with fatty acids, or phenolic acids, steryl glycosides, and acylated steryl glycosides (Gani et al. 2012). The most abundant plant sterol is β-sitosterol in Western diets (Kris-Etherton et al. 2002). Plant sterols and stanols are mainly present in oilseeds, vegetable oils, whole grains, legumes and nuts (Slavin 2004; Liu 2007). Moreover, cereals are recognized as significant plant sterol sources than vegetables (Gani et al. 2012). Among the cereals, the highest amounts of phytosterols are present in rye (80–90 mg/100 g) and following with wheat (70 mg/100 g), barley (70–80 mg/100 g) and oats (45–50 mg/100 g), respectively (Bartłomiej et al. 2012). Also, the phytosterol level is moderate in whole grain cereals but high in pearling fines, namely outer kernels of barley (Gangopadhyay et al. 2015).

In many researches, it is indicated that phytosterols reduced the absorption of serum cholesterol and it is attributed to a similar structure with cholesterol and phytosterols (Bartłomiej et al. 2012). Another study reported that less than 1 g/day, intakes of phytosterols have a significant effect on lowering cholesterol level and recommended consumption of 1–2 g phytosterols in a day (Slavin 2004). According to other epidemiological studies, ingestion of 1–3 g phytosterols in each day reduced the low-density lipoprotein (LDL) cholesterol level by 10% on average in blood serum, whereas triacylglycerols and the high-density lipoprotein (HDL) cholesterol levels and were not affected (Lagarda et al. 2006; Bartłomiej et al. 2012). Nevertheless, the estimated plant sterols content is about 200–300 mg/day in the Western diet (Slavin 2004). According to another source, the typical consumption of plant sterols is nearly 200–400 mg/day (Kris-Etherton et al. 2002). Also, it is stated that a non-vegetarian diet involves about 250 mg of unsaturated phytosterols whereas a vegetarian diet includes more than 500 mg (Abuajah et al. 2015). Therefore, it is difficult to fulfill the recommended phytosterol intake levels (Rebello et al. 2014). Yet, still, it is suggested that increased consumption of whole-grain implicitly total phytosterol intake contribute to a reduction in cholesterol (Slavin 2004) and potentially perform a protective effect on cardiovascular diseases (Rebello et al. 2014). Therefore, the beneficial health effects of plant sterols induce to the improvement of functional foods fortified with sterols. The eventuality of utilizing sterols as an adjunctive therapy to cholesterol decreasing pharmaceuticals has been recommended lately (Gangopadhyay et al. 2015).

γ-Oryzanol is a mixture of at least ten phytosteryl ferulates such as methyl sterols esterified to ferulic acid (Fardet et al. 2008). The γ-oryzanol is mainly specified in whole grain rice and particularly its bran fraction as 18–63 mg/100 g and 185–421 mg/100 g in the dry matter based, respectively (Fardet 2010). Another study is expressed that γ-oryzanol content of rice bran is corresponded to 3000 mg/kg following with wheat bran as ranges from 300 to 390 mg/kg (Ragaee et al. 2013). The content of γ-oryzanol is depended on several factors such as a variety of rice, milling procedure, extraction methods (Fardet 2010). γ-oryzanol is a strong inhibitor of iron driven hydroxyl radical formation. Antioxidant activity of γ-oryzanol has been proved not only in vitro (Juliano et al. 2005) but also in vivo studies (Suh et al. 2005). It is demonstrated that γ-oryzanol has favorable effects on CVD, hyperlipidemia and also reduction in cholesterol level and lipid peroxidation (Fardet 2010).

Avenanthramides (AVs) are a group of hydroxycinnamoyl anthranilate alkaloids. They have been found specifically in oats and particularly in oat groats and oat hulls. AVs are mostly localized in the oat bran when comparing with oat groat yet, more uniformly distributed throughout the oat groat (Gangopadhyay et al. 2015). Also, the content of oat flakes avenanthramides are higher than oat bran corresponds to 26–27 μg/g and 13 μg/g, respectively (Gani et al. 2012). The main avenanthramides are avenanthramide-1, avenanthramide-3, and avenanthramide-4, in other words, avenanthramide B, avenanthramide C and avenanthramide A, respectively. AVs have antioxidant antiatherogenic and anti-inflammatory properties (Gani et al. 2012).

Benzoxazinoids (BXs) is a group of nitrogen inclusive secondary metabolites is mainly stored as glucosides in plant cells. The presence of BXs in whole grain products discovered recently and it was stated that BXs are generally localized in the germ of wheat seeds, a part of the bran fraction. Yet, the content of total BXs in whole grain wheat products is very low correspond to about 5 μg/g in the dry matter based (Zhu and Sang 2017).

Whole Grain Phytochemicals and Effect on Health

Several metabolic diseases have an association with daily lifestyle, namely lifestyle disorders, being in the first place an unbalanced diet, intake of inadequate fiber and bioactive components as phytochemicals and micronutrients (Gani et al. 2012; Călinoiu and Vodnar 2018).

By-products of grain processing are cereal brans that source of nonstarch carbohydrates such as beta-glucan, arabinoxylan; phenolic acids mainly ferulic acid, flavonoids particularly anthocyanin and also tocols, carotenoids, folates, oligosaccharides, and sterols. Therefore, the bran parts obtained from cereals such as wheat, oats, barley, rye, rice, maize, millet, and sorghum have been qualified to provide with plenty of health-enhancing compounds are about antiatherogenic, hypoglycaemic, antihypertensive and antilipaemic features (Patel 2015). Epidemiological

prospective studies, randomized controlled trials and cohort studies on humans demonstrate that the consumption of whole grain and whole grain-based foods are preservative against to various health diseases which are related with an increment of oxidative stress. Kind of cardiovascular diseases, type 2 diabetes, obesity and some cancer types (Călinoiu and Vodnar 2018). These health benefits are acquired by way of several multifactorial physiological mechanisms subsuming antioxidant capacity and/or activity, procuration of hormones, improvement of the immune system and enable of substance transit via the digestive system, and absorbing and/or diluting of substances in the intestinal system (Gani et al. 2012).

It is generally agreed that the synergistic action of the components present in the bran and germ fraction of whole grain cereals have a preservative role because of whose antioxidant activities (Călinoiu and Vodnar 2018).

Cardiovascular Disease

Cardiovascular disease (CVD) involves mainly coronary heart disease (CHD), stroke, hypertension, peripheral vascular disease (Zhu and Sang 2017). CVD is a charge of one out of three mortality in all around the world, particularly in the industrialized world. The World Health Organization (WHO) is apprised of the increase of CVD in developing countries (Borneo and León 2012).

Whole grain consumption and total dietary fiber constantly related to preservation from coronary heart disease (CHD). It is indicated that the effect of fruit or vegetable fiber consumption was intermediate whereas whole grain cereal fiber intake had a lower frequency of substantial adverse association with CHD (Anderson et al. 2000).

The preservative effect of whole grain cereals CHD has come forward for nearly 60 years. Burkitt, Cleave, Trowell, and Walker lead the notion that highly refined foods are principal contributors to western diseases involving coronary artery disease. Over 45 years ago Trowell highlighted the fiber hypothesis linked with CHD. The first scientific research to corroborate the hypothesis was conducted by Morris et al. (1977) stated that higher cereal fiber intake resulted in a reduced rate of heart attacks in British men. After that several epidemiologic studies have the same view about this hypothesis and got strengthen in the following years. “Whole grain” hypothesis was supplanted by “dietary fiber” hypotheses. Burkitt also gave attention to wheat bran as a principal preservative food and support consumption of whole grain-based bread and bran cereals (Anderson et al. 2000). Trowell’s hypothesis was interpreted as a “fiber hypothesis” rather than as a “high-fiber food hypothesis” (Anderson 2004). They insisted on whole foods should get the emphasis. Recent findings of the “whole grain story” validate their vision (Anderson et al. 2000).

First of all, it is mentioned that high amounts of consumption natural starchy carbohydrates, with their whole fiber parts, is preservative versus ischemic heart disease and hyperlipidemia. After that, this notion was encouraged with another

study that interrelates intake of high levels of cereal-based dietary fiber and relation with reducing ratios of heart attacks among British men. This researches lead to affirmation by FDA and stated that diets rich in whole-grain foods could decrease the risk for heart disease. Furthermore, subsequent studies revealed that whole grain consumption has a stronger relationship with preservation from CVD than the intake of cereal fiber and other plant-based fibers as vegetables or fruits (Anderson et al. 2000). In 2010, the Dietary Guidelines Advisory Committee (DGAC) rated the evidence for the preservative association between whole grains and CVD as "moderate" (Harris and Kris-Etherton 2010).

Main risk factors of CVD are high levels of serum LDL cholesterol and fasting serum triacylglycerol, low levels of HDL-cholesterol, and also hypertension, diabetes, and obesity. Whole grain cereals and foods could be one of the healthiest preference to reduce the CVD risk. Epidemiological researches stated that high amounts of whole grain consumption could reduce the risk of CVD account for 29% than individuals with low levels of whole grain intake. Diets rich in whole grain foods are prone to reduce serum LDL-cholesterol and triacylglycerol levels also blood pressure whereas rising serum HDL-cholesterol levels (Anderson 2003).

Besides to dietary fiber several studies focused on relation between some bioactive compounds particularly carotenoids and cardiovascular health (Voutilainen et al. 2006; Giordano et al. 2012; Ciccone et al. 2013; Gammone et al. 2015; Di Pietro et al. 2016; Kulczyński et al. 2017) and also lignans (Peterson et al. 2010), ferulic acids (Alam et al. 2013).

Type 2 Diabetes

Whole grain intake is adversely related to type 2 diabetes risk, and this relationship is stronger for bran fraction than germ fraction. Results from prospective cohort studies constantly encourage the increased consumption of whole grain for the prevention of type 2 diabetes (De Munter et al. 2007).

Diets which are rich in whole grains are related with a nearly 20–30% decrease in risk of type 2 diabetes, that is accredited with whole grain components, particularly dietary fiber, phytochemicals, vitamins, and minerals. Major phytochemicals such as phenolics and flavanoids have antioxidant activity in vitro and have the potential to attenuate inflammation and oxidative stress which are involved in the pathogenesis of type 2 diabetes. However, their bioavailability is frequently limited because of these compounds are bound firmly to the cell wall. Clinical trials as postprandial and medium-term intake researches demonstrated that phytochemical compounds of cereals obtained a restricted benefit for prohibiting the development of type 2 diabetes. On the other hand, consumption of whole grains in the diet may cause an increment phenolic contents of postprandial plasma. Nonetheless, the magnitude of the response is generally impermanent and smidgeon. In addition, clinical relevance with the effect of whole grain cereals and their fractions are not improved biomarkers to decreasing risk of type 2 diabetes are still fewer (Belobrajdic and Bird 2013).

There are many possible mechanisms about the effect of whole grains and whose bioactive components on diabetes. These are related to the capability of reducing insulin resistance and enhance insulin sensitivity, improvement in glucose tolerance and the high levels of magnesium in whole grain. However, the exact mechanism is not identified yet, that is related to a low glycemic index of whole grains (Borneo and León 2012).

To detect the association of whole-grain consumption with glucose and insulin metabolism needs biomarkers. In this regard, the glycemic index (GI) could be a marker to compare the glycaemic response of foods (Slavin 2004). The glycemic index of whole grains such as barley, oats, corn, rice, rye, buckwheat, and wheat range between 36 and 81. Among them, barley and oats having the lowest glycemic indexes values. According to results, concentrations of blood glucose and insulin secretion decreased in subjects with and without diabetes mellitus who consumed a low-glycemic index diet (Slavin et al. 1999; Slavin 2004).

According to a report of Dietary Guidelines Advisory Committee (DGAC) in 2010, it is mentioned that there is a little or no relation between glycemic index, type 2 diabetes, weight loss, and cancer, also an inconclusive relation with cardiovascular disease (CVD) (Harris and Kris-Etherton 2010).

Andersson et al. (2011) studied on suppression effect of alkylresorcinols which is isolated from rye bran on hormone-sensitive lipase activity and adipocyte lipolysis. They pointed out that constantly high intake of ARs, in the format of whole grain rye, could cause to decrease lipolysis in vivo and so reduce the levels of circulating free fatty acids (FFAs). Liu et al. (2000) aimed to examine the relationship between consumption of whole grain or refined grain and the risk of type 2 diabetes mellitus in 75,521 U.S. women aged 38–63 years without a previous diagnosis of diabetes or cardiovascular disease. Throughout the 10 years follow up, results showed that replacement of refined grain products with whole grain-based products could reduce diabetes mellitus risk. Montonen et al. (2003) researched the association between the Consumption of whole grain and fiber and the incidence of type 2 diabetes in 2286 men and 2030 women aged 40–69 years without initially diabetes diagnosed. There is an adverse relationship between type 2 diabetes risk and persons with high whole-grain intake. This is attributed to cereal fiber intake and other bioactive components present in whole grain products, such as tocotrienols, lignans, phytic acids, and other antinutrients. The following research should address the mechanisms and which compounds are responsible for the effects, the exact amounts of whole grain needed to decrease type 2 diabetes risk (Murtaugh et al. 2003).

Obesity and Weight Management

Obesity arises from the consequence of a long-dated imbalance between energy expenditure and energy intake. To increase energy intake, one of the ways is raising the intake of whole grains (Mikušová et al. 2011). Epidemiological studies stated that there is an adverse relationship between consumption of dietary fibre with

weight gain and obesity. It is expressed that fibre dietary intake is related to reduced energy intake, increased satiety. Researches indicated that wheat bran could decrease in food consumption following a test meal with wheat bran (Stevenson et al. 2012). However, it is not clear yet whether this effect is long-lasting with regards to the management of obesity (Freeland et al. 2009; Stevenson et al. 2012).

Whole grain and whole grain-based foods reduce weight, body-mass index (BMI), the waist circumference and also waist-to-hip ratio by decreasing the amount of accumulated body fat due to whose dietary fiber content (Mikušová et al. 2011). It is hard and complex to identify a specific mechanism by which whole grains have a benefit on weight reduction or weight management. Moreover, intake of whole grains is also related to the acceptance of other healthy habits like increasing intake of fruits and vegetables and also physical activities (Borneo and León 2012). So, there is lack of accurate explanations of underlying mechanisms of the relation between them but it has been proven that the obesity risk could be diminished by substituting of refined cereal-based foods with, low glycaemic index and high-fiber content whole grains. The possible mechanisms are an enhancement of satiety and satiation, an extension of gastric emptying time while deceleration of nutrient absorption and impact on gut hormones like leptin, ghrelin, cholecystokinin, glucagon-peptide-1 (GLP-1) and peptide tyrosin-tyrosin (PYY). Moreover, the synergistic effect of dietary fiber between antioxidants could be a charge of slowing down the rate of glucose absorption, putting off insulin release and blunting glycaemic response, what may affect weight management (Mikušová et al. 2011). The reduction in markers of obesity such as insulin, leptin, and C-peptide are related to increased levels of whole grains ingestion (Borneo and León 2012). Diets which abound in whole grains pretend to affirmative effects on health comprising body weight management. Dietary recommendations declared that whole grains have several worthwhile bioactive compounds than refined grains. Many epidemiological researches verified invariably that intakes of whole grain instead of refined grains are related with lower body mass index (BMI). Nevertheless, some clinical trials are still inconsistent and/or incomplete to encourage the role of whole grain in enhance weight loss and/or weight control-regulation-maintenance (Karl and Saltzman 2012). In a study, wheat alkylresorcinols increased insulin sensitivity and also glucose tolerance by blunting intestinal cholesterol absorption and hepatic lipid accumulation, which subsequently suppresses diet-induced obesity (Oishi et al. 2015).

It was proven that effect of consuming whole grains decrease daily calorie intake, it was noted that merely randomized, placebo-controlled double-blind studies could show the evidence of phytochemicals and whose exact potential about weight loss and/or management at least up to 24 weeks of consuming (Tucci 2010). Observational studies highly recommend that consuming approximately three servings per day whole grain is related with lower body mass index, diminish weight gain, abdominal adiposity, increase in the dietary fiber and energy intake (Karl and Saltzman 2012). Moreover, it was mentioned that the people who intake more whole grains in their diet tend to have a healthier lifestyle as getting more dietary fiber intake but less fat intake, often exercise, more fewness of them smoke. For this reason, whole grains could be as a marker of healthy body weight but the underlying mechanism

of between them have inadequate proof to show up clear favorable effects of WG consumption on body weight management (Harland and Garton 2008).

Cancer

The guidelines of the American Institute for Cancer Research and the American Cancer Society, suggested consumption of whole grains instead of refined grains for as part of a comprehensive lifestyle approachment to decrease the incidence of cancer (Makarem et al. 2016). Case-control studies based on whole grain is the main reserve of dietary fiber, which associated with a decreased risk of several types of cancer, particularly associated with the digestive system (Xiao et al. 2018) such as colorectal, pancreatic, gastric and hormone-related cancer types (Mourouti et al. 2016). Potential preservative effect of whole grains and cereal fiber is limited to some cancer types such as head and neck cancers, renal cell carcinoma but these results require approval following researches (Makarem et al. 2016). Yet it was also demonstrated that intake of refined cereal-based products such as bread and pasta have been related with enhanced some kind of cancer types as the digestive tract, thyroid, larynx, pharynx, cancers (Gani et al. 2012).

Cell wall materials are resistant to upper gastrointestinal digestion, thus they could reach large intestine without digestion. Then, they could be fermented here by microflora and thus, bioactive components which have antioxidant activity are released (Gani et al. 2012). The antioxidant activity of insoluble phenolic acids could attribute the trapping oxidative components in the whole digestive system. For instance, a few amounts of ferulic acid (0.5–5%) which is typical phenolic acid in whole grain wheat, could be absorbed in the small bowel, majorly the soluble fraction and would perform possibly a major action for preservation from colon cancer (Gani et al. 2012). Kruk et al. (2017) reviewed that some in vitro and observational researches based on anticancer activities of natural or synthetic alkylresorcinols. According to in vitro studies, interception of some types of cancer cell lines as colon cancer, breast cancer, lung cancer and ovarian cancer at micromolar alkylresorcinols content. Prospective studies proved that there is an adverse relation between whole grains consumption and risk of colon cancer. According to prospective researches, approximate 52–66% reduction of distal colon cancer risk at nanomolar alkylresorcinols concentration in plasma whereas 40% increase in the risk of prostate cancer (Kruk et al. 2017). However, some meta-analyses stated an inverse relationship between dietary fiber and whole grain consumption and the risk of colorectal cancer. Some epidemiological studies have pointed out that a possible inverse relation, while other studies have found no clear relation between whole grain intake and risk of breast cancer (Xiao et al. 2018). The unsteady results could be due to some factors as different research designs, different assessment methods of dietary intake, the quantity of whole grain intake in different populations, and a range of contradicting factors that were adjusted in previous studies and were not place in forefront in early studies (Mourouti et al. 2016; Xiao et al. 2018).

The first meta-analysis to research a potential nonlinear relation of dietary fiber intake with breast cancer risk was conducted by Aune et al. (2012). Despite they could not find proof of nonlinearity with the statistical tests used, substantial inverse relations were monitored only among studies with a high level or large range of consumption. Farvid et al. (2016) examined the risk of breast cancer and lifelong grain consumption and remarked that consumption of higher amounts of whole grain foods could act a role in the hindrance of premenopausal breast cancer. Bakken et al. (2016) have investigated that intake of whole grain bread and colorectal cancer risk among Norwegian women. According to findings there was found no relation between whole grains consumption and colorectal cancer that supports the results of Swedish research. However, outcomes of study do not corroborate an early meta-analysis, which remarked that there could be an inverse relationship between the intake of whole-grains and the CRC risk. The differences between studies are attributed to CRC incidence, whole grains type, and whose contents. On the other hand, a recent study found no clear relation regarding the possibly different effects of grains as wheat, rye, or oats among Norwegians, Danes, and Swedes (Bakken et al. 2016). Mourouti et al. (2016) examined case-control research in women based on whole grain consumption and breast cancer. According to findings more than seven times whole grain consumption in a week was coherently related with decreased breast cancer risk, especially among premenopausal and normal weight women patients. Also, it was emphasized that randomized clinical and epidemiological trials are needed to approve or disprove the relationship between them (Mourouti et al. 2016). Also, there are controversial views in this regard. For instance, In the Danish Diet, Cancer and Health Cohort Study, the analysis of a cohort of Danish postmenopausal women consumption of whole grain products was not related to breast cancer risk (Mourouti et al. 2016).

There are many potential mechanisms for the conservation effect of whole grains against breast cancer. It could be first attributed to whose be sources of dietary fiber that enable to augment the amount of fecal bulk and reduce whose transition time. So it causes the decrease interaction fecal mutagens with the epithelial tissue of intestinal. In addition, dietary fibers could dilute or bind bile acids, which are thought to promote cell proliferation, so enabling an increased opportunity for mutations to occur. Besides, whole grains are rich in antioxidant content and phenolic compounds, which have a key role in cancer prevention. In addition, many antinutrients such as phytic acid, protease inhibitors, and saponins could also act as cancer preventers by counteracting the formation of carcinogens and via blocking the interaction of carcinogens with cells. Moreover, whole grains are important sources of phytoestrogens, which appear to have a role as natural cancer-protective composites, by whose antioxidant activity, ability to hinder cell proliferation and give rise to cell apoptosis. Furthermore, taking into consideration that in many epidemiological researches, it was revealed that higher serum insulin levels have been related with breast cancer an indirect way owing to whole grains have enabled to decrease insulin levels (Mourouti et al. 2016).

Processing Effect on Whole Grain Phytochemicals

In the beginning, recommendation and encouragement of whole grains consumption of cereals were related with whose only high dietary fiber contents which provided health benefits. Nonetheless, recent studies demonstrated that the phytochemicals of cereals are mainly located in the outer layers and so their removal would enable products that are less beneficial to health (Shahidi 2009). There are three main key factors affect the content of macronutrients and micronutrients in whole grains are variety and cultivars; growing conditions such as climate, weather and soil; processing type and conditions such as milling, baking, extruding (Jones et al. 2015). Among them, processing of foods mostly presents a major impact on their constituents consisting of their bioactivities (Shahidi 2009). Despite, generally known that the processing has a negative aspect of nutritional value, several factors encourage the significance of processing of grains to develop grain consumption (Slavin et al. 2001). In developed countries, like Europe and the USA, cereal grains are generally exposed to some processing types like milling, heat extraction, cooking, parboiling, or other technique. Commercial cereals are extruded, puffed, flaked, or modified (Slavin 2004) to get the desired product in general by optimizing appearance, texture, flavor, color, and shelf-stable end products (Slavin et al. 2001). Therefore the important thing is not only the content of phytochemicals in whole grains but also determination effect of the processing on end product with regard to pros or cons.

Processing could open up the food matrix, therefore enabling the unleash of strictly bound phytochemicals from the structure of grain (Slavin 2004). Food processing could lead give rise to the decrease phenolic components in the final products, but also food processing could determine physical or chemical modifications in food in the matter of increases the absorption and release of phenolic components in the gastrointestinal system (Călinoiu and Vodnar 2018). In the milling process, the nutritional quality of grain decreases as a result of separating bran and germ fractions from the endosperm. On the other hand, fiber and phytates could increase the bioavailability of minerals and vitamins (Harris and Kris-Etherton 2010) as a result of germination, fermentation and baking cause to phytate hydrolysis (Stevenson et al. 2012). Thermal processing such as cooking, baking, boiling, and parboiling could release antioxidants from the bran into the endosperm and so enhance the bioavailability of phytochemicals, and form resistant starch. The sourdough fermentation or the adding of organic acids to grains enhance glycemic response in the absence of fiber (Harris and Kris-Etherton 2010). In some rye-based studies, biologically active compounds are stable during food processing and also whose content may be increased with proper conditions (Slavin 2004). For instance, Liukkonen et al. (2003) examined the impact of some processes like milling, germinating and sourdough fermentation on whole grain rye. Results showed that after the 6-day germination at three different temperatures (5 °C, 10 °C, 25 °C) the amount of easily extractable phenolic compounds and folates increased and highest values were obtained in the highest temperatures. The amount of bioactive com-

pounds as easily extractable phenolic compounds, phenolic acids, lignans, sterols and alkylresorcinols slightly increased while tocols reduced after the fermentation process.

Despite some of these compounds could be substituted into the refined flour through compulsory enrichment policies, there is a prevalent view; that intake of the fortified product is not the same as intake of the original grain product with its more complicated structure. In the generality of modern milling methods, the individual compounds of the grain are removed, yet then re-constituted to re-form the whole grain flour (Seal 2006).

Germination

The main function of the endosperm is to provide energy to the seedling (Jacobs and Gallagher 2004). The germinated grain is a raw material as a fermentable sugar and nitrogen source eventuate in raising in bioactive compounds level. Also, grain germination commences with a soaking process which leading to synthesize several cell wall degrading enzymes to metabolize some molecules results synthesis of some bioactive compounds. Germinatination causes augmentation in the level of nutritional compounds and whose bioavailability and also, reduce in the content of antinutritional compounds (Alverez-Jubete and Tiwari 2013), such as phytic acid and tannins. Besides, germination improves the nutritional value of the grains by increasing the content of α-tocopherol and vitamin C as vitamins and β-carotene, polyphenols, ferulic acid, and vanillic acid as antioxidants (Yang et al. 2001; Schaffer-Lequart et al. 2017). Many in vitro studies supported that phenolic content and relevant with antioxidant capacity augments with germination process (Alverez-Jubete and Tiwari 2013). However, germination could also degrade β-glucan in oats and barley by decreasing the average molecular weight of β-glucan (Wolever et al. 2010; Schaffer-Lequart et al. 2017). The important point is the optimization of germination conditions as sprouting time and temperature to minimize lack of phytochemicals (Alverez-Jubete and Tiwari 2013). For instance, although it is declared that generally β-glucan is affected adversely during germination; optimize germination temperature and time as 72 h, 15 °C respectively could end up with higher retain β-glucan content (Wilhelmson et al. 2001).

Milling

Milling is a process of whole grains ends with refined flour (Slavin et al. 2013). Conventional milling of grains is based upon separate the endosperm, which generates white flour, from the bran and embryo fractions (Stevenson et al. 2012). In other words, the milling term generally refers to removing the germ and bran fraction (Alverez-Jubete and Tiwari 2013). The bran and germ fractions are

corresponded to 14% and 2.5% in whole wheat, respectively while lower than about 0.1% in refined wheat. Therefore, starch content is higher in refined grains than whole grains (Slavin et al. 1999).

Flour types could be defined by the extraction rate. In this regard, white flour is identified by 75% and/or less extraction rate whereas wholemeal or whole grain flour is specified by 100% extraction (Pedersen et al. 1989). Bran, along with germ is carried out for appearance, organoleptic properties and extend shelf life by discarding generally bran and germ fractions. These components have been characterized to include a large amount of dietary fibre, antioxidants, phenolic lipids as alkylresorcinol, phytosterols, vitamins, minerals and other phytochemicals (Patel 2015). Thus, the milling process causes remarkable losses as dietary fibre and other phytochemical compounds especially carotenoids, tocols and also phenolic acids, particularly ferulic acids (Alverez-Jubete and Tiwari 2013). For instance, refining wheat led to approximately 200–300-fold loss in phytochemical content (Craig 1997; Craig and Beck 1999).

Milling method, process parameters, the degree of milling are also had an effect on phytochemical contents, means high extraction rate flours related with high antioxidant capacity. Increasing the degree of milling is a particularly adverse effect on tocols (Alverez-Jubete and Tiwari 2013). For instance, whole grain wheat is possible to lose more than half of dietary fiber and folates about, 58% and 79%, respectively and also most of the minerals (Mg, Zn, Se) and vitamins (vitamin B3, vitamin E) (Fardet 2010). In other words, refined the grains not only have lower phytate and fiber contents but also lower mineral contents. The reducing availability of minerals is attributed to mineral-binding capabilities of phytic acid and fiber content. Consumption of whole grains and fiber in recommended amounts as 20–35 g in a day for fiber and three servings in a day for whole grains were not found to have any negative influences on mineral status (Slavin et al. 1999). Therefore it is known that consuming whole grains have more potential health benefits than refined flours (Alverez-Jubete and Tiwari 2013).

The particle size of the whole grain is a significant factor in determining the whose physiological effect. For instance, coarse wheat bran retards gastric emptying and small intestine transition by having a high faecal bulking effect than finely ground wheat bran. Thus, beyond composition differences coarse whole grains as has a unique physiological effect (Slavin 2004).

When comparing with refined wheat flour and whole grain wheat flour, it is determined that refined wheat flour lost whose 83% of total phenolics, 79% total flavonoids, 93% ferulic acids which is one of the phenolic acids compound, 78% zeaxanthin and 51% lutein content (Slavin et al. 2013). Conversely, some researches notified that operations as milling and the thermal process could increase bioaccessibility of phytochemicals (Slavin et al. 2013). Some researches notified that the phenolic content increased by the milling process. It is reported that the addition of 5% micronized fractions in the fermentation process to wheat flour based dough increased the content of dietary fiber, total phenols, antioxidant activities and free amino acids of dough and improved sensory properties of the end product in bread making process. Another experimental research reported that there is an adverse

relationship between the bioaccessibility of phenolic compounds and the bran particle size (Călinoiu and Vodnar 2018).

Fermentation

Sourdough fermentation is using traditional bread production to enhance nutritional value and some quality parameters as a flavor and textural properties in contribution to LAB and yeasts. Besides sourdough fermentation improves the bioavailability of minerals and also reduces the glycemic index. From the point of view phytochemical content, sourdough fermentation could affect positively or negatively based on the type of phytochemical compound and sourdough process. Studies mentioned that sourdough fermentation increases the content of folates and total free phenolic compounds, namely extractable phenolics thereby antioxidant capacity. In addition, decreasing in pH levels consequential of sourdough fermentation increases pronyl-L-lysine in end products. Whereas reduces β-glucan and also particularly oats and barley flours, and also tocols because of oxidation. Conversely, the fermentation process causes minimal decreasing in carotenoid content that is related to the preservative effect of bakery yeast by reason of consuming O_2 throughout fermentation which results in the inheritance of lipoxygenase related carotenoid degradation. In addition, it is reported that there could be a synergist effect between germination and fermentation processes on phytochemical content. In this context, the combination of these two processes increase folates, lignans, sterols and ferulic acids in whole grain rye. This could be explained by the same pH ranges of fermentation (pH: 4.5–6.0), and cell wall degradation which is carried out by indigenous grain or microorganisms enzymes levels (Alverez-Jubete and Tiwari 2013). Anti-nutritional compounds like phytic acid, tannins, and flatulence sugars could be reduced by fermentation (Soetan and Oyewole 2009; Schaffer-Lequart et al. 2017).

Thermal Process

Thermal processes as baking are generally causing to a reduction in the content of tocols, flavonoids, β-glucans, carotenoids and also antioxidant which results in decreasing antioxidant capacity in the final product. Loss of polyphenolic compounds is associated with extraction rate, substrate and baking process parameters. Reduction in tocol content is related to oxygenation throughout dough making and heat devastation throughout the baking process. Tocol content in the final product depends on several factors such as initial tocol content, tocol profile, other compounds which have antioxidant activity. As carotenoids polyphenolic compounds particularly flavonoids are heat sensitive and so adversely affected by thermal processing as baking. The whole breadmaking process affects the stability of carotenoids adversely. In this regard, the highest decrement is shown in dough making

due to oxygen inclusion during kneading and hereout eventuating carotenoid degradation, following by baking and then fermentation processes. From the point of carotenoid content view, the baking process cause to decrease in carotenoid content because of high sensitivity to heating and seen in more crust structure than crumb (Alverez-Jubete and Tiwari 2013). On the other hand, despite Maillard reaction and whose products are generally known as having a harmful influence on health, many researches have indicated that some of these compounds show antioxidant activities as well and so could affect the final antioxidative features of baked products, particularly in the crust part (Alverez-Jubete and Tiwari 2013; Slavin et al. 2001). For instance, when comparing with flour as a raw material or crust-free bread, it is remarked that the crust of white bread has double the antioxidant activity (Slavin 2004). Also, among boiling, microwave heating, and pressure cooking, roasting is the best cooking way to maintain and/or in some cases increase the phenolic compounds bioaccessibility in wheat, sorghum, finger millet and pearl millet (Călinoiu and Vodnar 2018).

Bioactive compounds are sensitive to extrusion process parameters and could be influenced in a negative or positive way. A number of researches noticed that content of phytochemicals, particularly tocols, anthocyanins, and phenolic acids are reducing during extrusion cooking in whole grain wheat, barley, oats and rye (Alverez-Jubete and Tiwari 2013). In a study, extrusion cooking treatment of wheat, oat, and brown rice were implied and the findings revealed that total antioxidant activity and total phenolic content stemming from the free forms were reduced whereas the total bound phenolic acids were significantly increased (Călinoiu and Vodnar 2018). In another study, extrusion cooking, which was applied between 120 and 200 °C, increased the phenolic content of oats and sorghum bran, while wheat, rye, and barley had an increment of 200–300% of ferulic acids, vanillic acids, and syringic acids as free forms suggesting that hydrothermal processing could raise the releasing rate of phenolic acids and whose derivatives. This increment could be a result of the combination of high temperature and water-stress (Călinoiu and Vodnar 2018). According to another study, dietary fiber through Klason lignin content of wheat increased after extrusion by catalyzing the Maillard reaction (Poutanen 2001). Zieliński et al. (2001) studied on the effect of extrusion cooking in three different temperatures (120 °C, 160 °C, 200 °C) on bioactive compounds of wheat, barley, rye, and oats. According to results, the content of tocopherols and tocotrienols decreased whereas phenolic acids which are free and released from ester bonds increased. On the other hand, also it was reported that the extrusion process caused degradation of the dietary fiber polysaccharides (Poutanen 2001). Also, the decrease in antioxidant activity and total phenolic content of end products as extrudates was reported. This decrease could be attributed to whose low resistance to heating and evaporating processes temperatures (Călinoiu and Vodnar 2018).

The extrusion process results in the mechanic breakdown of the glycosidic bonds and could cause increasing the amount of soluble dietary fiber. Actually, the mechanical stress through the extrusion process could be in charge of the breaking down polysaccharide glycosidic bonds, causing to the release of oligosaccharides. Because of this reason, it could increase the soluble dietary fiber. Nevertheless,

there are controversial opinions in some cases about increasing insoluble dietary fiber. These differences are attributed to the different process conditions as shape and speed and shape of the extrusion screws. In addition, both of the starch gelatinization and starch retrogradation, the formation of the protein-polysaccharide complex during Maillard reaction and also oxidation of dietary fiber-phenolic compounds could account for the increase of insoluble dietary fiber by the formation of diferulates and results in dietary fiber cross-linking (Vitaglione et al. 2008).

Parboiling is comprised of three different processes like soaking, heating, and drying, relatively. The effect of this hydrothermal process on phytochemical of whole grain rice is associated with stability and migration of carotenoids. Particularly brown rice parboiling decrease content of carotenoids to trace levels (Alverez-Jubete and Tiwari 2013).

Conclusion and Future Remarks

Cereals are one of the main raw materials for the staple foods in many countries. Also, awareness about the relationship between health and food is increasing day by day. In this regard, whole grains are rich in several phytochemicals involving dietary fibre, phenolic compounds and other phytochemicals as phytic acid, γ-oryzanol, avenanthramides, and benzoxazinoids which are linked with reducing some chronic diseases like cardiovascular disease, cancer, diabetes, and obesity. Therefore, raising awareness, education of consumers about the health benefits of whole grains and designated daily intake of whole grains is so important regarding public health policy, which should be mediated by government in collaboration with health organizations, media, academic researchers and industry. Moreover, one of the most significant factors for choosing foods is the sensory properties of foods. For this reason, future studies should focus on advanced processing techniques to develop palatability of wholegrain products, and so, increasing the consumption of whole grain-based baked foods. Further, while choosing the processing technology, its advantages and disadvantages on the end product should be taken into consideration. Besides, further epidemiological studies should enlighten the plausible mechanisms of the protection of these compounds and health problems.

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Chemical Hazards in Foods

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Abstract This extensive chapter focuses on chemical hazards that have increased dramatically because of the economic development in various sectors including agriculture, food processing, industry and transport. Chemical hazards in food chain pose a wide range of health risks varying from irritation to chronic diseases and cancer. Moreover, exposure to a combination of chemical hazards may be associated with additive, antagonistic, and synergistic interactions. Thus it is necessary to monitor their concentrations in food and reduce exposure to consumers. The well compiled chapter includes occurrence, detection, legislation, toxicity and risk assessment of a variety of chemicals of both natural and man-made origin.

Keywords Chemical hazards · Chemical contaminants · Food

Introduction

Food safety is a global concern and major issue for both manufacturers and consumers. Many toxic chemicals including natural occurring toxins, food additives, pesticides, adulterants, process contaminants, environmental contaminants, food contact materials, veterinary drugs, and others can be found in foods and feeds and may pose a risk to human and animal health. Among these chemical hazards, mycotoxins are of greatest concern in terms of human health as well as economics. While pesticides are important to control pests and diseases caused by pathogens and parasites, they can harm human and animal health when accumulate in agricultural products. Several chemical contaminants including acrylamide, furan, 3-MCPD, glycidyl fatty acid esters (GE), and polycyclic aromatic hydrocarbons (PAHs) can also be formed in food by cooking or other food processing methods. Acrylamide can form

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as a by-product during the heating of starchy food products like potato and bread to above 120 °C. 3-monochloropropane-1-2 diol (3-MCPD) and GE are other process contaminants that form during the refinement of edible oils and fats. The current knowledge on several chemical hazards that have been natural or man-made origin are discussed in this chapter.

Mycotoxins

Mycotoxins occur in agricultural products mainly the result of the activity of three genera: *Aspergillus*, *Penicillium* and *Fusarium* (Pitt et al. 2000). Fungi from genera *Fusarium* are mainly active in the field, while both *Aspergillus* and *Penicillium* species are considered the more likely storage fungi. Mycotoxins have been associated with severe toxic effects to animals and humans, from allergic responses to cancer and death, depending on a number of factors including intake levels, duration of exposure, toxin species, mechanisms of action, metabolism and defence mechanisms. The most dangerous and frequently found mycotoxins in nature are aflatoxins (AFs), ochratoxin A (OTA), trichothecenes (T-2/HT-2 toxin and deoxynivalenol (DON)) and fumonisins (FUMs) (Kabak et al. 2006). The chemical structures of frequently detected mycotoxins are illustrated in Fig. 1. The Scientific Commission of the European Community have regulated maximum limits (MLs) for certain mycotoxins including naturally occurring AFs, aflatoxin M_1 (AFM_1), OTA, FUMs (fumonisin B_1 (FB_1) + fumonisin B_2 (FB_2)), DON, zearalenone (ZEA) and patulin in foodstuffs due to their health hazards to human (European Commission 2006). Mycotoxins have been evaluated for their carcinogenic potential by International Agency for Research on Cancer (IARC) (IARC 1993). The carcinogenic potential of some mycotoxins have been shown in Table 1.

Aflatoxins

AFs are produced primarily by three species of toxigenic Aspergilli, *A. flavus*, *A. parasiticus* and rarely *A. nomius*. Fungal contamination and subsequently AFs synthesise can occur in commodities in the field, at harvest, during post-harvest and in storage. The main factors that affect the formation of AFs are temperature and humidity (EFSA 2004). Both *A. flavus* and *A. parasiticus* can grow at temperatures ranging from 10 to 43 °C, with an optimum temperature of 32–33 °C. However, AFs can be synthesised at 12–40 °C (Koehler et al. 1985).

AFs can be found a wide range of commodities (Table 2) including peanuts, tree nuts (hazelnuts, pistachios, almonds, walnuts, Brazil nuts), spices (*Capsicums* spp.), dried fruits (figs, raisins) and a range of cereals (especially maize). Although AFs contamination is generally considered to be a problem in tropical/subtropical regions of Africa, Asia and Latin America, it can also be produced in temperate countries of Europe and North America (EFSA 2004).

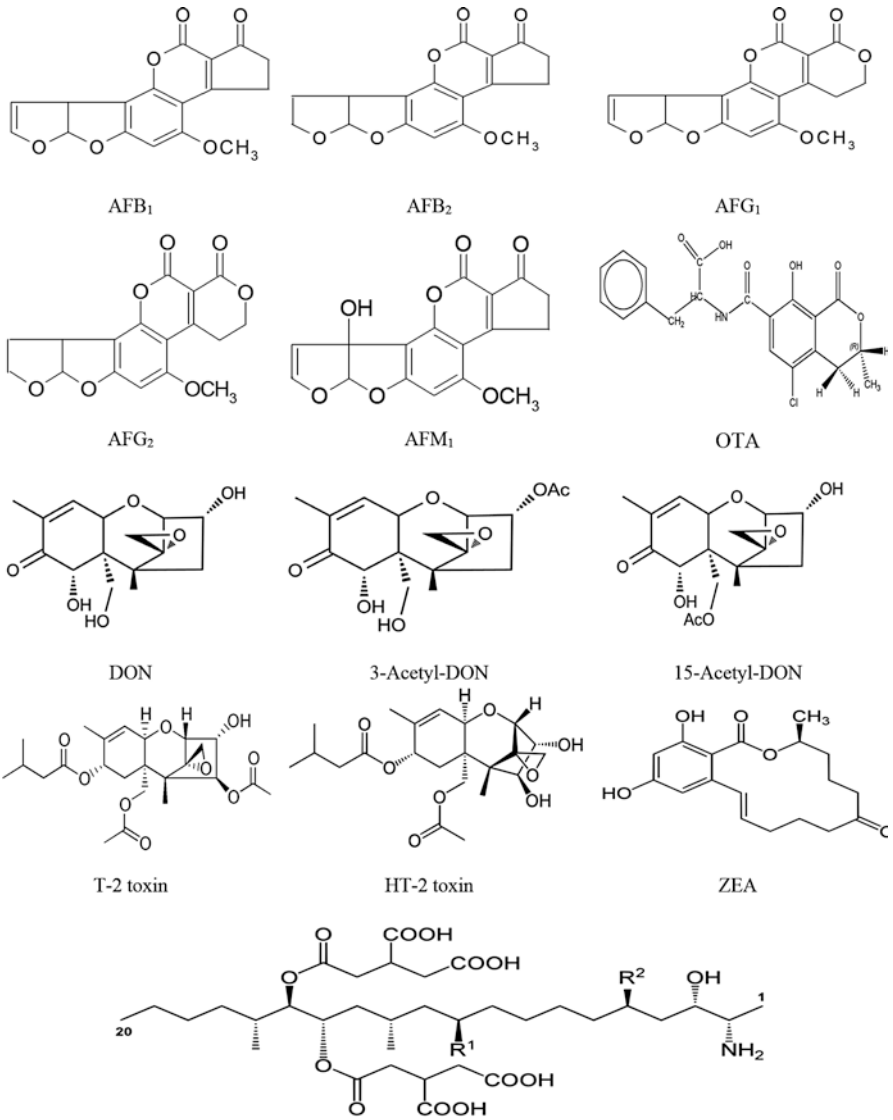


Fig. 1 Chemical structures of mycotoxins

Table 1 Mycotoxins classified by IARC

Mycotoxin	IARC Group	Evaluation
AFB_1 and naturally occurring AFs	Group 1	Human carcinogen
Aflatoxin M_1	Group 2B	Possibly carcinogenic to humans
Ochratoxin A	Group 2B	Possibly carcinogenic to humans
Fumonisin B_1	Group 2B	Possibly carcinogenic to humans
Sterigmatocystin	Group 2B	Possibly carcinogenic to humans
Deoxynivalenol	Group 3	It is not classifiable as to its carcinogenicity to humans
Zearalenone	Group 3	It is not classifiable as to its carcinogenicity to humans
Patulin	Group 3	It is not classifiable as to its carcinogenicity to humans

Table 2 Major mycotoxins, associated moulds and product of primary concern

Mycotoxin	Principal producing moulds	Product of primary concern
Aflatoxins	*Aspergillus flavus*, *A. parasiticus*, *A. nomius*	Peanuts, tree nuts (hazelnuts, pistachios, almonds etc.), figs, raisins, *Capsicums*, maize, cocoa beans
Ochratoxin A	*Aspergillus ochraceus*, *A. carbonarius*, *Penicillium verrucosum*.	Cereals (wheat, rice, etc.) and cereal-based products, *Capsicums*, raisins, figs, coffee and cocoa beans, wine and beer
Fumonisin B_1	*Fusarium moniliforme*, *F. proliferatum*	Maize and maize-based products
Deoxynivalenol	*Fusarium graminearum*, *F. culmorum*	Cereals (especially wheat, rice and barley) and cereal-based products
T-2/HT-2 toxin	*Fusarium sporotrichioides*, *F. equisetiiculmorum*, *F. poae*	Cereals and cereal-based products
Zearalenone	*Fusarium graminearum*, *F. culmorum*, *F. equiseti*, *F. sporotrichioides*	Maize and maize-based products
Moniliformin	*Fusarium moniliforme*, *F. oxysporum*, *F. fujikuroi*	Cereals and cereal-based products
Patulin	*Penicillium expansum*, *P. patulum*, *Aspergillus clavatus*, *Byssochlamys fulva*	Apple juice/concentrate

AFs occur in several chemical forms, approximately 19 different toxic derivatives of AFs have been reported. The four main naturally produced AFs (Fig. 1) are AFB_1, aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2). "B" and "G" refer to the blue and green fluorescent colours produced by these compounds under ultraviolet (UV) light on thin-layer chromatography plates, while subscript numbers 1 and 2 indicating major and minor compounds respectively. Following ingestion, AFB_1 is metabolized in the liver through the cytochrome P450 enzyme

system to various metabolites, including the endo- and exo-epoxides of AFB_1, the hydroxyl-metabolites AFM_1, aflatoxins M_2 and M_4, and aflatoxins P_1 and Q_1, as well as conjugated metabolites (McLean and Dutton 1995). The carcinogenicity of AFB_1 arises from its interaction with the DNA-guanine moiety to produce to the aflatoxin-N7-guanine adduct, whereas the acute toxicity of AFB_1 is believed to stem from interaction between the dihydrodiol and protein amino groups to produce Schiff base adducts (Pitt et al. 2000).

AFM_1, the 4-hydroxy metabolite of AFB_1, is the predominant metabolite of AFB_1 in milk. When lactating animals such as cows, goats and humans are fed with feedstuffs contaminated with AFB_1, this metabolite can be transferred to milk as AFM_1 in the range of 0.3–6.3% (Choudhary et al. 1998).

AFs are major class of mycotoxins produced by *Aspergillus* species which are both acutely and chronically toxic to various animal species, including humans, causing acute liver damage, liver cirrhosis, tumour induction and teratogenesis (Pitt 2000). AFs were first discovered in 1960s as a result of deaths of thousands of turkeys in the UK and this was referred to as "turkey × disease". Investigation led to the fact that outbreaks were associated with groundnut meal infected with *A. flavus* and contaminated by a compound which fluoresced blue under UV light (Moss 2002).

Exposure to AFs at high levels can led to acute human aflatoxicosis leading to jaundice, oedema, GI haemorrhage, and ultimately, death (Shephard 2008a). There have been reported several outbreaks of acute toxicity of AFs to humans, but not rarely. One of the most serious outbreak of aflatoxicosis occurred in the region of western India in 1974, leading to 397 recognized cases and 106 deaths. The outbreak was traced to consumption of maize heavily contaminated with AFs ranged between 6.25 and 15.6 mg kg^{-1}. Daily consumption of toxin by some of the patients was calculated to be 2–6 mg of AFs for several weeks (Krishnamachari et al. 1975). Other documented fatal human aflatoxicosis outbreaks have been reported in Kenya in 1981: 20 cases and 12 reported deaths (Ngindu et al. 1982); and Malaysia in 1988: 13 deaths (Lye et al. 1995). One of the largest outbreak of aflatoxicosis have happened in the eastern and central districts of Kenya in 2004 where 317 people were ill and 215 died, following the consumption of contaminated maize (up to 8 mg kg^{-1} AFs). More recently, a similar outbreak in the eastern districts of Kenya during 2005 resulted in 75 cases, with 32 deaths (Shephard 2008b).

Two human diseases, Kwashiorkor and Reye's syndromes may also associate with the ingestion of AFs-contaminated food. Kwashiorkor, a disease of children in Northern Africa, is usually attributed to nutritional deficiencies, but may also be related to AFs intake (Hendrickse et al. 1982). Similarly, Reye's syndrome, which is characterized by acute encephalopathy and fatty degeneration of the viscera has been linked to AFs because this mycotoxin has been detected in the organs of affected children in Thailand, Australia and New Zealand (Becroft and Webster 1972). AFs have been linked to hepatocellular carcinoma and classified as human carcinogens (IARC 1993).

Ochratoxin A

OTA is produced primarily by *Penicillium verrucosum*, *Aspergillus ochraceus* and *Aspergillus carbonarius* (EFSA 2006). While *P. verrucosum* is the main producer in cereals and cereal products for OTA in cooler regions of Northern Europe (Olsen et al. 2003) and Canada (JECFA 2001), *A. ochraceus* grows at moderate temperatures and it can infect a wide range of stored commodities including coffee beans, cocoa, cereals and edible nuts (EFSA 2006). *A. carbonarius* grows at high temperatures and is associated with maturing fruits, especially grapes (JECFA 2001).

Invasion with toxigenic species of *Aspergillus* and *Penicillium* has been reported worldwide and subsequently OTA can be found as a natural contaminant in cereals (wheat, maize, oat, millet, rye, barley and rice) cereal-based products, pulses, dried figs, raisins, wine, grape juice, beer, coffee beans, cocoa, as well as nuts (Table 2).

OTA has been reported to be carcinogenic (group 2B), teratogenic, immunotoxic, genotoxic and possibly neurotoxic to experimental animals (European Commission 2002). It is also a well-known nephrotoxic agent and has been associated with fatal human kidney disease, and with an increased incidence of tumours of the upper urinary effect (JECFA 2001).

Fumonisins

FUMs are produced primarily by *Fusarium moniliforme* and *Fusarium proliferatum*. These species cause *Fusarium* kernel rot of maize, an important disease in hot climates. As *F. moniliforme* and *F. proliferatum* grow over a wide range of temperature but only at relatively high water activities (above about 0.9), FUMs are formed in maize only before harvest or during the early stage of drying. Other FUMs producing species are *F. napiforme*, *F. anthophilum*, *F. dlamini* and *F. nygamai* (Musser and Plattner 1997).

FUMs can also be found in sorghum, asparagus, rice and mung beans. It has been also detected in several processed products such as beer, bread, breakfast cereals, chilli pickles, corn flakes, curry paste, maize muffin, maize pops cereals, maize starch, maize-based infant cereals, noodles (Arranz et al. 2004).

FUMs are characterized by a 19-20 carbon amino-polyhydroxyalkyl chain which is diesterified with propane-1,2,3-tricarboxylic acid. The first FUMs identified were FB_1 and FB_2 from cultures of *F. moniliforme* MRC 826, an isolate from South African maize (Gelderblom et al. 1988). The 28 FUMs analogs that have been characterized since 1988 can be separated into four main groups, identified as the fumonisin A, B, C, and P series (JECFA 2001). FB_1 has an empirical formula of $C_{34}H_{59}NO_{15}$ with a molecular weight of 721.838, and a melting point of 103–105 °C (Scott 1993).

FB_1 is the causative agent in the incidence of neurotoxic syndrome equine leukoencephalomalacia (ELEM) (Gelderblom et al. 1996). The ELEM, also known as "crazy horse disease" characterized by liquefactive necrotic lesions in the white

matter of the cerebral hemispheres of horses and other equine species. FB_1 also causes pulmonary oedema syndrome and hydrothorax in pigs (Ross et al. 1990); and it is also hepatotoxic and carcinogenic in rats (Gelderblom et al. 1988, 1991, 1996). The liver and kidney are the main target organs of FB_1 in mice and rats (Gelderblom et al. 1991). The FB_1-induced changes to cellular membranes, specifically those related to fatty acid changes in the major membrane phospholipid, and the altered fatty acid content of the hepatocytes are likely to be key events in explaining the cytotoxic effects and altered growth responses induced by FUMs in primary hepatocytes (Gelderblom et al. 1996).

FB_1 is not genotoxic and mutagenic (Gelderblom and Snyman 1991). There is some evidence *in vitro* for developmental toxicity, but except for chicken no teratogenic effects were reported in either *in vitro* or *in vivo* studies (SCF 2000). Other toxic effects of FB_1 such as neurotoxic and immunotoxic have also been reported (Stockmann-Juvala 2007).

Trichothecenes

Trichothecenes are a very large family of naturally occurring sesquiterpenoid metabolites produced by a number of fungal genera including *Fusarium*, *Stachybotrys*, *Myrothecium*, *Trichoderma*, *Cephalosporium*, *Verticimonosporium* and others. Although the number of trichothecenes runs into hundreds, only a few of them have been shown to be agriculturally important. Among Fusaria, *F. poae*, *F. sporotrichioides*, *F. moniliforme*, *F. culmorum* and *F. graminearum* are the most common trichothecene producers (Bennet and Klich 2003).

The trichothecene mycotoxins are non-volatile, low-molecular-weight (MW 250–500). The trichothecenes are colourless, mostly crystalline solids (European Commission 2003). All trichothecenes contain an olefinic bond at 9, 10 and an epoxide group at C-12, 13, characterized as 12, 13-epoxytrichothecene, and classified mainly as types A, B, C and D (Ciegler 1978) The most common type A trichothecenes are T-2 toxin, HT-2 toxin, neosolaniol, monoacetoxyscirpenol and diacetoxyscirpenol produced by mainly *F. sporotrichioides* and *F. poae*, while common type B trichothecenes include DON, and its 3-acetyl and 15-acetyl derivatives (3-AcDON and 15-AcDON, respectively), nivalenol (NIV) and fusarenon-X produced principally by *F. graminearum* and *F. culmorum* (Placinta et al. 1999). The type C trichothecenes are crotocin and crotocol (Ciegler 1978), while macrocyclic trichothences (type D) include satratoxins, verrucarins and roridins and produced by the members of the genus *Myrothecium* and *Stachybotrys* (Sudakin 2003).

Trichothecenes mainly occur in cereal grains such as wheat, barley, maize, oats, rice, soya beans and in derived products such as breakfast cereals, bread and beer. These compounds are also seldom detected in other food commodities including sorghum, potatoes, bananas, mustard seed, groundnuts, mangoes and sunflower seed. According to European Commission reports (2003), type B trichothecenes such as DON (57% of tested grain samples), 15-AcDON (20%), NIV (16%), fusarenon-X

(10%) and 3-AcDON (8%) are more frequent in European grain samples than type A trichothences. While T-2 toxin is the most common type A trichothecene (20% of tested samples), other toxins including HT-2 (14%), T-2 triol (6%), diacetoxyscirpenol (4%), monoacetoxyscirpenol (1%) and neosolaniol (1%) are less common.

T-2 Toxin and HT-2 Toxin

T-2 toxin and HT-2 toxin (type A trichothecenes) are produced by certain *Fusarium* species, especially *F. sporotrichioides*, *F. poae*, *F. equiseti* and *F. acuminatum* (JECFA 2001). T-2 and HT-2 toxins often occur together rarely in grains such as maize, wheat, barley, oats and rye as wells as in some cereal-based products including malt, beer and bread (SCF 2001a). Among type A trichothecenes, T-2 and HT-2 toxins were found to be two most frequent contaminants of cereal grains (3490 samples) from EU Member States. The frequency of occurrence of T-2 toxin and HT-2 toxin was 28% and 24% for maize, 21% and 12% for wheat (and wheat flour), 21% and 17% for rye (and rye flour), 16% and 41% for oats, and 3% and 5% for barley, respectively. The mean concentrations of T-2 toxin in positive samples were ranged from 3 to 255 μg kg^{-1} in maize, 2–160 μg kg^{-1} in wheat (and wheat flour), 10–193 μg kg^{-1} in rye (and rye flour), 10–550 μg kg^{-1} in oats, and 1.7–280 μg kg^{-1} in barley. The mean value of HT-2 toxin contamination has been reported to range from 3 to 120 μg kg^{-1} in maize, 3.3–50 μg kg^{-1} in wheat (and wheat flour), 10–70 μg kg^{-1} in rye (and rye flour), 10–1150 μg kg^{-1} in oats, and 1.7–287 μg kg^{-1} in barley (European Commission 2003). T-2 toxin and its metabolites may also be found in trace amount in animal products.

T-2 toxin can inhibit DNA, RNA and protein synthesis both *in vitro* and *in vivo* (Shinozuka et al. 2001). This compound also induces apoptosis both *in vitro* (at 10 ng ml^{-1} levels in HL-60 human promyelocytic leukemia cells and at 0.2 g ml^{-1} in mouse thymocytes) (Ueno et al. 1995; Shinozuka et al. 2001) and *in vivo* (at 10 mg kg^{-1} b.w. in intestinal crypt epithelial cells, and thymic and splenic lymphocytes in mice) (Li et al. 1997) in various organs. Moreover, T-2 toxin could inhibit the mitochondrial electron transport system, with succinic dehyrogenase as one site of action (Khachatourians 1990).

T-2 toxin is about 10 times more toxic than DON (Ueno et al. 1973). Acute effects of T-2 toxin occur after oral exposure to 0.06–10 mg kg^{-1} body weight (b.w.) in various species. The effects include non-specific symptoms like weight loss or poor weight gain, feed refusal or reduced feed intake, dermatitis, vomiting, diarrhoea, haemorrhages and necrosis of the epithelium of stomach and intestine, bone marrow, spleen, testis and ovary (SCF 2001a).

T-2 and HT-2 toxins have been associated with alimentary toxic aleukia (ATA) in humans. During World War II, a very severe human disease occurred in the former Soviet Union, particularly population in Orenburg. The disease, known as ATA is believed to be related to ingestion of over-wintered grains infected with *F. poae* and *F. sporotrichioides* that were milled into flour and made into bread. The most severe outbreak of the disease was in 1944, but outbreaks have also been reported in 1952,

1953, and 1955 particularly in people consuming over-wintered wheat (SCF 2001a). In an outbreak of toxicosis in China, 97 out of 165 persons fell ill who had consumed rice infected with *F. heterosporum* and *F. graminearum*. The level of T-2 toxin in these mould rice was up to 420 μg kg^{-1}. The symptoms were nausea, dizziness, vomiting, chills, abdominal distension, abdominal pain, thoracic stuffiness and diarrhea (Wang et al. 1993).

The FAO/WHO Joint Expert Committee on Food Additives (JECFA) proposed a provisional maximum daily intake (PMTDI) of 60 ng kg^{-1} b.w. per day for T-2 and HT-2 toxins, alone or in combination, using a safety factor 500 (JECFA 2001). A temporary TDI (t-TDI) of 0.06 μg kg^{-1} b.w. for the sum of T-2 and HT-2 toxins was set by the SCF of the European Commission (SCF 2001a). No regulations exist for T-2 and HT-2 toxins but EU plans regulatory limits for these mycotoxins in cereals and cereal products.

Deoxynivalenol

DON also known as vomitoxin belongs to the type B trichothecenes and is produced principally by *F. graminearum* (teleomorph *Gibberella zeae*) and *F. culmorum*. Both species are important plant pathogens, causing FHB in wheat and *Gibberella* ear rot in maize (JECFA 2001).

DON has been found as a natural contaminant in various cereal crops such as wheat, buckwheat, maize, barley, oats, rye, rice, sorghum and triticale. It has also been detected in processed cereal products including malt, beer, bread and breakfast cereals etc. In contaminated cereals 3-AcDON and 15-AcDON can in significant amounts (10–20%) occur concomitantly with DON (SCF 2000). Data were available for samples of 11,022 grains from EU Member States. DON was found to be the most frequent trichothecene in cereal grains such as wheat (and wheat flour) (6358 samples, 61% positive), maize (520 samples, 89% positive), barley (781 samples, 47% positive), rye (and rye flour) (271 samples, 41% positive), and oats (595 samples, 33% positive). The concentrations of DON in positive samples ranged from 2 to 50,000 μg kg^{-1} in wheat (and wheat flour), 7–8850 μg kg^{-1} in maize, 1.7–619 μg kg^{-1} in barley, 2–5004 μg kg^{-1} in oats and 2–595 μg kg^{-1} in rye (and rye flour). The frequency of occurrence of 3-AcDON and 15Ac-DON was 8% and 20% positive, with ranging from 1.7 μg kg^{-1} to 520 μg kg^{-1} and from 1.7 μg kg^{-1} to 1320 μg kg^{-1}, respectively (European Commission 2003). The transfer of DON from animal feed to meat and other animal products appears to be extremely small.

DON (12, 13-epoxy-3, 4,15-trihydroxytrichotec-9-en-8-one) has a molecular weight of 296.32 with the empirical formula of $C_{15}H_{20}O_6$ and a melting point of 131–135 °C. DON contains one primary and two secondary hydroxyl groups and is soluble in water and polar solvents such as methanol and acetonitrile.

DON may induce several detrimental health effects after acute, short-term, or long-term administration. The exposure to DON at low concentrations can result in a reduction in food consumption (anorexia), while higher doses induce vomiting (emesis). Although it has been suggested that chronic toxic effects of 15-AcDON

are similar to those of DON, 15-AcDON had twice the acute oral toxicity of DON. Acute doses of both DON (60–1000 mg kg^{-1}) and 15-AcDON (40–160 mg kg^{-1}) result in a variety of toxic signs ranging from necrosis of the gastrointestinal tract, bone marrow, lymphoid tissues, and focal lesions in kidney and cardiac tissue. The kidney of the mice appears to be 5–10 times more sensitive to 15-AcDON than DON, whereas the hearth was more sensitive to DON than 15-AcDON (Forsell et al. 1987).

Food Additives and Flavourings (FAF)

FAF are a variety of organic chemicals that are incorporated deliberately or by accident into the food during production/processing, which cannot normally be consumed as food (Deshpande 2002; Inetianbor et al. 2015; Martins et al. 2019). According to the Food and Drug Administration (FDA), food additives are any substance, the intended utilize of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise effecting the features of any foodstuff (FDA 1993). Direct food additives are substances which are deliberately supplemented to food for a specific aim, while indirect ones are the substances to which foods are exposed throughout manufacturing, processing, packing or storage, and found in foods at trace amounts (Al-Shammari et al. 2014; Pressman et al. 2017). FAF are used for various purposes (Deshpande 2002; Shibamoto and Bjeldanes 2009; Pressman et al. 2017). These are:

- Assisting in technological processes in the production of processed foods
- Preventing microbiological deterioration
- Increasing durability
- Maintain and protecting nutritive value and
- Making food attractive to consumers by improving and correcting sensory properties such as colour, appearance, taste, texture, and flavour.

Some FAF have been used in foods since ancient times. For example; vinegar and salt in the production of pickles, salting in bacon or fish, or sulphur dioxide in wines (Shibamoto and Bjeldanes 2009). Food additives used in the production of foodstuffs are toxic as dose-dependent (Ergun 2015). However, they do not have harmful effects on human health if they are used in accordance with the specified acceptable daily intake (ADI) values in the results of scientific research (Inetianbor et al. 2015).

Today, more than 100,000 chemical agents are used for various purposes and this number is increasing every year. Human health and the environment have been damaged by the mistakes made in the years when the chemicals were used intensively and relatively uncontrolled. As a result, fear and reaction to the use of chemicals in societies have emerged. However, toxicological investigations and the development of risk management practices for chemicals have enabled the use of safe chemicals. Today, drug, cosmetics, pesticides, chemicals used in industry, as well as the effects of FAF on human health are examined in detail, and the use of

unacceptably risky ones is not permitted. The developed international rules aim to prevent human health from being harmed by chemical use. In this process, countries do not make evaluation on their own related to chemicals. International organizations determine the rules of safe use. Food additives and its fragmentation are the most effectively controlled group of chemicals. The products must be absolutely nontoxic in the amounts used (Ergun 2015; Inetianbor et al. 2015).

The use of additives in foods is regulated by organizations responsible for food, medicine and health protection in most countries. Although regulations vary from country to country, they are all compatible with toxicological and technological applications (Ergun 2015). Each food additive has an internationally recognized number. In the European Community, the substances which are allowed to be used are given the E-code (number), which is the first letter of the word European. The E-code means that an additive is suitable for use in foods (Inetianbor et al. 2015; Shukla et al. 2017).

The quantity and type of food additives used must be approved by health institutions. On this area, the United States FDA is a very important organisation. On the other hand, they should not conceal processing and production defects, deceive the consumer and reduce the nutritional value of food. The use of food additives has been prohibited or restricted in certain foodstuffs because of the potential adverse effects on human health, sometimes because of the lack of technological necessity or misleading the consumer. The effects of food additives on health are only appear due to the fact that they are added to foods in very high doses, or as a result of one-way feeding for a long time (Ergun 2015).

Foodstuffs present in the market include distinct kinds and amounts of food additives, which may cause some health problems in humans (Inetianbor et al. 2015). The health problems of food additives on human beings can occur immediately, or if there is persistent exposure or accumulation in the body, they may have long-term detrimental effects. Acute adverse effects may occur in the form of headaches, energy reduction, allergic reactions and mental, behavioural or immune-related changes (Pandey and Upadhyay 2012). Synthetic preservatives and artificial colorants can exacerbate the symptoms of attention deficit and hyperactivity disorder. Moreover, food additives can also cause the reduction of nutritional value of foods. In this case, it may lead to subclinical malnutrition in people who are not adequate and balanced nutrition (Inetianbor et al. 2015).

Food Colours

Synthetic and natural food colours (from vegetables, minerals, or animals) include colour adjuncts, colour stabilizers, colour protective, colour retention agents, etc. Most food colours do not add any nutritional value to the food, but they provide to the food the appearance that consumers want (Pandey and Upadhyay 2012; Shukla et al. 2017). Red, blue and yellow colour pigments used in the production of foodstuffs and derived from natural sources belong to polyphenols and carotenoid family (Shukla et al. 2017). They are usually used in ripe olives, potatoes, candies, pastries,

sauces and syrups (Shibamoto and Bjeldanes 2009). Artificial dyes are one of the most frequently used contaminants in foods. The exposure to food colourants may results in a reduction in the learning ability of experimental animals (Aljaff et al. 2013).

Tartrazine is a monoazo dye that can be used as an additive in food products (Deshpande 2002). However, high amounts of tartrazine intake may cause various health problems in humans. Especially, it may have genotoxic effects against human lymphocytes and can also be straightly linked to DNA. Moreover, it may cause adverse effects on neurobehavioral parameters and may also lead to learning and memory deficiency (Inetianbor et al. 2015). Tartrazine can also create allergic problems, tissue swelling, hyperactivity, urticaria (hives), asthma, purpura, and itching in sensible persons (Deshpande 2002; Omaye 2004; Shibamoto and Bjeldanes 2009). Because of these toxic effects, it is of great importance to audit the concentration of tartrazine added in foodstuffs.

Curcumin is another colourant, which may cause iron deficiency in sensitive patients or mild nausea and diarrhoea when taken in high amounts (Inetianbor et al. 2015).

Annatto extract (bixin-based and norbixin-based, E160b), a carotenoid, is a native colouring substance acquired from the orange coloured external layer of the seeds of the tropical tree *Bixa orellana* L. (Deshpande 2002). It is allowed to use in certain products such as margarine, cheeses, appetizing snack products, coated nuts, extruded products and flavoured cereals, except for spices (Scotter 2009). However, it has been reported that annatto is the cause of rare food-related allergies in sensitive individuals. This dye can also cause anaphylactic shock and intestinal syndrome (Inetianbor et al. 2015).

Preservatives

Food preservatives are the class of food additives that may prevent, delay or inhibit the growth of food-spoilage microorganisms and pathogens (Al-Shammari et al. 2014). Preservatives can be divided into two groups: Class I (naturals such as salt and honey) and Class II (synthetics such as calcium propionate, sodium nitrite, sulphites and disodium EDTA) (Inetianbor et al. 2015). The effects of antimicrobial agents depend on type of food, storage conditions, target microorganism and its count, type and concentration of preservative, storage temperature and time, pH and buffering capacity of food. Among these substances sodium nitrite, sorbates, benzoates, propionates and sulphites are the most commonly used preservatives in food industry in the world (Shibamoto and Bjeldanes 2009; Inetianbor et al. 2015).

Nitrites and nitrates are considered to be a controversial additive (Al-Shammari et al. 2014). They are used especially in the production of meat and fish products since it prevents the development of *Clostridium botulinum* which is responsible for the production of *botulinum* neurotoxin. However, the use of these compounds may increase carcinogenic nitrosamine formation, especially in acidic mediums. It can

be induced tumours in a variety of organs such as liver, respiratory tract, kidney, oesophagus, stomach, and pancreas. Nitrosamines can cause not only carcinogens but also mutagenic effects (Omaye 2004). In individuals exposed to excessive nitrate exposure, nitrate changes its structure by binding to haemoglobin and causes a change in the so-called methaemoglobin which results in the skin turning blue (Omaye 2004; Inetianbor et al. 2015). Moreover, high levels of nitrate or nitrite exposure may increase the incidence of tumours of the brain tumours, leukaemia, nose and throat in children. It has been reported that high nitrate exposure induces the increased incidence of sudden infant death syndrome, risen risk of heart and nervous system imperfections (Inetianbor et al. 2015).

Benzoic acid has long been used as the antimicrobial agent in the food industry, especially in the production of carbonated and other drinks, fruit salads, jam and jellies, canned foods, mincemeat, margarine, assorted desserts, relishes, pies, and soy sauce. In these foods, its sodium salt is generally used. The sodium salt of benzoic acid is lesser toxic than the acid form. Adverse effects in experimental animals may occur in the form of the allergies (skin rashes and asthma), weight loss, diarrhoea, internal bleeding, destruction of the inner membranes, liver and kidney-related problems, hypersensitivity, brain damage and paralysis resulting in death (Shibamoto and Bjeldanes 2009; Pandey and Upadhyay 2012).

Sorbic acid, propionic acid and their salts are used primarily to inhibit mould and yeast (Deshpande 2002; Shibamoto and Bjeldanes 2009). Sorbates and propionates, the safest antimicrobials used to increase the shelf life of foods, are generally non-toxic at the permitted concentrations, and have no adverse effects even at high concentrations. In contrast, sorbate-containing pharmaceuticals or cosmetics may cause allergic reactions by damaging mucous layers and skin at high concentrations. Moreover, 6 g of sodium propionate taken by daily diet shows local antihistaminic effect in adults (Deshpande 2002). The JECFA has established an ADI of 0–25 mg kg^{-1} b.w. for sorbic acid and 0–5 mg kg^{-1} b.w. for benzoic acid (JECFA 1996).

Sulphur dioxide (gas form) and its salts (sodium sulphite, potassium sulphite, and potassium metabisulphite) are among the oldest antimicrobials used in foods and beverages. The usage of sulphide in foods such as meats and fish was constricted or prohibited in the USA because it destructs the vitamin B_1. In contrast, they are commonly used in fruit and vegetable drying and wine production. It may cause some adverse effects such as inflammation of polyuria, visceral organ atrophy, irritates bronchial tubes, bone marrow atrophy, limited growth, and spectacle eyes in experimental animals at high doses (Deshpande 2002; Inetianbor et al. 2015).

Antioxidants

Antioxidants are substances added to oil and fat containing foods to protect peroxidation or oxidative rancidity and thus maintain their integrity, palatability, and shelf life (Pandey and Upadhyay 2012; Inetianbor et al. 2015; Shukla et al. 2017). They

are divided into two groups; natural or synthetic. As the first group is a relatively weak antioxidant characters, synthetic antioxidants are often used in the production of foods (Shibamoto and Bjeldanes 2009). Antioxidants are also divided into three groups according to their effect types: Anti-browning agent, Antioxidant and Antioxidant synergist (Pandey and Upadhyay 2012). Most important antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate, tertiary-butylhydroquinone, thiodipropionic acid, and dilauryl thiodipropionate (Inetianbor et al. 2015; Shukla et al. 2017).

BHT and BHA, which are synthetic phenolic compounds, are widely used in the production of several foods such as margarine, oils, crisps and cheese due to their antioxidant characteristics (Deshpande 2002; Pandey and Upadhyay 2012). When taken in high doses, they cause adverse effects such as chronic allergic reactions, malformations and damage to the metabolic system in experimental animals (Shibamoto and Bjeldanes 2009). Moreover, BHA and BHT are suspected to have a detrimental effect on the lung, liver and kidney and most significantly increase the risk of cancer. These antioxidants can have a carcinogenic (stomach) and cytotoxic effect when taken in high doses (Inetianbor et al. 2015).

Propyl gallate is the most effective antioxidant among various gallates and is commonly used in a wide variety of foods such as vegetable oils and butter (Omaye 2004). When exposure to 1% or higher doses of dietary propyl gallate of experimental animals, it causes a decrease in weight gain, delayed growth, anaemia, and kidney damage. It can induce kidney damage in rats and cause 40% death in the first month following consumption (Deshpande 2002; Shibamoto and Bjeldanes 2009).

Emulsifiers and Stabilizers

Emulsifiers are substances used to obtain fixed liquid mixtures, to stabilize gas-in-liquid and gas-in-solid mixtures (Inetianbor et al. 2015). They also support stability, extend shelf life, and audit rancidity reactions, viscosity, and texture. About three-fourths of the emulsifiers used in the world are mono- and diacyl glycerides and their derivatives (Martins et al. 2019). Stabilizers improve and stabilize the tissue of foods, emulsions, suspensions, and foams. Moreover, they prevent the formation of crystals, and decrease the stickiness of icing in baked products. The most commonly used stabilizers are gum arabic, agar-agar, alginates, casein, carrageenan, carboxymethyl cellulose and its sodium salts, starch and derivatives, xanthan, guar and pectin (Inetianbor et al. 2015; Martins et al. 2019).

Acidity Regulators

pH regulators control the pH of the food. They affect the odour, flavour, viscosity, texture and, primarily shelf life by straight influencing on the oxidation/enzymatic reactions and prevention growth of microorganisms. pH agents are acids, alkaline

chemicals, conjugated salts and buffers (Inetianbor et al. 2015). They can be used alone or in combination with buffers such as salts of the phosphates, lactates and citrates. The most common acidity regulators are acetic, citric, malic, benzoic and formic acid (Martins et al. 2019).

Succinic acid has been reported to be used as a pH regulator and also flavouring and neutralizing agent in foods. However, it can be moderately toxic to the skin, in particular, can also cause eye irritation (Deshpande 2002).

Sweeteners

Sweeteners are used to condense the sweetness and keep the energy value of the food (calories) low (Inetianbor et al. 2015). These sweeteners, which are alternative to sugar, are artificial and have no nutritive values. Sweeteners such as fructose, isomalt, sugar alcohols, and maltodextrin have fewer calories than sugars, but are never calorie free. Sweeteners are chemically evaluated in five different categories; peptides, sulphonamides (acesulfame, cyclamates and saccharin), low-calorie sweeteners, chlorosaccharides and polyols (Martins et al. 2019).

Artificial sweeteners such as saccharin, aspartame, sucralose and acesulfame K may cause health problems when taken in high quantities (Inetianbor et al. 2015). Saccharin is a non-nutritive artificial sweetener that is 300–500 times sweeter than saccharides (Omaye 2004; Shibamoto and Bjeldanes 2009). Saccharin with very low toxicity may cause allergic reactions such as a headache, breathing difficulty, skin rashes, diarrhoea, and bladder tumours (Deshpande 2002). It is also thought to cause this sweetener irritability and muscle dysfunction.

Aspartame, one of the controversial sweeteners found in products such as cheese, chocolate, citrus, fatty foods, ice creams, wines and beers, is one of the substances that induce migraines. Symptoms related to complaints from foods containing aspartame include headache, giddiness, mood swings, arthrosis soreness, inexplicable depression, spew or nausea, abdominal spasm and cramping, diarrhoea, memory problems, and weariness (Inetianbor et al. 2015).

Flavour Enhancers

Flavour enhancers are substances which impart characteristic flavours when added to foods and beverages, and enhance the flavours of the other substance by acting synergistically (Deshpande 2002; Shibamoto and Bjeldanes 2009). Natural flavourings are not abundant and they cause high costs. For this reason, flavourings obtained by chemical means, which are known as nature identical, are used in the production of foods (Shukla et al. 2017). Some of the flavourings can cause dizziness and palpitations (Ergun 2015).

Monosodium glutamate (MSG), sodium salt of glutamic acid, is one of the most intensively used and controversial flavour enhancers (Deshpande 2002). MSG is generally produced by a natural fermentation process in industry (Al-Shammari et al. 2014). It is accountable for the fifth basic taste known as Umami (Deshpande 2002). Although MSG is generally known as a safe additive, some studies with young mice have been found to cause brain damage (Omaye 2004). In addition, some individuals may usually have symptoms similar to heart attacks and a feeling of pressure in the upper body when consuming foods containing high amounts of MSG. The use of MSG supplements has been banned in baby foods (Inetianbor et al. 2015).

Myristicin has been reported to induce several health problems such as headache, nausea, abdominal pain, hypotension and dizziness. Allyl isothiocyanate is an important flavouring that can be used in foods and gives the food a mustard and horseradish flavour. It may cause epithelial hyperplasia and stomach ulcers in the liver (in dogs) and may also induce mitotic activity and toxicity in experimental animals in high concentrations. Cinnamyl-2-aminobenzoate is one of the most used synthetic flavourings in drinks, confectioneries, puddings, gums, and bakery products. It gives food an aroma of grapes or cherries. The National Cancer Institute has reported that it causes cancer in experimental animals when taken at 15,000 μg kg^{-1} or greater with a diet (Deshpande 2002).

Acrylamide

Acrylamide (AA) is an industrial chemical which is odourless, colourless and crystalline at room temperature (Jin et al. 2013). This organic compound is highly soluble in water and polar solvents, and has a low molecular weight (71.08) vinyl compound (Besaratinia and Pfeifer 2007; Zamani et al. 2017). However, it is usually not-soluble in carbon tetrachloride which is a non-polar (EFSA 2015). The molecular formula of AA is CH_2=CH-CO-NH_2 (C_3H_5NO) (Chen et al. 2012; Shibamoto and Bjeldanes 2009). Synonyms are 2-propenamide, acrylic amide and ethylene carboxamide (EFSA 2015). It has a boiling point of 125 °C and a thaw point of 84.5 °C with a density of 1.27 g ml^{-1} at 25 °C (Gaikwad et al. 2016; Zamani et al. 2017).

There are two forms of AA, monomeric and polymeric. Monomer form is form found in foods (Arusoglu 2015). It is highly reactive in the air and can quickly polymerize. Namely, monomers can bind together and then form new polymers of AA (polyacrylamide) with new properties (Besaratinia and Pfeifer 2007). Nowadays, that polyacrylamide compound is widely used compound for different goals (flocculants, coagulant, sealants, soil stabilizers, binders and additives/adhesives/fixatives) in different industries. It is generally used for drinking water, sewage and wastewater treatment, construction of dams and roadways, soap making, recovery of enriched oil, and in paper and pulp industry. In addition, it has recently been used in chromatography and electrophoresis applications such as protein separation and

purification (Arusoglu 2015; Shibamoto and Bjeldanes 2009). Furthermore, cigarette (especially in smoke) contains AA (1–2 μg/cigarette) (Claeys et al. 2016). It can also be found in high amounts (up to several milligram per kilogram) in commonly consumed everyday foods and is a proven carcinogen (Besaratinia and Pfeifer 2007; Bongers et al. 2012).

AA is absent in the raw materials but, is formed during processing of some foods at high temperature (Gaikwad et al. 2016). The AA formation were first determined in the raw materials of plant origin which was subjected to high-temperature heat treatment in 2002 (Halford et al. 2012; Claeys et al. 2016). An increase in public health concern for AA has been observed following the provocative disclosure of the presence of AA in certain foods (such as in cooked, fried, toasting, roasted and baked high carbohydrate foods) prepared at temperatures above 120 °C and widely consumed throughout the world (Chen et al. 2012; EFSA 2015; Besaratinia and Pfeifer 2007). The formation, quantities, and toxicity of AA in foods have attracted the attention of many government agencies and national authorities because of this public concern and the potential risk to public health (Chen et al. 2012; Claeys et al. 2016).

AA is formed during Maillard reactions. During these reactions, especially the asparagine reacts with reducing sugars (glucose and fructose) during heating processes at temperatures higher than 120 °C (Bongers et al. 2012; Jin et al. 2013; Claeys et al. 2016; Zamani et al. 2017). On the other hand, the combination of the temperature and heating period to which the foodstuffs are exposed upon the formation of AA are very important (EFSA 2015). When the temperature is increased to 180 °C, AA formation reaches the highest level (Arusoglu 2015). The temperature at which AA begins to form in a food depends on the moisture content of that food. Less than 20 μg kg^{-1} AA is formed, when cooking process is applied to the wet potatoes at 120 °C under pressure. However, about 10,000 μg kg^{-1} AA occurs when dry potato powder is heating (EFSA 2015). AA is usually not found in large amounts in boiled foods, but can be found in significant amounts, especially in foods produced by deep frying or frying/roasting (Kopanska et al. 2017). Not only the temperature, cooking time and moisture content but also the method of cooking, immersion in different solutions (NaCl, $CaCl_2$, citric acid) before the heat treatment, pH, frying oil, water activity, type of food (presence and concentration of precursor molecules such as asparagine and reducing sugars), affect also the formation and amount of AA (EFSA 2015; Gaikwad et al. 2016). This is the cause of differences in the AA content of the same foods produced by different companies, and even among the batches of the same brand of food (Bongers et al. 2012).

AA may also be composed of 3-amino-propionamide (beta-alanine amide, $C_3H_8N_2O$) which is present in different amounts in various potato varieties (Zyzak et al. 2003; Jin et al. 2013). This compound is a potent precursor compound in the formation of AA and forms as a transient intermediate during the thermal degradation of asparagine (Jin et al. 2013). There are also AA formation routes that do not require asparagine. AA can be composed of acrolein (it turns into acrylic acid with the oxidation reaction) and acrylic acid (reacts with ammonium) in foods containing lipid under high temperatures (Shibamoto and Bjeldanes 2009; Kopanska et al. 2017), or it can also occur from gluten (Halford et al. 2012).

According to each country's tradition and unique food preparation methods, the amount of AA in food may be different (Zamani et al. 2017). AA has been detected in several foods such as potato products, chips, French fries and roasted corn flakes, coffee (brewed or not brewed, coffee substitutes etc.) and bakery products (pastry, biscuits, bread, rolls etc.) (Bongers et al. 2012; EFSA 2015). In addition, humans are exposed to AA indirectly by food packaging containing polyacrylamide (Zamani et al. 2017). Amount of AA in some raw and processed food products is given in Table 3.

According to WHO, daily intake of AA is 0.3–2.0 μg kg^{-1} b.w. It is estimated that daily intake of AA in children (infants, toddlers and other children) is 2–3 times higher than in adults (Besaratinia and Pfeifer 2007; EFSA 2015). This is due to the fact that children have a higher calorie intake than adults, and a higher feeding rate with AA-rich foodstuffs such as French fried potatoes and soft breads (Halford et al. 2012; Gaikwad et al. 2016; Zamani et al. 2017). The presence of soft bread with low AA content in the products on this list may be surprising, however, too much bread is consumed in Europe and especially in Turkey, and this is normally increasing the concentration of the exposure to AA (Halford et al. 2012; Arusoglu 2015). Other foods that cause total AA exposure of children and adolescents are breakfast cereals, pastry, biscuits, crackers, crisp bread, rolls and other products based on cereals. All of these food groups together with coffee are the basic contributors for adults, elderly and very elderly (EFSA 2015). The mean intakes of AA for men and woman are 0.36 μg kg^{-1} per day and 0.33 μg kg^{-1} per day, respectively. The highest intake of AA in males occurs between the ages of 16 and 30. Whereas, 13-old boys and girls intake less amount of AA then adult (0.52 μg kg^{-1} per day and 0.49 μg kg^{-1} per day, respectively) (Zamani et al. 2017).

Parameters Influencing the Formation of AA

The main agent of AA-forming is free asparagine amount. Asparagine is an amino acid that can be found in very different concentration range, depending on the type of plant and year of harvest (Shibamoto and Bjeldanes 2009; Halford et al. 2012). Especially asparagine combined with glucose and fructose is very significant agent in the AA formation (EFSA 2015). When the level of reducing sugar is high, the formation of the AA increases accordingly, whereas when the sugar levels are low, the formation of the AA is proportional to the amounts of the precursor amino acids (Halford et al. 2012). A significant decrease in AA concentrations can be achieved by controlling glucose and fructose amounts in potato varieties (Biedermann-Brem et al. 2003; EFSA 2015). In addition to choosing the right variety, crop varieties such as potato cultivar with low-reducing sugars (manipulation of the metabolic regulator) and/or free asparagine can be obtained through hybridization, genetic modification and other genetic techniques such as the identification of quantitative traits (Noti et al. 2003; Halford et al. 2012; EFSA 2015). At the same time, the ratio of fructose to glucose affects AA concentration of fried potato strips. Increased

Table 3 Amount of AA in some raw and processed food products (Besaratinia and Pfeifer 2007; Arusoglu 2015; EFSA 2015)

Category of food	Foodstuff	Mean concentration (μg kg^{-1})	Maximum concentration (μg kg^{-1})
Cereals/cereal-based products	Cereal-based products	343	7834
	Raw/boiled cereals and pasta	15	47
	Processed cereals/pasta (toasted, fried, grilled) Soft bread Gingerbread	123 42 1000	820 – –
	Bread and rolls	446	3436
	Pastry and biscuits	350	7834
	Cereals for breakfast (crispy)	96	1346
	Pizza	33	763
Fish and other seafood	Breaded, fried, baked foods	25	233
Meat and offal products	Coated, cooked, fried foods Flaked meat *Adana kebap*	19 57 127	313 63 250
Milk and dairy products		6	36
Nuts and oil seeds		84	1925
Legumes		51	320
Roots and tubers of plant	Roots and tubers of plant	477	5312
	Potato purees (mashed or boiled)	16	69
	Baked potato	169	1270
	Potato crisps (chips/french fries)	110	5312
Stimulants/other analogues		509	7300
	Coffee (Roasted/brewed/non-brewed) Coffee powder Turkish coffee	13 200 25	1291 230 266
	Coffee extracts	1100	4948
	Coffee substitutes	845	7300
	Products of cocoa	220	909
	Roasted green tea	306	660
Sugars and honey		24	112

(continued)

Table 3 (continued)

Category of food	Foodstuff	Mean concentration (μg kg^{-1})	Maximum concentration (μg kg^{-1})
Vegetables	Raw, boiled and canned vegetables	4	25
	Toasted, baked, fried or grilled vegetables	59	202
Fruits	Dried or fried fruits	131	770
Other processed products	Alcoholic beverages (beer, gin, wine)	7	46
	Baby food (canned, jarred, dry powder)	16	121
	Baby food (cereal-based, biscuits etc.)	73	1217
	Dried food	121	1184
	Chocolate Powder	75	100

fructose ratios support the formation of AA (Mestdagh et al. 2008). On the other hand, proper storage circumstances are also important in the formation of AA (Noti et al. 2003). The storage of potatoes has seasonal effects on the amount of AA (Powers et al. 2013; EFSA 2015).

Temperature is one of the important factors in the formation and degradation of AA. The effect of food processing on the formation of AA and their reduction strategies are given in the following:

- The main factors affecting the AA levels in coffee as well as in potato and cereal products are time and roasting degrees. In the production of French fries, the increase of temperature may increase the amount of AA more pronounced than the rising of duration at the constant temperature frying process. Oil frying temperature above 170–175 °C may produce high amounts of AA in the final product. Even the highest temperature and the longest processing time in the convection oven contains less AA than the fried potato patties prepared in the stove (215 °C for 6.5 min). Because of AA generally occurs towards the end of the frying process, the temperature is very important towards the end of process. Dropping to the temperature 140–145 °C towards the end of the process reduces AA formation to significant levels (EFSA 2015).
- The type of vegetable oil used in the frying process also affects the level of AA. For example, sweet potatoes fried in palm olein contain less concentration of AA than fried in soya bean oil. Moreover, the most effective way to reduce the amount of AA in frying is by immersion in the citric acid solution (1 g L^{-1}). This process reduces AA by about 77% (EFSA 2015).
- Peeling of potato causes a decrease in AA level in the production of chips. Because reducing sugars can be found at higher level near the peel. In addition, sliced potatoes are usually washed with water at ambient temperature before frying. During the washing process, significant levels of the primary AA precursors

in the potato is away with water, thereby reducing the production of AA in fried slices (Gaikwad et al. 2016).

- The formation of AA is higher in the crust of the food than in the interior; therefore, reducing the surface area of the food can reduce AA in baked products (Gaikwad et al. 2016).
- The addition of a food-grade colouring agent in the production of frozen French fries may result in a reduction in AA level. Because, the dark coloured product is requested by the consumers, however, it increases the formation of AA. With the addition of food-grade colouring agent, both the desired colour is obtained and the formation of AA can be reduced (Gaikwad et al. 2016).
- Some amino acids (e.g., glycine, taurine, lysine, and cysteine) and organic acids (acetic acid, ascorbic acid, citric acid, monosodium citrate, sodium citrate, lactic acid) can suppress to the formation of AA in potato products (Jin et al. 2013; Gaikwad et al. 2016). Especially, glycine can reduce the formation of AA by up to 50%, but may create unwanted odour (Arusoglu 2015). It has been stated that application of a small amount of citric acid before cooking and/or frying can greatly reduce the formation of acrylamide in fried and baked potato fries (Jung et al. 2003).
- The reduction of asparagine levels using asparaginase enzyme prior to the heat treatment of food was successfully used in some food products such as cereal-based products. However, it is not a process that can be applied to all foods because it is ineffective to some products or causes unacceptable changes in the product. This enzyme hydrolyses asparagine to aspartic acid (EFSA 2015). It has been reported that asparaginase can reduce acrylamide formation by 80% in fried potatoes (Mahajan et al. 2012).
- The adding of ammonium carbonate and bicarbonate to foods may result in high amounts of AA formation (EFSA 2015).
- The prolongation of the fermentation time in the production of fermented products leads to some decrease in the amount of AA. This is because; yeast is the use of free asparagine in metabolism. On the other hand, AA is often found in the bread crust, while the AA is not found in the bread (Arusoglu 2015). AA formation in French fries may be adequately reduced by lactic acid fermentation of the potatoes before deep-frying (Baardseth et al. 2006).
- The flour type and toasting time may also affect the formation of AA. After toasting, higher amounts of AA forms in bread made from potato flour than wheat, rye or multi-grain flour (EFSA 2015).

Toxicological Evaluation

Its low molecular weight and high water solubility allow the AA to readily pass through different biological membranes. The chemical structure and the ability to undergo metabolic transformation of AA allows it to react easily with various cellular targets (Besaratinia and Pfeifer 2007). Toxicological studies on AA are per-

formed in rats, mice, monkeys, cats, and dogs using different doses and exposure routes (oral, intravenous etc.). Oral LD_{50} values of AA for rats, mice and rabbits are >150 mg kg^{-1} b.w., 107 mg kg^{-1} b.w. and 150–180 mg kg^{-1} b.w., respectively (EFSA 2015).

AA has genotoxic, neurotoxic, reproductive toxic and immunotoxic effects (Gaikwad et al. 2016). AA has been classified as Group 2A (probable carcinogen) by IARC, and as a Category 2 carcinogen and mutagen by the European Union (IARC 1994; Halford et al. 2012). AA has a structure similar to vinyl carbamate and acrylonitrile, known to be carcinogenic. However, multiple tumours may develop in animals when taken in large amounts through potable water. AA may also increase the risk in some types of cancer such as kidney and breast cancer especially after menopause (Zamani et al. 2017).

AA is a strong and effective lethal neurotoxic matter. The toxic effect of AA on both human occupational exposure and animals is neurotoxicity. For example, during the construction of the railway tunnel, Swedish workers had signs of deterioration in nerve function. It has been observed that it was due to a private gel called "Rhoca Gel", which contains AA used against water leaks in the tunnel wall (Kopanska et al. 2017; Zamani et al. 2017).

AA may damage DNA at a dose of 10, 20 and 30 mg kg^{-1}. There is insufficient evidence for the effect of AA on reproductive toxicity in humans. However, it has been demonstrated that the adverse effects of AA on male reproductive parameters including reduced sperm counts in rats with a No Observed Adverse Effect Level (NOAEL) of 2 mg kg^{-1} per day (EFSA 2015).

Recently, AA has been stated to induce oxidative stress (Kopanska et al. 2017; Zamani et al. 2017). AA may cause formation of reactive oxygen species by affecting cellular redox chain. AA and glycidamide may interact with the group of nucleophiles in cells. Oxidative stress is an important step in the induction of various types of cell death types such as apoptosis which stated as blobbing of membranes, shrinkage of cells, and DNA fragmentation It has also an immunotoxic effect, and may reduce the final body weight, spleen and thymus weights, and lymphocyte count in experimental animals (Zamani et al. 2017).

The intake of AA may rise the rate of lung and skin adenoma and carcinoma in mice, and may also stimulate scrotal mesotheliomas, thyroid gland adenomas and/or mammary gland tumours. It can stimulate adenocarcinomas, central nervous system tumours, clitoral gland adenomas and oral papillomas, tumour risk of the uterus, colon and clitoral gland in rats (Besaratinia and Pfeifer 2007; Chen et al. 2012).

The half-life of AA is between 2.4 and 7.0 h according to the toxicokinetic researches in humans. More than 60% of AA can be excreted from the body with the urine which is the major route of excretion (Besaratinia and Pfeifer 2007; Zamani et al. 2017). Only 4% of AA with stool is discarded after 7 days (EFSA 2015). AA is often metabolized by conjugation with glutathione. It can be also metabolised by epoxidation by cytochrome P450 2E1 (CYP2E1) into an epoxy derivative (glycidamide), which is widely distributed into tissues (Bongers et al. 2012; Kopanska et al. 2017). This metabolite is more reactive than AA, the parent compound against DNA and proteins (Besaratinia and Pfeifer 2007). After recruitment into the human

body, the AA and its metabolite, primarily conjugated with glutathione, and then converted to by-products of mercapturic acid, which are excreted in urine (Zamani et al. 2017). Mercapturic acid is an important compound and is the major metabolite of AA and glycidamide. Urinary excretion levels of these metabolites are used as biomarkers of AA exposure. The formation of glycidamide is important because it represents the pathway underlying the genotoxicity and carcinogenicity of AA (EFSA 2015).

Furan

Furan (C_4H_4O) is an uncoloured chemical that is associated with the flavour character of foodstuffs (particularly undesirable flavour) (Nerín et al. 2016; Santonicola and Mercogliano 2016). It is a mini cyclic ether with high volatility which molecular weight is 68.07 g mol^{-1}, and it starts to boil at 31.04 °C. Furan and its derivatives such as methylfuran were first detected in thermal treated foodstuffs (food and beverages) about 50–60 years ago. Only furan may occur in foods or may occur together with derivatives (Knutsen et al. 2017). In 2004, FDA released a notification on the presence of furan and its derivatives (2-methylfuran, 2-alkylfuran, 2-ethylfuran, 2-pentylfuran, 2,5-dimethylfuran, 2-butylfuran, 2,3-benzofuran) in many of the heat-treated foodstuffs such as canned and jarred foods (Fromberg et al. 2014; Santonicola and Mercogliano 2016). Furan and its derivatives has also been detected in tobacco (Xu et al. 2017), industrial wastes and exhaust gas from diesel and gasoline engines (Bas et al. 2016; Knutsen et al. 2017).

Furan compounds resulting from heat treatment and dioxin-like furan compounds (polychlorinated dibenzo-furans, PCDF) are different from each other. In both, however, the main compound is furan and used as a solvent for rosin and polishes. In addition, it is used in the preparation of organic compounds and pharmaceuticals (Vranová and Ciesarová 2009). Dioxin and dioxin-like furan are highly chemically stable organochlorine compounds and are considered among the major food contaminants arising from environmental pollution (Ergun 2015).

People are generally exposed to furan or its derivatives formed by thermal applications via hot-air dried, baked, fried, grilled and roasted foodstuffs. Various factors such as temperature and time used in production, pH, water activity, storage temperature and duration, the amino acid to sugar ratio, oxygen, presence of metals, presence/absence of inhibitors and activators can affect furan formation and amounts in foodstuffs (Nie et al. 2013; Santonicola and Mercogliano 2016). Among these, processing temperature and time, pH and the amino acid to reducing sugar ratio play important role in the formation of furan (Knutsen et al. 2017).

Furan and its derivatives are among the Maillard reaction products that occur during processes such as heating and browning (Bogdanova et al. 2018). Their formation by Maillard reactions depends on activators such as amino acids and sugars (Santonicola and Mercogliano 2016). Pasteurization applications applied to foods cause lower furan formation than sterilization applications (Knutsen et al. 2017).

Similarly, higher levels of furan may occur in frying than in baking. In productions performed industrially, furan amounts in foods rise with rising temperature, especially, up to 200 °C (Nie et al. 2013). However, when the process temperature overruns 200 °C, the amount of the furan may vary without depending on the temperatures (Santonicola and Mercogliano 2016). Fromberg et al. (2014) stated that there were no differences with regard to furan amounts between French fries fried at 160 °C and 175 °C. However, they reported that an important rise in the amount of furan when the frying temperature rose to 190 °C and more browning on the surfaces of potato chips. The researchers also determined that the colour increased from the brown to darker as it continued to toasting in the making of toasted bread, and with the browning increased, it rose of the furan amount in the product.

pH can affect the amount of furan at temperatures above 110 °C. For example, in a study performed by Nie et al. (2013) at pH 7.00 (30 min and at 150 °C), it was stated that a much higher amount of furan was formed than pH 9.4 and 4.2. However, at the same pH, as the temperature increases the amount of furan increases significantly. For example, when the temperature rises from 120 to 150 °C at pH 7.0, the amount of furan increases from 34 to 304 ng mL^{-1}.

The main mechanisms for formation of furan and its derivatives can be originated from (i) thermal degradations of reducing sugars and Maillard browning reactions in the presence of reducing sugars and amino acids; (ii) disruption of some amino acids due to heat treatment; (iii) thermal lipid oxidation of unsaturated fatty acids or triglycerides; (iv) the thermal oxidation of some compounds such as carotenoids; (v) the decomposition of ascorbic acid foods; and (vi) heating of unsaturated aldehydes (Vranová and Ciesarová 2009; Fromberg et al. 2014).

As mentioned above, the main source of the furans in foodstuffs is the deterioration of carbohydrates (glucose, fructose or lactose) found in high amounts in the food due to heat treatment (Fromberg et al. 2014; Santonicola and Mercogliano 2016). According to FDA, carbohydrates and amino acid mixtures or alanine, serine, cysteine, casein, ascorbic acid, unsaturated/polyunsaturated fatty acids are multiple precursor compounds affect the presence and amount of furan in foods (Vranová and Ciesarová 2009; Nerín et al. 2016). Furan may also occur as a result of interaction with some non-precursor substances such as starch (Knutsen et al. 2017).

Furan may occur if amino acids such as serine, cysteine are subject to heat treatment without the need for any other source. However, furan is not form if alanine, threonine and aspartic acid are alone. In addition to the heat treatment, reducing sugar, serine or cysteine is required for the formation of furan from these amino acids. In the roasting process applied to the foods that do not contain amino acids, the furan mainly consists of intact sugars (Vranová and Ciesarová 2009; Knutsen et al. 2017). The presence of alanine, threonine or serine can support the formation of furan, which can result from both sugars and amino acids. The parent furan may be composed of pentose sugars such as ribose (Santonicola and Mercogliano 2016).

Ascorbic acid is also effective in furan formation. The ascorbic acid present in the food is first oxidized to dehydroascorbic acid, followed by hydrolysis to produce 2,3-diketogulonic acid. This compound is first converted to aldotetrose and then to

furan. Nevertheless, ascorbic acid does not undergo oxidation under non-oxidative conditions and 2,3-diketogulonic acid is not produced. Instead, it undergoes hydrolysis followed by decarboxylation, and then furan can form by following the ribose pathway (Vranová and Ciesarová 2009). Moreover, ascorbic acid alone causes a certain amount of furan formation, while less furan is formed when it is subjected to heat treatment with other substances (glycine, serine, erythrose or linoleic) in the mixture (Santonicola and Mercogliano 2016).

Mono-unsaturated acids such as oleic acid are specified not to form furans. The furan is composed of unsaturated fatty acids and, as the degree of unsaturation increases, furan formation increases (Santonicola and Mercogliano 2016). On the other hand, antioxidants such as tocopherol acetate can greatly reduce furan formation (70%) (Vranová and Ciesarová 2009). Ionized radiation of apple and orange juices causes furan formation (Fan 2005).

Not only industrial products, but also home-made cooked foods may contain furan (Vranová and Ciesarová 2009; Fromberg et al. 2014). It has been detected in coffee (47–5982 μg kg^{-1}), canned foods (1–105 μg kg^{-1}), baby food (4–224 μg kg^{-1}) and breakfast cereals (2–387 μg kg^{-1}) (Nerín et al. 2016; Santonicola and Mercogliano 2016). Furan has also been determined in soups, fruits and dried fruit products such as raisin, plum and banana, cereal based products, snack foods, cooked chicken, sodium caseinate, hazelnut, fruit and vegetable juices, tin containing meat and pasta, plum beverage, soy protein, rapeseed protein and caramel (Vranová and Ciesarová 2009; Fromberg et al. 2014). In a recent study, furan was detected in 100% of beer samples collected from different supermarkets in Riga, Latvia, with the levels ranging from 1.4 to 32.5 μg kg^{-1} (Bogdanova et al. 2018). However, furan was not occurred in some of foodstuffs produced in home such as omelette, pancakes, fruit compotes, cakes and cookies do not include furan (Fromberg et al. 2014).

However, the amount of toxic furan formed by heat treatment in foodstuffs is far below the amounts that can cause harmful effects in humans (Vranová and Ciesarová 2009). Furan content can be considerably reduced in some foods such as foods applied heating or cooking process. But, approximately 50% of the furan may remain in the product (Fromberg et al. 2014). For example, furan can be volatilizing by stirring during heating of canned or jarred foodstuffs in an open stove. Up to 85% reduction can be achieved in open container by stirring a time of 5.5 h in boiling water (Santonicola and Mercogliano 2016). On the other hand, volatilization of furans during thermal processes highly depends on the contents of foodstuff. For example, lipophilic substances such as fats cause furan to be retention considerably (Fromberg et al. 2014).

Furan has been classified as a possible carcinogen (group 2B) (IARC 1995) and can induce a dosage-dependent increment in hepatocellular adenomas and carcinomas in both rat and mice. Furan was added to the list of carcinogens in tobacco (Xu et al. 2017).

Furan causes tumour-inducing effect in experimental animals. The most striking effect is the induction of hepatic cholangiocarcinomas (Vranová and Ciesarová 2009). Furan causes ATP loss resulting in activation of cytotoxic enzymes including

endonucleases. Furan can also trigger gene mutations, chromosome deflections and sister chromatid alterations in mammalian cells, chromosomal aberrations in bone marrow cells in mice, and formation of liver tumours in infant male mice (Knutsen et al. 2017). In addition, furan can cause severe histopathological changes in rats (Bas et al. 2016), detrimental impacts in the pancreas and adrenal cortex (Karacaoglu et al. 2012), and also reduce the albumin amount by causing liver dysfunction (Bas et al. 2016). Furan also has a moderate nephrotoxic effect in rodents when administered orally. On the other hand, it induces histological alters in testes, prostate gland and seminal vesicles in rats (Knutsen et al. 2017).

No limitation on the amount of furan present in foods has been specified so far and, still not also covered by European Union Regulation (Knutsen et al. 2017). As furan causes a carcinogenic effect, the amounts in foods should be kept as low as possible. Depending on the food consumed, children between the ages of 4 and 6 receive an average of 1.5 μg furan per day, and adults 27 μg (Santonicola and Mercogliano 2016). Because of their low polarity, furan and its derivatives are rapidly and intensely absorbed from the intestine and lung both in the human body and in experimental animals to an extent of at least 80%, and then quickly metabolized with cytochrome P450 2E1 (CYP2E1) enzymes (Knutsen et al. 2017; Bogdanova et al. 2018). It then goes to various organs through which it can accumulate or act through biological membranes. While the accumulation in the liver and kidneys increases due to repeated furan doses, less accumulation may be in the stomach, blood and lungs (Santonicola and Mercogliano 2016; Knutsen et al. 2017).

3-MCPD and Glycidyl Fatty Acid Esters

Glycerol-based process contaminants are found in vegetable oils, fats and some processed foods that raise potential health concerns for people who consume high amount of these products. 3-Monochloropropane-1,2-diol (3-MCPD), 2-monochloropropane-1,3-diol (2-MCPD) and their fatty acid esters are process contaminants and may found in vegetable oils, mainly in palm oil, and numerous heated foods (EFSA 2013). They are among non-volatile chloropropanols, was discovered by Velísek et al. (1978) in the late 1970s in the composition of acid-hydrolysed vegetable protein (acid-HVP) used for savoury flavour-enhancing food ingredient such as soya (EFSA 2016).

Glycidyl fatty acid esters (GE) are food processing contaminants and they are formed during the physical refining process of vegetable oils and fats at high temperatures (over 200 °C), i.e. in the deodorization step. GE are hydrolysed in glycidol in the gastrointestinal tract (EFSA 2016).

Free 3-MCPD or free 2-MCPD can be occur when foods that contain both fat and salt are exposed to high temperatures during production. The fatty acid esters of 3- and 2-MCPD are formed during the refining of vegetable oils and fats either from acylglycerols (Freudenstein et al. 2013) or from triacylglycerol (Destaillats et al. 2012). The formation and level of 3-MCPD esters depend on the level of acylglyc-

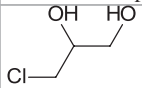
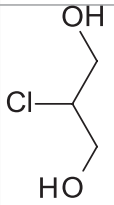
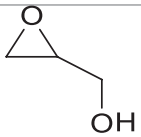
erols (tri-, di- and monoacylglycerols), the concentration of chlorine, pH, the deodorization temperature and time (EFSA 2013).

GE are formed mainly from diacetylglycerol during the deodorization step of physical refining of oils (in excess of 200 °C). The highest levels of GE, as well as 3-MCPD and 2-MCPD were detected in palm oil, which can have a high diacetylglycerol (4–12%) content (EFSA 2016).

Chemical Characteristics

3- and 2-MCPD belong to the group of chloropropanols which are chlorinated derivatives of glycerol. While the chlorine atom is in position 3 in 3-MCPD, it is in position 2 in 2-MCPD molecule. Glycidol has not only glycerol as chloropropanols but also an epoxide structure. GE are substances consisting of glycidol esterified with a fatty acid (BfR 2016). The chemical and physical properties of 3-MCPD, 2-MCPD and glycidol compounds are shown in Table 4. It has been also reported that fatty acid esters of 3- and 2-MCPD, and glycidol have similar properties, with slightly lower melting points (Hamlet et al. 2011).

Table 4 Chemical and physical properties of 3- and 2-MCPD, and glycidol

Parameter	3-MCPD	2-MCPD	Glycidol
Name	3-monochloropropane-1,2-diol	3-monochloropropane-1,3-diol	Glycidol
IUPAC systematic name	3-chloropropane-1,2-diol	2-chloropropane-1,3-diol	oxiranylmethanol
CAS Number	96-24-2	497-04-1	556-52-5
Empirical formula	$C_3H_7ClO_2$	$C_3H_7ClO_2$	$C_3H_6O_2$
Molecular weight (g mol^{-1})	110.5	110.5	74.08
Density (g ml^{-1})	1.32	1.32	1.12
Melting point	−40 °C	−40 °C	−45 °C
Boiling point	213 °C (at 760 mmHg)	213 °C (at 760 mmHg)	167 °C (at 760 mmHg)
Solubility	Soluble in water, alcohol and ether	Soluble in water, alcohol and ether	Soluble in water, alcohol and ether
Colour	Colourless or pale yellow	Colourless or pale yellow	Colourless
Chemical structure			

Since glycidol has only one hydroxyl group, it can form monoesters. However, 3- and 2-MCPD can each form monoesters and diesters with fatty acids under high temperatures during the refining of vegetable oils and fats. Lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid are known the major esterifying fatty acids (EFSA 2016).

Analytical Methods

There are several analytical methods for the determination of chlorinated propanols and their esters in refined oils, fats and numerous foods. The analytical methods used for chloropropanols have been largely described by Hamlet et al. (2011). The determination of 3- and 2-MCPD is usually carried out by gas chromatography-mass spectrometry (GC-MS) methods. To enhance volatility and mass detector response, derivatisation step is needed. The derivatisation reagents have been applied including heptafluorobutyrylimidazole (HFBI), *N*,*O*-bis(trimethylsily) trifluoroacetamide (BSTFA), heptafluorobutyric anhydride (HFBA) or more recently using of phenylboronic acid (PBA) (Platinga et al. 1991). The limit of quantifications (LOQs) for free 3-MCPD and 2-MCPD are up to levels of 15 μg kg^{-1} and 10 μg kg^{-1}, respectively. However, currently there is no available method for the determination of free glycidol substance.

Methods for analysing ester-bound 3- and 2-MCPD and glycidol are commonly based on two different approaches, direct and indirect analysis. The indirect methods for esters of 3- and 2-MCPD and glycidol in processed foods are well characterised in various foods, using t-butyl methyl ether, t-butyl methyl ether/hexane or diethyl ether/hexane as extraction solvents. Three methods adopted by the American Oil Chemists' Society (AOCS) have been developed for quantification of fatty acid esters of 3- and 2-MCPD and glycidol, and show LOQs in the range 15–30 μg kg^{-1} (EFSA 2016).

Occurrence Data

A number of studies have been conducted on the levels of 3-MCPD, 2-MCPD, glycidol and their esters in vegetable oils and fats within the last two decades. The results of these studies are summarised in Table 5.

In 2006, a comprehensive report on the risks for human health related to the presence of 3- and 2-MCPD, and their fatty acid esters, and GE in food has been published by EFSA. In this exposure assessment, 7175 occurrence data on 3- and 2-MCPD and glycidol in soy sauce, HVP and similar products (702 data points), oils and fats (4754 data points), and various foods including infant formula, cereal-based products and potato crisps (1719 data points) have been evaluated. Within the categories of oils and fats, palm oils/fats had the highest concentrations of 3-MCPD

Table 5 Content of 3-MCPD, 2-MCPD, glycidol and their esters in vegetable oils and fats

Substance	Food	No. of samples	Incidence *n* (%)	Range (min-max), µg kg^{-1}	Reference
3-MCPD	Cold pressed/ refined safflower oils	11	5 (45.5)	<100–3218	WeißHaar (2008)
Free 3-MCPD	Virgin seed oils	9	–	<9–12	Zelinková et al. (2006)
	Refined seed oils	5	–	<3–<9	Zelinková et al. (2006)
	Virgin olive oils	4	–	<3–<9	Zelinková et al. (2006)
	Refined olive oils	5	–	<9	Zelinková et al. (2006)
Bound 3-MCPD	Virgin seed oils	9	–	<100–337	Zelinková et al. (2006)
	Refined seed oils	5	–	<300–1234	Zelinková et al. (2006)
	Virgin olive oils	4	–	<100–<300	Zelinková et al. (2006)
	Refined olive oils	5	–	<300–2462	Zelinková et al. (2006)
	Edible oils	27	3 (11)	260–300	Jedrkiewicz et al. (2016)
	Margarines	5	5 (100)	1300–7300	Jedrkiewicz et al. (2016)
	Fish oils	5	5 (100)	1500–5500	Jedrkiewicz et al. (2016)
	Soybean oil	5	–	<100–500	Kuhlmann (2011)
	Rapeseed oil	5	–	<100–1000	Kuhlmann (2011)
	Sunflower oil	5	–	100–2100	Kuhlmann (2011)
	Palm oil	20	–	1100–10,000	Kuhlmann (2011)
	Vegetable oil fat mixes	11	11 (100)	897–2435	Seefelder et al. (2008)
Bound 2-MCPD	Edible oils	27	3 (11)	180–230	Jedrkiewicz et al. (2016)
	Margarines	5	5 (100)	630–1700	Jedrkiewicz et al. (2016)
	Soybean oil	5	–	<LOQ[a]–100	Kuhlmann (2011)
	Rapeseed oil	5	–	<LOQ–300	Kuhlmann (2011)

(continued)

Table 5 (continued)

Substance	Food	No. of samples	Incidence *n* (%)	Range (min-max), μg kg^{-1}	Reference
	Sunflower oil	5	–	<LOQ–300	Kuhlmann (2011)
	Palm oil	20	–	200–5900	Kuhlmann (2011)
Bound glycidol	Soybean oil	5	–	<100–600	Kuhlmann (2011)
	Rapeseed oil	5	–	<100–300	Kuhlmann (2011)
	Sunflower oil	5	–	<100–400	Kuhlmann (2011)
	Palm oil	20	–	300–18,000	Kuhlmann (2011)

[a]*LOQ* limit of quantification

(from esters), 2-MCPD (from esters) and glycidol (from esters), with the middle bound (MB) levels of 2912 μg kg^{-1}, 1565 μg kg^{-1} and 3955 μg kg^{-1}, respectively. The MB levels of 3-MCPD, 2-MCPD and glycidol from esters in vegetable fats/oils varied from 48 μg kg^{-1} to 608 μg kg^{-1}, 86–270 μg kg^{-1} and 15–650 μg kg^{-1}, respectively. With regard to margarine and similar products, the MB levels were 181–668 μg kg^{-1} for 3-MCPD from esters, 80–236 μg kg^{-1} for 2-MCPD from esters, and 114–582 μg kg^{-1} for glycidol from esters (EFSA 2016).

According to that report, potato crisps, hot surface cooked pastries, shortcrusts and cookies had the highest levels of 3- and 2-MCPD and glycidol from esters among the food groups other than fats and oils. The MB levels were varied from 154 to 247 μg kg^{-1} for total 3-MCPD, from 79 to 135 μg kg^{-1} for total 2-MCPD, and from 110 to 149 μg kg^{-1} for glycidol from esters in these products.

There are also several studies focusing on the occurrence of chlorinated propanols in baby formula in the scientific literature. Zelinková et al. (2009) analysed a total of 14 infant and baby food products for the presence of free and bound 3-MCPD. While the authors were not detected free 3-MCPD in any of the samples, the levels of bound 3-MCPD in baby foods ranged from 62 to 588 μg kg^{-1}. EFSA (2016) reported the mean levels of 3-MCPD, 2-MCPD and bound glycidol in infant formulas (powder) of 108, 44 and 87 μg kg^{-1}. In a recent study, Arisseto et al. (2017) found 3-MCPD and GE in 37.5% (15 out of 40 samples) and 42.5% (17 out of 40 samples) of infant formula available in the Brazilian market up to levels of 600 μg kg^{-1} and 750 μg kg^{-1}, respectively. In another study conducted in the United States, infant formulas containing palm/palm olein contained bound 3-MCPD and glycidol at levels from 21 μg kg^{-1} to 920 μg kg^{-1}, and from <LOQ to 400 μg kg^{-1}, respectively. However, palm/palm olein-free infant formulas contained bound 3-MCPD and glycidol in the range of 72–160 μg kg^{-1}, and 5–150 μg kg^{-1}, respectively (Leigh and MacMahon 2017).

Toxicological Evaluation

The main target organ is kidney and 3-MCPD can induce nephropathy, renal tubular hyperplasia and adenomas in the chronic exposure. This compound has been classified by IARC as a "possible human carcinogen (group 2B)" (IARC 2012). In 2001, the European Union Scientific Committee on Food (SCF) set a tolerable daily intake (TDI) of 2 μg kg^{-1} b.w. (SCF 2001b). However, a TDI of 0.8 μg kg^{-1} per day for 3-MCPD and its fatty acid esters has been established by EFSA CONTAM Panel (EFSA 2006). It is important to note that no-health based guidance value for 2-MCPD has been established due to the lack of sufficient toxicological data.

The toxicological effect of GE has not been fully elucidated yet and there is no data in vivo. On the other hand, it is stated that GE has been demolished by up to 100% as a result of digestion process in humans and turned into glycidol. There is strong evidence that glycidol is a genotoxic compound as a result of *in vitro* and *in vivo* studies. Glycidol was also classified by IARC as "probable human carcinogen (group 2A)" (IARC 2000).

Legislation

Commission Regulation (EC) No. 1881/2006 sets maximum levels (MLs) for certain contaminants in foodstuffs (European Commission 2006), which has been amended and replaced with new regulation to revise legal limits for 3-MCPD and GE. In the European Union (EU), maximum level (ML) of 20 μg kg^{-1} 3-MCPD has been established for HVP and soy sauce for liquid products containing 40% dry matter, corresponding to a maximum of 50 μg kg^{-1} in the dry matter. With regard to GE expressed as glycidol, the EU has set a ML of 1000 μg kg^{-1} for vegetable oils and fats, and 500 μg kg^{-1} for vegetable oils and fats destined for the production of baby food or processed cereal-based food for infants and young children. The Commission has also established ML of 50 μg kg^{-1} (as from July 1, 2019) for GE (expressed as glycidol) in the infant formula, follow-on formula and foods for special medical purposes intended for infants and young children (powder form), while the legal limit of 6 μg kg^{-1} (as from July 1, 2019) for liquid form (European Commission 2018).

Polycyclic Aromatic Hydrocarbons

While, PAHs are a very large family of organic compounds that consist of about ten thousand individual chemical substances, a few of which occur in considerable amounts in the environment and foodstuffs. They are composed of two or more fused aromatic rings and do not contain heteroatoms. When the PAHs have contain

up to four fused benzene rings, called as “light PAHs”. The PAHs containing more than four benzene rings are known as “heavy PAHs”, which are more stable and more toxic than light ones (Wenzl et al. 2006).

PAHs are formed primarily by incomplete burning of carbon-containing organic materials, such as coal, crude oil, gas, wood, garbage or tobacco, and also formed during various industrial processes such as in the cracking of petroleum, or in internal–combustion engines in vehicles. Humans are exposed to PAHs through different routes, mainly via intake of food, inhaled air and drinking water. Food can be contaminated with PAHs that are present in soil, air or water, and mostly by industrial food processing methods and home food preparation applications. The main source of contamination of meat and dairy products with PAHs is heat processing, such as grilling, roasting, drying and smoking processes (EFSA 2008).

Chemical Characteristics

PAHs are stable, fat-soluble, high boiling and melting points and low vapour pressure (EFSA 2008) Some chemical, physical and carcinogenic properties of the 16 PAHs (15 EU priority PAHs + 1) are summarised in Table 6. The chemical structures of 16 PAHs are illustrated in Fig. 2.

Occurrence Data

A large number of studies have been performed for the determination of PAHs in various foods. The results of these studies conducted on the last decade are summarised in Tables 7 and 8. As can be seen in Table 7, the incidence of PAH4 in vegetable oils are quite high. The levels of PAHs in vegetable oils were ranging from 0.1 μg kg^{-1} to 7.5 μg kg^{-1}, 0.01–6.9 μg kg^{-1}, 0.3–8.6 μg kg^{-1} and 0.5–13.1 μg kg^{-1} for BaP, BaA, BbFA and CHR, respectively. The highest concentration of BaP (7.5 μg kg^{-1}) was detected in maize oil originating from Brazil. For oils and fats, the legal limits were set at 2 μg BaP and 10 μg PAH4 per kg product, respectively (European Commission 2011).

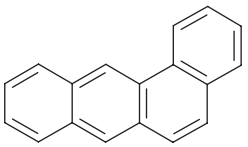
While there are large differences in the individual PAHs content of various consumer products (Table 8), high concentrations of PAHs have been reported in barbecued/grilled and smoked products.

The individual concentrations of BaP, BaA, BbFA and CHR in grilled/barbecued and smoked samples (meat, chicken or fish samples) are up to levels of 17.5 μg kg^{-1}, 36.5 μg kg^{-1}, 13.8 μg kg^{-1}, and 27.8 μg kg^{-1}, respectively. In EFSA report, results of 9714 food samples in 33 food categories on the occurrence of one or more of the 16 PAHs submitted by the 18 European countries were evaluated. Data for the full set of PAH15 were reported for 1375 food samples. According to results, 31.8% of the samples did not contain any PAH analysed above the limit of detection. The

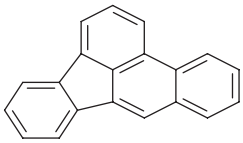
Table 6 Chemical, physical and carcinogenic properties of 16 PAHs

PAH	Abbreviation	CAS Number	Emperical formula	Molecular weight (g mol^{-1})	Melting point (°C)	IARC group
Benzo[*a*] anthracene	BaA	56-55-3	$C_{18}H_{12}$	228.3	160.7	2A
Benzo[*b*] fluoranthene	BbFA	205-99-2	$C_{20}H_{12}$	252.3	168.3	2B
Benzo[*j*] fluoranthene	BjFA	205-82-3	$C_{20}H_{12}$	252.3	165.4	2B
Benzo[*k*] fluoranthene	BkFA	207-08-9	$C_{20}H_{12}$	252.3	215.7	2B
Benzo[*c*]fluorene	BcFL	205-12-9	$C_{17}H_{12}$	216.3	126.5	3
Benzo[*ghi*] perylene	BghiP	191-24-2	$C_{22}H_{12}$	276.3	278.3	3
Benzo[*a*]pyrene	BaP	50-32-8	$C_{20}H_{12}$	252.3	178.1	2A
Chrysene	CHR	218-01-9	$C_{18}H_{12}$	228.3	253.8	3
Cyclopenta[*cd*] pyrene	CPP	27208-37-3	$C_{18}H_{10}$	226.3	170.0	3
Dibenz[*a,h*] anthracene	DBahA	53-70-3	$C_{22}H_{14}$	278.3	266.6	2A
Dibenzo[*a,e*] pyrene	DBaeP	192-65-4	$C_{24}H_{14}$	302.3	232.0	2B
Dibenzo[*a,h*] pyrene	DBahP	189-64-0	$C_{24}H_{14}$	302.3	317.0	2B
Dibenzo[*a,i*] pyrene	DBaiP	189-55-9	$C_{24}H_{14}$	302.3	282.0	2B
Dibenzo[*a,l*] pyrene	DBalP	191-30-0	$C_{24}H_{14}$	302.3	162.4	2B
Indeno[*1,2,3-cd*] pyrene	IP	193-39-5	$C_{22}H_{12}$	276.3	163.6	2B
5-methylchrysene	MCH	3697-24-3	$C_{19}H_{14}$	242.3	117.1	2B

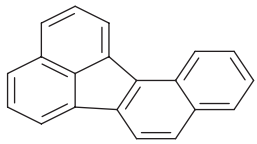
incidence of individual PAH15 in 1375 food products ranged from 3.6% to 77.8%. CHR was the most prevalent PAH in food products, followed by BaA (70.3%), BbFA (63.1%), benzo[*ghi*]perylene (56.4%), and BaP (54.9%). The mean concentration of BaP in all food products was 0.8 μg kg^{-1}. However, BaP was detected in 100% of barbecued meat samples, with a mean level of 1.92 μg kg^{-1}. In this EFSA report, the median dietary exposure of BaP across European countries was calculated both for mean and high dietary consumers and varied between 3.9 ng kg^{-1} b.w. day^{-1} and 6.5 ng kg^{-1} b.w. day^{-1}, respectively. The median dietary exposure to PAH4 was calculated as 19.5 ng kg^{-1} b.w. day^{-1} for mean consumers, and 34.5 ng kg^{-1} b.w. day^{-1} for high consumers. It has been also concluded that estimates intakes of BaP and PAH4, based on available exposure data, were of low concern for human health (Margin of Exposure (MoE) >10,000) (EFSA 2008).



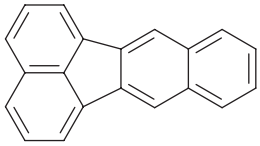
Benzo[*a*]anthracene



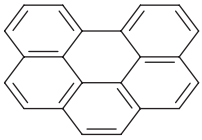
Benzo[*b*]fluoranthene



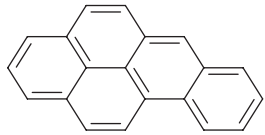
Benzo[*j*]fluoranthene



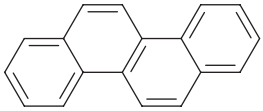
Benzo[*k*]fluoranthene



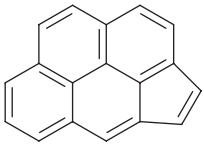
Benzo[*ghi*]perylene



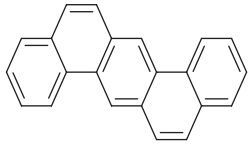
Benzo[*a*]pyrene



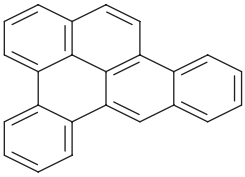
Chrysene



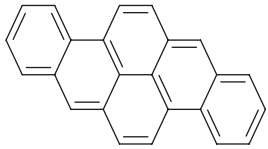
Cyclopenta[*cd*]pyrene



Dibenz[*a,h*]anthracene



Dibenzo[*a,e*]pyrene



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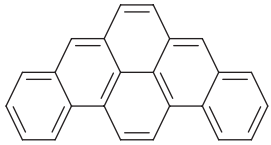
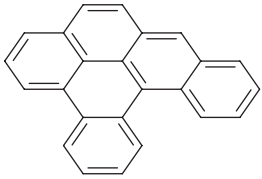
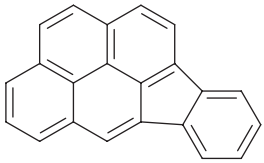


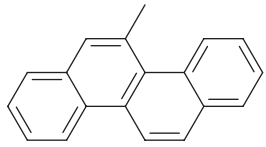
Dibenzo[*a,i*]pyrene



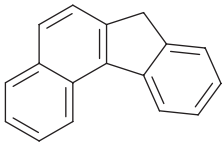
Dibenzo[*a,l*]pyrene



Indeno[*1,2,3-cd*]pyrene



5-methylchrysene



Benzo[*c*]fluorene

Fig. 2 Chemical structures of PAHs16

Table 7 Occurrence and levels of PAH4 in vegetable oils

Food	Country	BaP[a]		BaA[b]		BbFA[c]		CHR[d]		Reference
		Incidence/No. of samples	Range (min-max), μg kg^{-1}	Incidence/No. of samples	Range (min-max), μg kg^{-1}	Incidence/No. of samples	Range (min-max), μg kg^{-1}	Incidence/No. of samples	Range (min-max), μg kg^{-1}	
Blended oil	Iran	13/13	0.9–3.7	–	–	–	–	–	–	Yousefi et al. (2018)
Canola oil	Brazil	NR[e]/23	<0.1–6.3	NR/23	<0.1–4.7	NR/23	<0.1–4.8	NR/23	<0.1–7.9	Molle et al. (2017)
Cottonseed oil	China	5/5	0.2–6.3	5/5	0.3–6.4	5/5	1.5–7.9	5/5	1.1–8.1	Shi et al. (2016)
Frying oil	Iran	14/14	0.9–5.2	–	–	–	–	–	–	Yousefi et al. (2018)
Maize oil	China	12/12	0.4–1.6	12/12	0.7–2.1	12/12	0.9–2.6	12/12	1.1–3.7	Shi et al. (2016)
Maize oil	Brazil	26/26	0.3–7.5	26/26	0.4–6.7	26/26	0.6–5.4	26/26	1.1–13.1	Molle et al. (2017)
Maize oil	Iran	6/6	0.9–5.0	–	–	–	–	–	–	Yousefi et al. (2018)
Olive oil	China	8/8	1.3–0.9	8/8	0.01–1.0	8/8	0.4–1.2	8/8	0.8–1.9	Shi et al. (2016)
Peanut oil	China	10/10	0.7–4.7	10/10	0.7–6.9	10/10	0.3–8.6	10/10	0.6–8.6	Shi et al. (2016)
Rapeseed oil	China	10/10	1.1–2.2	10/10	0.3–2.4	10/10	1.8–3.1	10/10	0.5–3.0	Shi et al. (2016)
Sesame oil	China	10/10	0.1–2.6	10/10	0.1–2.7	10/10	0.5–4.9	10/10	0.8–4.5	Shi et al. (2016)
Soybean oil	China	15/15	0.4–1.5	15/15	0.4–2.5	15/15	1.1–2.8	15/15	1.1–3.6	Shi et al. (2016)

(continued)

Table 7 (continued)

Food	Country	BaP[a]		BaA[b]		BbFA[c]		CHR[d]		Reference
		Incidence/No. of samples	Range (min-max), μg kg^{-1}	Incidence/No. of samples	Range (min-max), μg kg^{-1}	Incidence/No. of samples	Range (min-max), μg kg^{-1}	Incidence/No. of samples	Range (min-max), μg kg^{-1}	
Sunflower oil	China	10/10	0.7–1.7	10/10	0.6–1.1	10/10	1.4–2.9	10/10	1.0–2.2	Shi et al. (2016)
Sunflower oil	Brazil	26/26	0.3–6.3	NR/26	<0.2–3.4	26/26	0.3–2.5	NR/26	<0.3–6.4	Molle et al. (2017)
Sunflower oil	Iran	5/5	0.9–1.1	–	–	–	–	–	–	Yousefi et al. (2018)

[a]BaP: Benzo[*a*]pyrene
[b]BaA: Benzo[*a*]anthracene
[c]BbFA: Benzo[*b*]fluoranthene
[d]CHR: Chrysene
[e]*NR* not reported

Table 8 Occurrence and levels of PAH4 in various food products

Food	Country	BaP[a]		BaA[b]		BbFA[c]		CHR[d]		Reference
		Incidence/ No. of samples	Range (min-max), μg kg^{-1}	Incidence/ No. of samples	Range (min-max), μg kg^{-1}	Incidence/ No. of samples	Range (min-max), μg kg^{-1}	Incidence/ No. of samples	Range (min-max), μg kg^{-1}	
Basil	India	25/25	0.85–2.9	NR/25	<0.09–3.1	25/25	1.6–9.3	25/25	1.6–9.7	Rozentāle et al. (2018a)
Thyme	Poland/ China	25/25	1.3–5.7	25/25	0.81–7.6	25/25	1.8–11.5	25/25	2.95–18.2	Rozentāle et al. (2018a)
Oregano	Turkey	NR[e]/25	<0.05–1.6	NR/25	<0.09–2.7	NR/25	<0.04–1.5	NR/25	<0.09–8.4	Rozentāle et al. (2018a)
Black tea	Argentina	27/27	0.2–92.5	27/27	0.2–62.8	27/27	0.1–67.6	27/27	2.5–109.1	Londoño et al. (2015)
Green tea	Argentina	14/14	0.4–61.3	14/14	0.7–74.4	14/14	0.2–66.6	14/14	4.6–153.7	Londoño et al. (2015)
Bread	Turkey	20/20	0.11–0.25	20/20	0.01–0.09	11/20	<0.01–0.08	20/20	0.01–0.11	Kacmaz (2016)
Breakfast cereals	Turkey	20/20	0.09–0.30	20/20	0.03–0.23	10/20	<0.01–0.14	20/20	0.03–0.25	Kacmaz (2016)
Mixed vegetables in olive oil	Italy	0/4	<0.01	3/4	0.07–0.14	4/4	0.07–0.11	4/4	0.18–0.30	Sannino (2016)
Mushrooms in sunflower oil	Italy	5/5	0.08–0.10	5/5	0.15–0.29	5/5	0.16–0.27	5/5	0.35–0.51	Sannino (2016)
Mayonnaise	Italy	6/6	0.04–0.10	6/6	0.08–0.26	6/6	0.09–0.16	6/6	0.14–0.44	Sannino (2016)
Soybean	Brazil	1/39	3.49	3/39	0.5–58.8	2/39	1.4–18.5	30/39	0.9–103.9	Garcia et al. (2017)

(continued)

Table 8 (continued)

Food	Country	BaP[a]		BaA[b]		BbFA[c]		CHR[d]		Reference
		Incidence/ No. of samples	Range (min-max), μg kg^{-1}	Incidence/ No. of samples	Range (min-max), μg kg^{-1}	Incidence/ No. of samples	Range (min-max), μg kg^{-1}	Incidence/ No. of samples	Range (min-max), μg kg^{-1}	
Milk powders	Uruguay	NR/44	<0.001–11.5	NR/44	<0.005–4.1	NR/44	<0.001–2.7	NR/44	<0.004–8.7	Londoño et al. (2017)
Milk powders	Argentina/ Brazil	28/31	0.01–0.57	30/31	0.02–2.46	25/31	0.07–1.49	24/31	0.19–5.88	Londoño et al. (2013)
Barbecued beef	Denmark	29/91	<0.3–17.5	–	–	–	–	–	–	Duedahl-Olesen et al. (2015)
Barbecued pork	Denmark	16/54	<0.3–8.4	–	–	–	–	–	–	Duedahl-Olesen et al. (2015)
Barbecued chicken	Denmark	3/30	<0.3–0.6	–	–	–	–	–	–	Duedahl-Olesen et al. (2015)
Canned/ smoked fishes	Poland	NR/60	<0.18–4.8	NR/60	<0.18–36.5	NR/60	<0.18–3.9	NR/60	<0.18–27.8	Zachara et al. (2017)
Chicken fillets	Poland	NR/15	<0.18–2.3	NR/15	<0.18–1.2	NR/15	<0.18–3.2	NR/15	<0.18–2.7	Zachara et al. (2017)
Grilled meat	Malaysia	NR/162	<0.03–12.5	–	–	NR/162	<0.03–13.8	–	–	Farhadian et al. (2010)
Grilled meat (gas grilled)	Iran	80/80	0.28–5.0	NR/80	<0.15–4.3	NR/80	<0.15–1.0	NR/80	<0.15–4.3	Gorji et al. (2016)

		Incidence/ No. of samples	Range (min-max), μg kg^{-1}	Incidence/ No. of samples	Range (min-max), μg kg^{-1}	Incidence/ No. of samples	Range (min-max), μg kg^{-1}	Incidence/ No. of samples	Range (min-max), μg kg^{-1}	
Grilled meat (choarcoal grilled)	Iran	80/80	0.45–5.8	NR/80	<0.15–5.2	NR/80	<0.15–5.2	NR/80	<0.15–4.1	Gorji et al. (2016)
Pork hams	Poland	NR/25	<0.18–2.7	NR/25	<0.18–10.2	NR/25	<0.18–4.1	NR/25	<0.18–9.6	Zachara et al. (2017)
Sausages	Poland	NR/20	<0.18–6.2	20/20	0.81–15.0	NR/20	<0.18–5.2	NR/20	<0.18–12.4	Zachara et al. (2017)
Smoked meat products	Latvia	NR/128	<0.05–6.0	NR/128	0.05–14.2	NR/128	<0.05–4.6	NR/128	0.1–14.5	Rozentāle et al. (2018b)

[a]BaP: Benzo[*a*]pyrene
[b]BaA: Benzo[*a*]anthracene
[c]BbFA: Benzo[*b*]fluoranthene
[d]CHR: Chrysene
[e]*NR* not reported

Toxicological Evaluation

In long-term studies, PAHs caused kidney and liver damage, and cataracts, jaundice and skin tumours. Among, PAHs, benzo[*a*]pyrene (BaP) is the most common PAH, which could be used as a marker of exposure. However, BaP constitutes only 1–20% of the total concentration of carcinogenic PAHs (Wenzl et al. 2006). BaP is also considered as a human mutagen (EU Category M2) and a human reprotoxic compound (EU Category M2) (BfR 2009). The SCF concluded that 15 PAHs, namely benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, benzo[*a*]pyrene, chrysene, cyclopenta[*cd*]pyrene, dibenz[*a,h*]anthracene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, indeno[*1,2,3-cd*]pyrene and 5-methylchrysene caused mutagenicity/genotoxicity in somatic cells in experimental animals *in vivo*. There is also strong evidence from *in vivo* data that these PAHs with the exception of benzo[*ghi*]perylene have carcinogenic effects in experimental animals (EFSA 2008). In addition, benzo[*c*]fluorene is also identified as a priority PAHs by JECFA.

Legislation

Specific ML of BaP and sum of BaP, benz(a)anthracene, benzo(b)fluoranthene and chrysene were set by EU for foodstuffs containing oils and fats, smoked foods and foods where environmental pollution might cause high levels of contamination. While the legislative limit of BaP varies from 1 μg kg^{-1} for foods for infants and young children to 10 μg kg^{-1} for *Bivalve molluscs*, the ML of sum of BaP, benz(a)anthracene, benzo(b)fluoranthene and chrysene in various foods of between 1 μg kg^{-1} (for foods for infants and young children) and 35 μg kg^{-1} (for Bivalve molluscs) (European Commission 2011).

Chlorinated Organic Compounds

Chlorinated organic compounds are divided into three groups: polychlorobiphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs, dioxin), polychlorinated dibenzofurans (PCDFs, furan) (Ergun 2015; González et al. 2019). PCBs are a general name used to express different chlorinated derivatives of biphenyl and consist of a very heterogeneous group of chemicals. PCBs with very low solubility in water are heat stable, non-flammable, and do not break down easily (Shibamoto and Bjeldanes 2009). However, they may be oxidized to dibenzo dioxin and dibenzo furans under specific conditions (Ayaz and Yurttagul 2008).

PCBs are materials commonly used in as coolants and lubricants in transformers and capacitors, and as hydraulic fluids and heat exchange fluids in electrical/elec-

tronic equipment (Arvanitoyannis et al. 2014; Thompson and Darwish 2019). In addition, they are also used in plasticizers and paints (Shibamoto and Bjeldanes 2009). They can be transmitted to food in various ways and large amounts. PCBs were detected frequently in fishes and seafood caught at high concentrations in large lakes (Shibamoto and Bjeldanes 2009; Arvanitoyannis et al. 2014). They are also found in organic and conventional meat, oils, milk and cheese (González et al. 2019).

More than 90% of PCBs are absorbed from the gastrointestinal tract, and accumulated mostly in adipose tissue in the skin, adrenal glands, and aorta. The amount of PCBs in the adipose tissue decreases more slowly than in the blood. It is usually excreted with faeces from the body. Via urinary excretion is in low amounts but, human milk excretion is very low compared to urine (Shibamoto and Bjeldanes 2009).

PCBs may cause acute and chronic effects in various animals (Omaye 2004; Thompson and Darwish 2019). They have carcinogenic, teratogenic and neurotoxic effects (Thompson and Darwish 2019). It leads to progress rather than initiating cancer formation. In addition, they can cause harmful effects by entering the phase of metabolic enzymes such as oxidases, reductases and conjugates. The most important effects in humans are stubborn acnes in the head and chest skin (Ayaz and Yurttagul 2008). Moreover, they may cause a number of problems in language delay, mental and motor development in humans (Thompson and Darwish 2019).

Dioxin is a highly chemically stable organochlorine compound and is considered one of the major food contaminant arising from environmental pollution (Ergun 2015). Dioxin is a toxic substance that has attracted the attention of the world after the incident known as “Seveso Disaster”. In most of the chemicals (chlorophenols, phenoxyacid herbicides, chlorinated biphenyls and aromatic hydrocarbons) commonly used in industry, dioxin is present as impurity (Ayaz and Yurttagul 2008).

Dioxin sources can be listed as follows:

- Dioxin can be transmitted to the environment from the paper manufacturing industry. Especially, the chlorination stage in the bleaching units of the paper industry leads to the formation of dioxins,
- It can be produced as a by-product during the production of chlorophenols used as herbicides, fungicides, insecticides and bactericides,
- It can be found in most of the pharmaceuticals that we use frequently in our daily lives,
- Plastics, which constitute a significant part of local wastes such as polyvinyl chloride, also cause PCDD formation,
- It may occur in exhaust gases of automobiles using leaded fuel, and
- It may occur during the burning of wood and also formed by burning chlorine-based chemical compounds with hydrocarbons (Ayaz and Yurttagul 2008; Shibamoto and Bjeldanes 2009).

Dioxin can be taken from food, beverages, respiration and/or skin to the human body (Ayaz and Yurttagul 2008). The most important cause of dioxin exposure of humans is their diets (Shibamoto and Bjeldanes 2009). People are exposed to dioxin in particular with animal foods (Ergun 2015) such as milk and dairy products, fish and meat (Deshpande 2002; Shibamoto and Bjeldanes 2009).

Even small amounts of dioxin, which can be found in foods (fish and seafood), air, soil and water, can adversely affect human health (Arvanitoyannis et al. 2014). Dioxin containing 1–3 chlorine atoms is considered to be the lowest toxic effect, while chlorine containing dioxin at 2,3,7,8 (tetrachlorodibenzodioxin, TCDD) is considered to be the most toxic (Omaye 2004; Ayaz and Yurttagul 2008). That dioxin is one of the most potent teratogens known (Deshpande 2002). On the other hand, TCDD has been reported to be a more potent carcinogen than AFB_1 in studies done in female rats. The toxic effect of dioxins, and in particular TCDD, can vary from live to alive (Shibamoto and Bjeldanes 2009). However, health problems generally caused by dioxin include loss of appetite, changes in pigmentation in the skin, liver disorders, psychological abnormalities, neurological problems, high blood pressure, increased blood lipid and cholesterol levels. In addition, there are reports about the occurrence of congenital disorders such as reproductive disorders, palate cleft and defective kidney formation and the formation of soft tissue cancers (Ayaz and Yurttagul 2008).

Heavy Metals

Heavy metals (HMs) are used for metals with a specific gravity greater than 4.0 g/cm^3 (Raikwar et al. 2008) and as having an atomic number over 20 (El-Kady and Abdel-Wahhab 2018). HMs are not formed as organic pollutants, they are naturally present in the earth's crust (El-Kady and Abdel-Wahhab 2018). Living all organisms need trace amounts of HMs to sustain various physiological functions. These HMs are cobalt (Co), iron (Fe), copper (Cu), manganese (Mn), molybdenum (Mo), vanadium (V), strontium (Sr), and zinc (Zn). There are also metals that are not necessary for metabolism. The most important ones are cadmium (Cd), mercury (Hg), lead (Pb), arsenic (As), Uranium (U), aluminum (Al) and tin (Sn) (Shibamoto and Bjeldanes 2009; Nfon et al. 2009).

HMs are one of the oldest toxic substances known since ancient times (Deshpande 2002). People are exposed to HMs in different ways, such as inhalation, and ingestion of foods or beverages (Iheanacho et al. 2017; Hejna et al. 2018). Volcanic eruptions, continental weathering, forest fires and mining-related operations are important sources of HMs (El-Kady and Abdel-Wahhab 2018). Depending on the developing technology, as well as sewage, agricultural chemicals (pesticides, fertilizers), domestic and untreated industrial effluents, the contamination of HMs is increasing and the residues and wastes of the related industries pollute the environment, plants, human and foods (Jan et al. 2015). Since they do not degradable and have high water soluble properties, they remain in the environment for a long time. HM pollution is increasing day by day in worldwide, especially in developing countries (Tasrina et al. 2015) As a result, certain amounts of toxic HMs in foods can exceed allowable limits.

HMs, which are dangerous, can be exceedingly poisonous even in low exposure doses and can be transmitted to foods (El-Kady and Abdel-Wahhab 2018).

Especially, foods can be exposed to metallic contamination at various stages of the production (such as harvest, handling, processing storage etc) and consumption chain. In the breeding and harvesting stage, contamination may occur from the soil, air, water and agricultural activities (pesticides, fertilizers), and in the processing stage from metal equipment and packaging materials (canned, plastic) (Deshpande 2002; Ayaz and Yurttagul 2008). Pollution of surface waters and groundwater by industrial wastes is another important cause of the HMs that people and animals are exposed (Deshpande 2002). Raw materials type and quality, distance of the raw material to the roadside, additives, soil eating and inadvertent contamination can affect dietary intake of HMs (Hejna et al. 2018). An important amount of heavy metals can be transferred to the edible parts of plants grown in heavy metal-rich soils (Tasrina et al. 2015).

HMs can cause cancer, kidney damage, endocrine disruption, cardiovascular, renal and immunological problems, nervous system damage and even death at very high concentrations in humans (Hejna et al. 2018; Edelstein and Ben-Hur 2018). IARC has included some heavy metals in the group of substances that can be carcinogenic to humans (Hejna et al. 2018). Factors affecting the toxicity of metals; metabolic interactions of metals, formation of metal-protein complexes, and routes of exposure, chemical form or type of HM (Deshpande 2002), as well as the age, gender, lifestyle, genetics and nutritional status of the exposed individuals (Hejna et al. 2018). Especially infants, children and adolescents are more susceptible to heavy metal-borne infections than adults. Today, to avoid heavy metal poisoning, some arrangements have been made in many countries about the maximum amounts of heavy metals that can be found in foods and beverages (Edelstein and Ben-Hur 2018).

An important part of the HMs gets strongly attached to the blood cells and tissues. Then, they are slowly excreted from the body via in the urine and lesser extent via gastrointestinal tract (Dorne et al. 2011). Therefore, they accumulate in some organs such as blood, liver, kidney, and hair (Deshpande 2002; Hejna et al. 2018). Some of the heavy metals which are more dangerous to human and animals are summarised below:

Arsenic

Arsenic, an inherently comprising and, distributed metal ubiquitous in the environment is present in low amounts in foods (Jan et al. 2015). Arsenic is contaminated to the environment from volcanic explosions, mining activities, steel production and coal and fossil fuels, as well as agricultural activities such as the use of pesticides (El-Kady and Abdel-Wahhab 2018). People can be exposed to arsenic from soil, water, air and food (Jan et al. 2015).

Both various organic and inorganic form of arsenic may be found in foods and environment. Organic arsenic can accumulate in fish and sea products such as shellfish, oysters, mussels and shrimp in high amounts (Shibamoto and Bjeldanes 2009).

While a small amount (10%) of arsenic found in fish and seafood is inorganic, most of the arsenic (almost 90%) in all other foods such as rice is inorganic (Dorne et al. 2011; El-Kady and Abdel-Wahhab 2018). The amount of arsenic in plants may vary depending on various factors such as soil content, water contamination, air pollution and/or fertilizer use (Ayaz and Yurttagul 2008).

Arsenic is readily absorbed from the foodstuffs in the GI system and transferred to all organs and tissues. It accumulates mostly on the skin, hair and nails. In a lesser proportion accumulates in the bones and muscles (Deshpande 2002).

Unlike mercury and lead, inorganic arsenic and its compounds are more toxic for humans than their organic forms (Deshpande 2002; Dorne et al. 2011). Intake of high amounts of arsenic can cause acute intoxication, and accordingly nausea, muscle weakness, vomiting, edema neurotoxic problems, and severe diarrhoea may occur (Shibamoto and Bjeldanes 2009; Edelstein and Ben-Hur 2018). Loss of appetite, weight loss, gastrointestinal disorders, liver enlargement, anemia, reduction of white blood cells, peripheral neuritis, hyperkeratosis and skin melanosis can occur in chronic intoxications (Deshpande 2002). Moreover, arsenic may lead to gastrointestinal problems and also skin, liver, and kidney cancers by affecting numerous organs (Jan et al. 2015).

Cadmium

Cadmium is a naturally found and widely distributed heavy metal in the environment. It is widely used in technological processes and form as most by-products. For these reason, cadmium can be found in soil, air, water, vegetation, tobacco, dust and food sources (Shibamoto and Bjeldanes 2009). Foods contain mostly inorganic salts of cadmium (Deshpande 2002). Moreover, cadmium can be found in the aquatic environments due to industrial and agrochemical wastes (El-Kady and Abdel-Wahhab 2018).

Cadmium can be found in several foods with a wide range of contamination levels (Deshpande 2002). A 30% of the daily intake of cadmium is provided from animal foods, while 70% is provided from herbal sources such as especially vegetables (El-Kady and Abdel-Wahhab 2018). Vegetarian people who consume foods such as cereals, nuts, oilseeds and pulses in high amounts may be exposure up to 5.4 mg kg^{-1} b.w. per week to cadmium (Dorne et al. 2011). Genetic factors, age and nutritional factors affect the absorption of cadmium taken with foods (Deshpande 2002). Significant amounts of cadmium may accumulate in shellfish and some animals' kidneys. It can be transmitted to herbal foods by irrigation water (Ayaz and Yurttagul 2008). Other sources of cadmium contamination include cadmium-containing food machinery and equipment, zinc galvanized equipment, glass, porcelain, rechargeable batteries, anticorrosion agents (Deshpande 2002; El-Kady and Abdel-Wahhab 2018).

Cadmium has been classified as group 1 (human carcinogen) (Deshpande 2002). Dietary high amounts of cadmium increase the risk of various cancers such as lung

cancer in humans (Hejna et al. 2018). It has toxic effects on the cardiovascular and skeletal system (Gomiero 2018). It causes slow and irreversible liver and kidney damages, dermal irritation, ulcer, inactivation of some enzymes in humans (Dorne et al. 2011; Hejna et al. 2018). Chronic intoxications occur in the form of growth retardation, reproductive disorders (Ayaz and Yurttagul 2008) and osteoporosis (Gomiero 2018). The high amounts of cadmium intake may also lead to *itai*, a bone disease that can lead to death (Raikwar et al. 2008).

Lead

Lead is one of the most plenty natural metals in the world because of it can be easily separated from the ore. It has been widely used for a variety of purpose in the industry in mining, smelting, refining, battery manufacturing, and so on (Dafaelseed et al. 2007; Jan et al. 2015). Thus, it can be found in air, water, soil and food (Raikwar et al. 2008).

One of the sources of lead contamination is tetraethyl lead, which is added to increase the octane rating to gasoline (Deshpande 2002; Jan et al. 2015). Half of the lead in the exhaust gas released from the vehicles spreads on both sides of the highways. As a result of this, lead content increases in plants growing in soil and in this soil. On the other hand, lead values are reduced when the distance away from the highway, and also as the soil depth increase (Ayaz and Yurttagul 2008).

People can be exposed to lead contamination from a wide variety of sources (Jan et al. 2015). The most important sources of non-industrial contamination are foods (more than 90%) and water (Shibamoto and Bjeldanes 2009). The amount in water may vary depending on the source of the lead. The use of lead-containing pipes and tanks in water distribution, especially if the water is soft and acidic, leads to an increase in lead content in water. This is because the acid in the water dissolves the lead in the pipes and therefore increases the concentration. Another important source of lead contamination is the glaze in ceramic containers. The amount of lead in acidic foods stored in such containers may increase (Ayaz and Yurttagul 2008). Other sources of contamination are lead used for soldering, canned food, lead-containing substances used to hunt animals such as birds and rabbits, leaded crystal cups, bottles and containers, storage batteries, insecticides, and newspaper which used for food packaging purposes (Deshpande 2002).

Foods with the highest lead content are usually shellfish. There is less lead in milk, fruits, vegetables, cereal products, potatoes, fish, meat, and tap water (Deshpande 2002; Dorne et al. 2011). Most of lead remains in the roots of plants (El-Kady and Abdel-Wahhab 2018). However, children can also be exposed to significant amounts of lead, especially from paints in dust, soil and toys in the house (Dorne et al. 2011). Lead is more easily absorbed in children (approx. 40%) than adults (approx. 10%) (Deshpande 2002).

There are several factors affecting the absorption of lead from the gastrointestinal tract (Shibamoto and Bjeldanes 2009). Absorbed lead acts as calcium in the

body and accumulates in various organs (Raikwar et al. 2008). 95% of the absorbed lead is distributed in hard tissue (bones, teeth, hair, and nails), 2% in blood and 3% in soft tissues in the liver, kidneys, aorta, muscle and brain (Deshpande 2002; Shibamoto and Bjeldanes 2009).

Organic form of lead is more dangerous than the inorganic one (Ayaz and Yurttagul 2008). One of the clinical symptoms of lead poisoning in the body is the interaction with some enzyme systems which are necessary for biosynthesis, and which lead to anaemia (El-Kady and Abdel-Wahhab 2018). Acute lead exposure may cause health problems such as loss of appetency, headache, hypertension, kidney failure, abdominal ache, lassitude, sleeplessness, arthritis, hallucinations and vertigo. Chronic intoxications may result in mental insufficiency, birth faults, allergy, weight losses, hyperactivity, paralysis, brawn weakness, brain damage, kidney detriments and even deaths (Jan et al. 2015). Lead can also induce anorexia, dyspepsia, constipation, paroxysmal abdominal pain and colic attack (Deshpande 2002). Intake to high amounts of lead in pregnant women may lead problems such as miscarriage, stillbirth, preterm delivery, low birth weight (Iheanacho et al. 2017).

Mercury

Mercury is mainly used in various activities such as thermometer, battery, fluorescent lamp, dye and fungicide production. In addition, it is used as amalgams in dental preparations (Deshpande 2002; Jan et al. 2015).

Mercury can be found in meat products, plants and especially in fish and other seafood (Shibamoto and Bjeldanes 2009). This is due to the fact that more than 3/4 of the total mercury in fish is in the form of methyl mercury produced by microorganisms in the sea. Especially in fishes such as swordfish, tuna, whale, shark and dolphin, mercury level can reach to 6 $\mu g\ g^{-1}$. Plants absorb only a limited amount of mercury with their roots even in soils contaminated with high levels of mercury. While mercury cannot be detected in potatoes, beans, olive oil and rice, in some cereal and cereal-based foods can be found up to 0.57 $\mu g\ g^{-1}$ (El-Kady and Abdel-Wahhab 2018). The majority of the mercury found in plants is due to surface contamination with the atmosphere and to a lesser extent from the soil (Ayaz and Yurttagul 2008). The reason for the high content of mercury in the soil is the intensive use of pesticides and fungicides which include high amounts of mercury in their compositions (El-Kady and Abdel-Wahhab 2018).

Mercury has three different chemical forms: (i) elemental (metallic, HgO), (ii) organic (phenyl mercuric salts and alkyl mercuric compounds) and (iii) inorganic (monovalent and divalent). These chemical forms of mercury have its own toxicity and have negative impacts on health. The toxicity of mercury can vary related to chemical forms and its distribution in the human body. Because of its lipophilic properties, the organic mercury form readily absorbed after ingestion is more dangerous than the other two forms on health (Deshpande 2002; Jan et al. 2015). Organic mercury (methyl mercury) causes adverse effects on the central nervous

system, and these affects are caused by the accumulation of mercury in the motor regions of brain and central nervous system (Jan et al. 2015).

Foods can include both organic and inorganic forms. Inorganic forms are mercury chloride (HgCl), mercury bromide (HgBr) and mercury oxide (HgO), and organic forms methyl mercury (El-Kady and Abdel-Wahhab 2018). Inorganic mercury compounds, which have a half-life of about 40 days, damage the gastrointestinal tract, liver and kidneys when taken with food (Shibamoto and Bjeldanes 2009; Jan et al. 2015). They cause acute kidney failure and neurotoxicity in humans (Hejna et al. 2018). Moreover, acute inorganic mercury poisoning may cause gastrointestinal discomfort, abdominal pain, nausea, vomiting and bloody diarrhoea (Shibamoto and Bjeldanes 2009). Consumption of marine products containing high amounts of mercury (MeHg) during pregnancy causes the birth of children with neurological problems (El-Kady and Abdel-Wahhab 2018). On the other hand, in humans consuming high amounts of mercury-containing fish and crustaceans, a disease called *Minamata* occurs. *Minamata* is mercury poisoning that occurs in the form of decreased appetite, weakness, blindness, stagnation, inaction (Raikwar et al. 2008). Methyl mercury, one of the six most dangerous toxic chemicals in the environment, is neurotoxic for the adult and fetus because it accumulates in the brain (Deshpande 2002).

Conclusions

People are routinely exposed to many chemicals including naturally occurring substances, unapproved food additives and adulterants, agrochemicals, food processing contaminants, migration from food packaging materials and environmental/industrial pollutants. Some of these chemical hazards are not only toxic but also remain in the environment for a long time, and building up to high levels in the food chain and accumulate in the body tissue.

The strict regulations for certain chemical hazards have been established by the national governments and international bodies such as European Commission and Codex Alimentarius. It is recommended to monitor chemical hazards in food chain routinely and the preventive actions must be conducted in order to reduce exposure to chemical substances. However, the risk assessment of chemical hazards in the food chain is a non-static process and it needs to be flexible without long consistency. It should be needed forthcoming analyses including the assessment of multiple substance–exposure scenarios.

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Risk Management of Chemical Hazards Arising During Food Manufacturing

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Abstract The three main types of food contaminants are physical, chemical and microbiological. Since ancient times chemical hazards are known as potential food safety concerns. These hazards may be transmitted into food at all phases in the processing, packaging, transportation, supply chain, including production and trade. The chemical hazards can arise in distinct forms and as a result of various events. With the advancement in the technology, detection of such contaminants becomes easier. Risk management of chemical hazard requires a vigorous management of food safety system that has focus on operational prerequisite programs, such as trader quality assurance and inventory control as well as Hazard Analysis and Critical Control Point (HACCP). This chapter highlights various groups of food contaminants, their occurrence in the food chain. Other part of the chapter mainly focuses on food process toxicants, food additives and nutrients, and approaches to be employed to solve the risk they pose to the consumer and prevent them from health hazards.

Keywords Risk management · Chemical hazards · Food safety · Food processing

Introduction

Since ancient times chemical hazards are known as potential food safety problems in terms of guideline (Sumar and Ismail 1995) and procedures of analysis to detect them (Accum 1820). These hazards are major cause of contamination of food and in extreme situation lead to outbreaks of food-borne diseases (Faille et al. 2018). Accidental or intentional contamination of food is an unfortunate act having serious consequences on human health. Food contamination was recorded in history

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8000 years ago, however, with the advent in the agri-food industry and globalization, this problem has been spread all over the world (Robertson et al. 2014). The contamination in food may be caused during various stages of procurement, handling, processing, packaging and transportation.

There are several reasons of food contamination (Ingelfinger 2008). Preparation of food is subjected to a series of processing, where each phase is a possible source of contamination by hazardous chemicals. For improving the shelf life of a food product, some chemicals are intentionally added during the food preparation. Chemical hazards in food generally consist of food processing contaminants, environmental contaminants, unapproved adulterants and food additives (Mastovska 2013). Environmental contaminants refer to impurities added by humans or naturally occurring in air, water or soil. Food processing contaminants are produced in food during cooking, heating, roasting, canning, hydrolysis or fermentation (Schrenk 2004). In this chapter various groups of food contaminants and the principles that underlie the effective management of risks related to the chemical hazards by agribusinesses has been discussed.

Risk Management of Chemical Hazards

All over the world regulatory agencies have recognized the risks that chemical contaminants pose to the food supply. As a result, they have established the limits for many of these compounds in different food categories in order to protect the people from exposures to contaminants level which may pose adverse health effects. The growing complexity of global food trade (Ercsey-Ravasz et al. 2012) combined with the patchwork of regulatory requirements, has created additional challenges in ensuring both the security of the food supply and its full compliance with regulatory requirements.

Food marketing and manufacturing in different parts of the world also requires companies for ensuring safety of food for human consumption and to meet regulatory requirements. In the European Union, regulations also require companies to take steps to ensure that the food ingredients they use also conform with all applicable regulations on chemical contamination (European Parliament 2006). However, the application of a food safety standard that ensures compliance with different national regulations on chemical contaminants presents many challenges. Because of the need to prioritize regulations for chemical contaminants and complexity of the number of different foods in the food supply, national regulations do not set limits for all relevant chemical contaminants for all possible categories of foods. The importance of setting limits for chemical contaminants in ingredients and products is particularly important in view of the fact that many chemical contaminants are inherent in the environment and therefore remain in trace amount in the food supply. The challenge for global agribusinesses is to create a chemical contaminants control program that ensures product safety and regulatory compliance. Establishing a science-based, risk-based approach to define target lists and limits for chemical

contaminants in food categories, which would allow companies to reduce the risk of ingredients and products exceeding national regulations. In addition, such an approach would enhance public confidence in the food supply safety and inform regulators that the risks associated with contaminants are being addressed.

Risk management is defined as "coordinated activities to direct and control an organization with regard to risk" (British Standards 2002). Chemical hazard's risk management starts with the recognition of the Paracelsus statement (1493-1541) "the dose makes the poison" (Borzelleca 2000). Paracelsus dictum indicates that toxic effect of a substance depends both on the amount and frequency of intake. In general, therefore, toxicity may be acute when a response is induced after a single or small number of (relatively) high doses (e.g., paralytic shellfish poisoning), or chronic, when an adverse event occurs after long-term exposure at (relatively) low doses (e.g., dietary arsenic-induced skin cancer). In terms of risk management, the Paracelsus dictum delivers a limit value, which distinguishes among safe and dangerous consumption. These values are established using risk assessment procedures (Benford 2012). Then the values can be used to update regulatory limits.

International best practice believes that this is best achieved through risk-based management approach. In addition, where this is unavoidable, as a general principle and even in the absence of appreciable risk to the consumer, the limits for chemical contaminants must be consistent with the ALARA (as low as reasonably achievable) principle (Food and Agriculture Organization 1997). The principle generally employed to the setting of regulatory limits for certain chemicals and is frequently included in the Regulation for contaminants for which limit does not exists (Council of the European Communities 1993).

The reviews of BS ISO 31000:2009 (British Standards 2009) indicates that, in a commercial (food) context, risk management considers not only aspects of the hazard and its probability; but also all the business operations and mechanisms necessary to ensure that the risk can be maintained at an acceptable level. For this a farm-to-table approach is required. Alldrick (2012) suggested that such an approach needs know-how of the way and where the contaminants happen within the food chain; the effects of industrial transformation; handling of foods after the purchase and ultimately; who ends up consuming it. Therefore, risk management of chemical hazards needs a robust food safety management system and also operational prerequisite applications, like trader quality assurance and record control as well as the implementation of Hazard Analysis and Critical Control Point system.

Chemical Hazards in Food

Essentially, potential chemical contaminants in foods (Fig. 1) can be classified into the following categories:

1. natural toxins, for example plant toxins, phycotoxins and mycotoxins.
2. environmental contaminants, for example heavy metals.

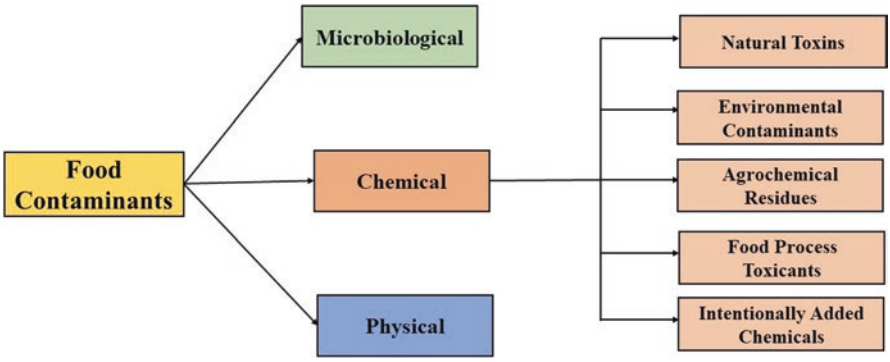

Fig. 1 Chemical hazards associated with food

3. agrochemical residues, for example pesticides and veterinary drugs.
4. processing toxicants, for example heterocyclic aromatic amines, acrylamide.
5. internationally added chemicals, for example food additive and nutrients.

Natural Toxins

Phytotoxins

Several plant foods have pharmacologically active elements that sometimes can be precisely eaten for the properties they bring (example, caffeine in coffee). In other cases, herbal foods may comprise pharmacologically active substances that at even comparatively low concentration can result in disease or death. These compounds are termed as phytotoxins and are frequently secondary metabolites (for example, alkaloids, phenolics and terpenes); but it can also be proteins such as lectins of certain legumes (D'Mello et al. 1992). Pyrrolizidine alkaloids are an example of how consumers may be exposed to phytotoxins as a result of consuming foods in which the producing plant is accidentally mixed or bioaccumulated. The 1, 2-unsaturated alkaloids of pyrrolizidine are known for causing hepatic veno-occlusive disease which, in its acute form led to high mortality incidence and in its chronic form results in liver cirrhosis (WHO 1988). The poisoning of these compounds is related with the cereal crops contamination with *Heliotropium* species seeds in different portions of the world. Research work in Germany have shown the presence of common groundsel (*Senecio vulgaris*) leaves and flowers, that may also have pyrrolizidine alkaloids as a contaminant of mixed salad preparations, and Bundesinstitut fur Risikoberwertung (BfR) has suggested the limits of these compounds from *S. vulgaris* must be maintained as low as possible (Dussemund et al. 2010). Food Safety and Standards (Contaminants, toxins and Residues) Regulations, 2011 has given permitted limits of some natural toxins (Table 1).

Table 1 Permissible limits of some natural toxins in foods

Name of the chemical contaminant	Description of food	Limit µg/kg
Aflatoxin	Cereals and cereal products	15
	Pulses	15
	Ready to eat nuts	10
	Spices	30
Aflatoxin M1	Milk	0.5
Ochratoxin A	Barley, wheat, rye	20
Patulin	Apple juice and apple juice ingredients in other beverages	50

The European Food Safety Authority (EFSA) assessed the risks associated with the occurrence of phytotoxins in honey (European Food Safety Authority 2011) and suggested that although, in general, there is no harm to the majority of the population but young children and babies who consume a lot of honey are likely to worry about their health. Therefore, agri-food companies must follow their food safety management systems considering the raw material and the target market product. Hence based on the situation, various risk management strategies will be taken into account. When phytotoxins are directly linked with the plant being used, risk management may involve following methods: vertical selection of cultivars having lesser levels of chemical contaminants; post-harvest culture and handling of the plant to minimize the production of phytotoxins or removing contaminated plant; also the treatment for denaturing or removing contaminants from the food. For example, the maintenance of low levels of glycoalkaloid (solanine and chaconine) in potatoes by minimizing the light exposure during tubers developing and post-harvest storage (Potato Council 2015); thermal denaturation of lectins (phytohemagglutinins) in some legumes (Almeida et al. 1991). In general, for many phytotoxins-plant combinations, no limit is set neither in the Codex Alimentarius nor at a regulatory level. Therefore, food businesses should be conscious of the potential of plant foods to include these compounds and ensure, through the approaches described above, that their concentrations are as low as reasonably achievable (ALARA principle) to avoid any risk of contamination.

Phycotoxins

Phycotoxins are produced by cyanobacteria. They have various chemical structure and pathologies. Bioaccumulation of phycotoxins in both aquatic animals that consume algae and in their predators, which ultimately enter the food chain thus posing a risk to the consumer (Etheridge 2010). Some examples of phycotoxins are paralytic toxins in molluscs (PSPs) (saxitoxins), diarrheal toxins (DSPs) (okadaic acid) and neurotoxic toxins (NSPs) (Brevetoxins). Phycotoxins can also be a problem in sea fish, such as ciguatoxins. Phycotoxin contamination in drinking water led to

toxicosis in farmed animals (Fitzgerald and Poppenga 1993), in humans (Pouria et al. 1998) and in their pets (Lürling and Faassen 2013).

For protecting the consumer from harmful properties of phycotoxins and other health threats, risk management has defined laws. Regulatory authority regularly monitors the fishery. Such as the Food Standards Agency in England and Wales conducts weekly investigation of fisheries for determining phycotoxins levels in shellfish and algae (Food Standards Agency 2015). These monitoring systems allow control authorities to inform producers of the risk of unacceptable contamination and the possibility of closing the fishery if the limits are exceeded. Among other risk management measures, it is recommended that consumers avoid or limit fish consumption and avoid fish body parts like liver, eggs and head, intestines, known to have a particularly high rate of ciguatoxins (Ansdel 2014). For microcystins in drinking water, WHO has proposed an indicative limit of 1 μg/L (World Health Organization 2011). Guidance on the management and elimination of toxin in drinking water sources is also available (US Environmental Protection Agency 2014).

Mycotoxins

The toxic metabolite produced by some fungi like molds, that infect and multiply in agricultural products (eg. corn and wheat, fruits, peanuts and nuts) in the field and during storage are defined as mycotoxins. Mycotoxins include aflatoxin, fumonisin, deoxynivalenol (vomitoxin), ochratoxin and patulin. They can produce several toxicological effects. Some mycotoxins are mutagenic, carcinogenic or teratogenic, in susceptible animal species and are known for causing different diseases in livestock and humans. Their occurrence in human and animal foods is inevitable. Environmental factors like temperature, humidity and rainfall during the pre-harvest, harvest and post-harvest periods play an important role in their occurrence in food products. Mycotoxins are contaminating about 25% of world's agricultural production (Charmley et al. 1995) and these are the widely produced and controlled food contaminants in the world (Van Egmond and Jonker 2004). With regard to their toxic and other biological effects, the aflatoxins are very interesting compounds. Acute or subacute poisoning resulted in animals by feeding aflatoxin-contaminated diets or by treating with purified preparations of toxins. Levels of Aflatoxin in the feed to 10–100 ppm or less resulted in poisoning in most domestic animals. Although cattle tolerate relatively high levels of the toxin, they secrete in milk aflatoxin M_1, a derivative that is also toxic. Aflatoxin B_1 is among the most potent chemical carcinogens known, and it is this property that has provided an important stimulus for research on these mycotoxins. The presence of these toxigenic molds in human foods presents an obvious potential risk to public health, which provides strong motivation for implementation of all available techniques for minimizing contamination of foods by mycotoxins.

Based on the strategy of prevention concept the codes of practice were developed (Battaglia et al. 1996). Both internationally and nationally the codes of practice include: patulin in apple juice and related products (Codex Alimentarius Commission

2003a); mycotoxins in cereals (Codex Alimentarius Commission 2003b), aflatoxins in peanuts (Codex Alimentarius Commission 2004) and nuts (Codex Alimentarius Commission 2005a) and ochratoxin A in wine (Codex Alimentarius Commission 2005b) and coffee (Codex Alimentarius Commission 2009).

Environmental Contaminants

Heavy Metals

Heavy metals including lead, cadmium, arsenic and mercury, may be of concern in some foods due to agricultural practices (use of pesticides containing heavy metals etc.), industrial waste or the leaching of the heavy metals contained in equipment, containers or utensils in contact with food. Their toxicity depends on their molecular form such as inorganic arsenic is more toxic than the organic form (European Food Safety Authority 2009); while organo-mercury compounds are known for more toxicity (European Food Safety Authority 2012). The consumption of foods contaminated with heavy metals have adverse health consequences. Exposure to lead can hinder cognitive development in children (FDA 2006). Inorganic arsenic consumption resulted in skin lesions, cancer, cardiovascular disease, developmental effects, neurotoxicity and diabetes in humans (JEFCA 2010). Bandara et al. (2010) suggested that rice contaminated with cadmium is due to the use of fertilizers. The dietary exposure to arsenic is related with contamination of groundwater with high levels of arsenic. Sources of human exposure are thus drinking contaminated water or consuming contaminated crops irrigated with groundwater (World Health Organization 2012). Permissible limits of some heavy metals in food and food products according to Food Safety and Standards (Contaminants, toxins and Residues) Regulations, 2011 are given in Table 2.

The adoption of stricter environmental protection regulations will result in reduction in levels of heavy metals in the environment which also resulted in simultaneous decline in the concentrations of heavy metals in plant based foods (Kabata-Pendias and Mukherjee 2007). When a heavy metal that requires preventive control is identified by hazard analysis, the type of control depends on entrance of heavy metal in the food product. If the food product contains a food crop contaminated with heavy metal through contaminated soil, a preventive control such as a supply chain control with a verification program to ensure that the grower performs an evaluation of the growing area before the use of it for agriculture may be appropriate.

Table 2 Acceptable limits of some heavy metals in foods

Name of the chemical contaminant	Description of food	Maximum permitted concentration in parts per million
Lead	Edible oils and fats	0.5
	Infant milk substitute and infant food	0.2
	Fruit and vegetable juices	1
	Corned beef, cooked ham, luncheon meat, chopped meat, canned chicken, canned mutton and goat meat	2.5
	Berries and other small fruits	0.2
	Citrus fruits/pome fruits/stone fruits	0.1
	Fruiting vegetables	0.1
	Legume vegetables	0.2
	Pulses	0.2
	Poultry meat	0.1
	Fish	0.3
Copper	Infant milk substitute and infant food	15
	Juice of grape, orange, tomato, apple, lemon and pineapple	5
	Tea	150
	Pulp and pulp products of any fruit	5
	Foods not specified	30
Arsenic	Milk	0.1
	Infant milk substitute and infant food	0.05
	Juice of grape, orange, tomato, apple, lemon and pineapple	0.2
	Pulp and pulp products of any fruit	0.2
	Fish and crustaceans	76
	Foods not specified	1.1
Cadmium	Infant milk substitute and infant food	0.1
	Fruiting vegetables	0.05
	Leafy vegetables	0.2
	Legume vegetables	0.1
	Pulses	0.1
	Rice, polished	0.4
	Wheat	0.2
	Fish	0.3
Mercury	Fish	0.5
	Other foods	1
	Non-predatory fish, crustaceans, cephalopods, molluscs	0.5
	Predatory fish (tuna, marlin, sword fish, elasmobranch)	1
Methyl mercury	All foods	0.25
Chromium	Cereals and vegetables	1
	Meat of animal and poultry	1
	All fishery products	1

Agrochemical Residues

Pesticide Residues

Presence of pesticide residues is of concern in food crops and in foods of animal origin (due to pesticide residues in animal feed). "The term pesticide is used for products such as insecticides, fungicides, rodenticides, insect repellents, herbicides or weed killers, and some antimicrobials intended to prevent, destroy, repel or reduce all types of pests" (EPA 2015). Bioaccumulation of some pesticides can also occur via food chain, such as in eggs (Holmes et al., 1969) and milk (Salas et al. 2003). Food products adulteration with pesticide residues happens through improper handling of raw materials containing registered pesticides and raw materials exposed to banned pesticides.

For regulation of pesticides three federal agencies have share the responsibilities. Those pesticides that have been approved by the US Environmental Protection Agency (EPA) can be applied directly to crops according to the labelling instructions. The EPA sets a tolerance, i.e. the maximum amount of pesticide residues allowed in or on a food for registered pesticide. The responsibility of FDA is to implement pesticide tolerances for foods other than meat, poultry and certain egg products, which is the responsibility of the US Department of Agriculture Food Safety and Inspection Service (USDA FSIS) (FDA 2012). Regulations such as EU Regulation 396/2005 (European Parliament and Council 2005) postulate that which pesticides may be applied to which crops and what residual quantities are authorized in food stuffs anticipated for human consumption. Given the variety of possible combinations of pesticide crops and pesticides, these are supported by a database to facilitate regulatory agreement (European Commission 2015). The risk of pesticide use is reduced partly by advising the accountable makers of pesticides to the end-users. It specifies the plants for which the pesticide may be used, and suggested rates of application.

Animal Drug Residues

Drug residues in animals may be a problem for foods of animal origin (muscle meat, organ meat, fat/skin, eggs, honey and milk). These occur after the giving medicines to livestock. Medications are commonly administered for any of these reasons: therapeutic (for curing animal from a particular disease), prophylactic (to prevent the contraction of a certain disease) or directly for growth promotion. The first danger to the consumer is the inadvertent use of pharmacologically active residues with possible harm. Depending on the chemical properties of the drug, residues of certain drugs may concentrate during food preparation and processing. For example, if a fat soluble, heat stable drug residue is present in raw milk, the drug may concentrate when the milk is converted to full fat cheese (Cerkvenik et al. 2004; Imperiale et al. 2004). An example of unauthorized drug residue that adulterated foods is fluo-

roquinolone, an antibiotic that is not approved for use in honeybees in the US and has been detected in honey products from certain non-US regions (FDA 2015).

Issues related to the risk management of veterinary medicines generally indicate one or more of the following: failure in best practice; imports of meat inclosing drug residues authorized in the exporting country but not in the importing country; or the use of unauthorized medicines to gain an unfair economical profit.

Legal regulations have been made in many countries that require veterinary medicines to be allowed only by the European Union, the European Medicines Agency (such as the European Parliament and Council 2009) and that their residues in food should not surpass the levels of special regulation. In some jurisdictions, usage of some drugs in all or in some animal groups is strictly prohibited. The EU has prepared a list of banned drugs that do not have a maximum residual limit (MRL) (European Commission 2010).

Food Process Toxicants

Food process toxicants are chemical hazards that arise directly during food processing (Lineback and Stadler 2009). Generally, these arise in consequence of chemical reactions that occur due to thermal processing or activities of microbes occurring in processing and/or storage (e.g. histamine in fish and ethyl carbamate in fermented products). Some of them also occur as environmental pollutants like polycyclic aromatic hydrocarbons (PAHs) and if occur either in feed or drinking water, they can be bioaccumulated in substantial amounts in meat, fish, and other animal products (eggs, milk).

The heat usage in the making process is additional cause of contamination. Usage of high temperatures during cooking in households and in industry is the commonly used process for the processing of food. The practice of a high cooking temperature coupled with external factors may result in the toxic compounds formation which affect food safety and quality. Harmful compounds like acrylamide, nitrosamines, chlorophenols, PAHs, furans are formed during processing food operations such as roasting, heating, grilling, baking, preserving, fermenting or hydrolysing (Nerín et al. 2016). Deep frying is a major basis for the production of number of toxic compounds in food manufacturing processes (Roccato et al. 2015). These thermally-made compounds occur due to two general mechanisms. Firstly, the partial burning of cooking fuel (PAHs), during smoking (Gomaa et al. 1993), or in domestic cooking, for example, the burning of fuel during grilling (Lijinsky and Ross 1967). Second mechanism is the result of chemical reactions that occur during cooking process. Examples include chloropropanols, acrylamide and heterocyclic aromatic amines.

The risk management of food process toxicants is partly based on their chemistry of formation and the impact of processing on these reactions. Some combinations of chemicals and food have legal limits. For example, limits have been set in the European Union for the occurrence of chloropropanol-3-monochloropropane-1,2-

diol (3-MCPD) in hydrolysed vegetable protein and soya sauce; and dioxins in animal origin products (eggs and milk, meat) and fish origin (European Commission 2006). Similarly, there are legal limits on the existence of histamine in fish products (European Commission 2013) and certain heterocyclic aromatic amines in meat flavors (European Parliament and Council 2008),

Another example of food process toxicants is of acrylamide. Important sources of acrylamide in the diet are high carbohydrate contented foods (coffee, baked pastries and fried potato products) produced at high temperatures (Svensson et al. 2003). For various foods of potential high-risk, extensive research helped in the identification of mitigation measures. One such measure is the "Acrylamide Toolbox" (Food Drink Europe 2014). The toolbox works on the ALARP principle. It addresses issues of how precursors can be formed or removed earlier for processing, also processing alternatives to lessen formation of acrylamide.

Intentionally Added Chemicals

Food Additives

Food additives and nutrients have been added to foods since prehistoric times. Although staple foods do not contain additives as food are processed to be converted into a variety of products, more and more additives usually are used. With the technical advancement in food processing many different additives are used. For achieving the desired effect more than 3000 diverse additives are deliberately added to foods. A food additive is a substance whose anticipated usage causes it to be incorporated into the food or affect the food properties. The additives usually offer some profit to the food manufacturers, processors or consumers. For consumers, additives can enhance the organoleptic properties of foods, increase their nutritional value otherwise facilitate the preparation of ingredients and meals. For the food manufacturer or processor these additives improve product quality, variety safety and shelf life.

Additives can occur in different amounts in foods, play various tasks in foods and work synergistically with other additives. Important functions of these additives are (a) conserving or improving nutritional quality, (b) maintaining or improving product safety or quality, (c) assisting in preparation and processing (d) improving sensory properties (FDA 1979, 1992). Additives affecting nutrient value are mainly minerals and vitamins. Sometimes they are added for enriching foods or replacing the nutritional loss occurred during processing. For some foods, minerals and vitamins are added to fortify them for supplementing nutrients which are missing in the diet. To prevent growth of bacteria and fungi in food, antimicrobials or preservatives are used. The additives may delay spoilage or prolong the shelf life of foods by suspending lipid oxidation or rancidity. Additives used as processing or cooking aids generally affect the texture of ingredients and ready meals. Some of them are categorized as stabilizers, emulsifiers, thickeners, blowing agents, and anti-caking

agents. The fourth main role of these is improving color or taste of foods in order to make them attractive. Natural and artificial colors are used to enhance the optical food appeal, to differentiate the taste of foods, to increase natural color intensity, or to restore color loss.

Direct toxicological effects of additives are of great concern. Short-term acute effects from additives are unlikely. A small number of additives are used at levels that result in direct toxicological impact. Some additives even used at acceptable limits may have severe effect on sensitive individuals. The reactions to sulfites and other additives, are examples of such a problem. With proper labeling, however, sensitive individuals should be able to avoid potential allergens. Cancer and reproductive problems are not reported in humans. However, some animal studies showed potential problems of some additives.

For ensuring the safety and efficiency of food additives many federal agencies, laws and regulations working together. The Food, Drug and Cosmetic Act of 1938 gives the US Food and Drug administration the power to regulate food, ingredients and their labelling. The amendment to the FD & C Act of 1958 on food additives requires FDA consent for the usage of new additives before their addition in food. This amendment also involves the additives manufacturer to demonstrate the safety of an additive for the recommended use until 1958 (e.g. Sodium nitrite for meat preservation).

Amount of food and color additives may be limited by Current Good Manufacturing Practice (CGMP) regulations. The Codex Alimentarius Commission Committee on Food Additives and Contaminants has established an international numbering system (INS) for food additives based on the E system (Codex Alimentarius Commission 2001). The INS system is planned as an identification system for food additives permitted for use in one or more countries.

Nutrient Additives

Recently there is increase in the use of nutrient additives. Grains and cereal products are often supplemented with vitamins for restoring the nutritional loss during processing or to increase the overall nutritional value. Minerals like iodine and iron are extremely important to prevent malnutrition. Like vitamins, minerals are mainly used in cereal products. In foods, amino acids and proteinaceous materials are not usually used, but to improve protein quality lysine sometimes added to grain.

Several processed foods have additives and/or nutritional additives (vitamins and minerals). Their usage is commonly governed by laws that vary according to jurisdiction. Restriction on the use of food additives may exist to avoid deception or for safety reasons. Sometimes food additives may be pharmacologically active when consumed in enough amounts. Such as the laxative effect of polyols and the adverse effect of aspartame (a source of phenylalanine) on persons suffering from phenylketoneurea. Therefore, foods containing them may need to contain cautions for consumers (European Parliament and Council 2008); minerals and vitamins also pose safety concerns, as the Paracelsus principle also applies. Vitamin-associated dis-

eases are usually related with deficiency (e.g. scurvy); excessive intake of minerals or vitamins may also result in health problems. Therefore, there are indications as to which tolerable upper limits could apply to intake (European Food Safety Authority 2006).

Conclusions

Food contamination by chemicals is a serious issue. Generally, food contamination happens through environmental pollutants and naturally occurring toxins or during food processing and preparation. With the advancement in technology, these contaminants are easily detectable. But still there are many unknown contaminants hence research is required. For minimizing the individual exposure to food contaminants, the governments have taken suitable steps and measures are still needed to decrease the health risks related with chemical food contamination. Risk management of chemical hazards includes two objectives: (a) food production that ensures chemical contaminants are regulated (b) when contamination is unavoidable, the contamination level should not only be safe but also follow ALARA. Effective risk management of chemical hazards involves consideration of Paracelsus' dictum for ensuring suitable limits be set, a complete knowledge of farm-to-fork scale, together with the suitable tools for responding to an ever-changing environment.

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Assessment of the Risk of Probiotics in Terms of the Food Safety and Human Health

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Abstract Probiotics are often referred to as microorganisms (bacteria or yeasts) that generally provide health benefits. There is great interest in probiotics for various medical reasons and millions of people around the world consume probiotic microorganisms daily with the perception that it is beneficial for health. Members of the *genus Lactococcus* and *Lactobacillus, Streptococcus, Enterococcus* strains, and some other LAB strains are generally accepted as safe (GRAS) status, although they contain some opportunistic pathogens. In addition, some of the spore forming bacteria have been researched and used as probiotics. However, nowadays theoretical concerns and side effects are discussed with regard to the safety of probiotics. Systemic infections, the risk of harmful metabolic activities, risk of adjuvant side effects, immunomodulation and gene transfer risk are among the theoretical concerns discussed. The most common side effects of probiotic microorganisms include gastrointestinal disorders such as nausea, diarrhea, bloating, abdominal pain and dyspepsia. Other side effects include respiratory tract infections, abscess, allergic reactions and severe medical conditions such as sepsis, endocarditis and fungemia. The safety of probiotics is related to the potential vulnerability of the consumer or the patient, the dose of use, duration of consumption and the frequency of consumption. The significance of negative probiotic effects will be better understood by understanding of the probiotic interaction mechanisms with host and colonizing microbes. In this chapter, the evaluation of the risk associated with the consumption of probiotic products has been discussed, based on epidemiological data and infected cases.

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Introduction

The term "probiotic" originates from the Greek words "pro" and "bio" and it means "for life". It was defined first by Lilly and Stillwell in 1965 as "the metabolites that are secreted by a microorganism and helps in the development of another living organism". In 1971, Sperti used this term for the tissue extracts contributing to the microbial reproduction (Yiğit 2009; Ebners et al. 2014). The most recent meaning of probiotics was used firstly in 1974 by Parker and it was defined as the microorganisms and metabolites protecting the intestinal microbial balance. Removing the term "metabolites" from the definition made by Parker, the current definition of the probiotic is obtained (Lee and Salminen 2009). According to Guarner and Schaafsma, the probiotics are the living microorganisms positively contributing to the health when taken at sufficient amount together with the probiotic foods, in addition to the nourishment (Guarner and Schaafsma 1998). The probiotics can be used as national support both in a healthy development and in the treatment of diseases. These bacteria compete against the harmful microorganisms for the nutrients and colonize on the intestinal surface. Besides that, they also positively contribute to the activities in the gastrointestinal system and the health. Moreover, in research of Metchnikoff carried out between 1845 and 1916, it is known that the probiotics have positive effects on the health by ensuring the microbial ecosystem balance in intestines (Metchnikoff 2004). The probiotics are defined as the living microorganisms positively contributing to the host's health when orally taken at sufficient amount (Özden 2005).

Some strains of *Lactobacillus* spp. (*Lactobacillus acidophilus*, *L. casei*, *L. brevis*, *L. bulgaricus*, *L. cellebiosus*, *L. delbrueckii*, *L. johnsonii*, *L. lactis*, *L. reuteri*, *L. rhamnosus*, *L. plantarum*, *L. fermentum*, *L. helveticus*, *L. curvatus*, *L. salivarius*, *L. gasseri*), *Bifidobacterium* spp. (*Bifidobacterium adolescentis*, *B. bifidum*, *B. breve*, *B. longum*, *B. infantis*, *B. thermophilum*), *Pediococcus* spp. (*P. cerevisiae*, *P. acidilactici*, *P. pentosaceus*), *Streptococcus* spp. (*S. cremoris*, *S. thermophilus*, *S. intermedius*, *S. lactis*, *S. diacetilactis*), *Bacillus* spp. (*B. subtilis*, *B. pumilus*, *B. lentus*, *B. licheniformis*, *B. coagulans*, *B. cereus*), *Bacteroides* spp. (*B. capillus*, *B. suis*, *B. ruminicola*, *B. amylophilus*), *Propionibacterium* spp. (*P. shermanii*, *P. freudenreichii*), *Leuconostoc* spp. (*L. mesenteroides*), some yeasts (*Saccharomyces cerevisiae*, *S. boulardii*, *Candida torulopsis*) and molds (*Aspergillus niger*, *A. oryzae*) are used in probiotic foodstuffs (Bozkurt and Aslım 2004; Toprak Kavas 2007).

The prebiotics are the nutrients improving the host health by positively contributing to the reproduction of gastrointestinal system bacteria and passing directly to the large intestines without being digested (Ceyhan and Alıç 2012). Some of the compounds having prebiotic character are inulin, fructo-oligosaccharides, lactulose, galacto-oligosaccharides, soya oligosaccharides, gluco-oligosaccharides and isomalto-oligosaccharides. Many plants synthesize the inulin. Onion, garlic, wheat, leek, and banana contain inulin (Gülmez and Güven 2002). The breast milk contains more than 130 sorts of oligosaccharides (Çoşkun 2006). The symbiotic is the com-

bined form of probiotics and prebiotics. The postbiotics are the biologically active by-products of the probiotic cultures and they are the materials such as short-chain fatty acid, which have positive effects on the health when added to the nutrients (Ceyhan and Alıç 2012).

The probiotic bacteria are of Gram-positive, asporogenic, and basil form, and they develop at 35–38 °C temperature and pH range of 5.5–6.0. *L. acidophilus* is an anaerobic or facultative anaerobic bacterium. The optimum temperature for the bifidobacteria is 37–43 °C, whereas the pH range is 6.5–7.0. Their development slows when ambient pH decreases below 4.5–5 or increases above 8–8.5. The bifidobacteria transform glucose into acetic acid and lactic acid. Thus, they are heterofermentative. An enzyme of this special mechanism, which is fructose-6-phosphate phosphoketolase (F6PKK), is routinely used in distinguishing the bifidobacteria from the other microorganisms (Ceyhan and Alıç 2012).

The probiotic bacteria can resist to the gastric acidity more than the other bacteria can. It is more resistant to the bile salt and lysozyme enzyme. The probiotic bacteria control the reproduction rate of the undesired bacteria in the intestines by producing antimicrobial materials such as lactic acid, acetic acid, and bacteriocin (Ceyhan and Alıç 2012).

Moreover, the expected characteristics of microorganism used as probiotic are as follows:

- It must be from the intestinal system microflora of a normal human.
- It must be easily metabolized in the intestinal system without being affected by the negative environmental factors such as low pH and bile salts.
- It must be able to live on intestinal epithelial cell surfaces and it must be able to colonize.
- The number of living cells on the intestinal surface must be at a high level.
- It must be capable of maintaining its vitality and activity during the production and storage.
- It must be capable of stimulating the immune system of the host.
- It must be safe and it must have no adverse effect,
- It must be capable of negatively influencing the carcinogenic and pathogenic bacteria.
- It must produce antimicrobial material.
- It has to have no pathogenic feature (Timmerman et al. 2004; Friedman 2005; Gönülateş 2008).

The retention of the probiotic bacteria on the epithelial and mucosal surfaces of the gastrointestinal system was reported to be the most important and essential characteristic in order for them to have a biological effect (Sağdıç et al. 2004). In order for the probiotics taken from the nutrients to show the expected benefit, they must resist to the bile salts and gastric acidity and bile salts, reach at the gastrointestinal system in living form, and they must be capable of living on and colonizing the epithelial cell surfaces of the gastrointestinal mucosa (Otles and Cagindi 2003). The food biotechnology aims to investigate and improve the tolerance of probiotics against the acidity and bile salts, as well as their capacity to retain on the intestinal

surface, their proteolytic characteristics, and their capacity of secreting lactic acid. These characteristics are also the most important characteristics of the probiotics (Maragkoudakis et al. 2006; Ranadheera et al. 2014).

Nowadays, the probiotics became very popular because of their protective effects against the diseases. As the probiotics become more popular, the studies on developing new probiotic products also gained speed. In many countries, the innovative probiotic products developed as a result of biotechnological studies compete with each other in order to dominate the biotechnology market. In the studies on probiotic market and consumption, it was reported that the 28 million USD has been spent in the USA in 2011 (Tall 2016). Yogurt, yogurt derived products, dairy based milks, dietary supplements are the nutrients having probiotic properties and being popularly consumed nowadays. *Lactobacillus* spp., (*L. johnsonii*, *L. paracasei*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. rhamnosus*) GG, *Bifidobacterium* spp. (*B. bifidum*, *B. animalis*, and *B. longum*), *S. thermophilus* are most common microorganisms found in the yogurt and yogurt-derived products (milk or soy based). Predominantly single strains, such as *L. casei* Shirota and *L. lactis* are commonly found in dairy-based drinks. Same as yogurt, generally single strains found in cheese, buttermilk and other dairy products. Same as yogurts, frequently single strains are found in non-dairy products such as fermented cereals, fruit drinks and raw sausages. Single strains of various species are found in dietary supplements (tablets, powders, drops an capsules) usually in combinations with vitamins. Single strains, such as *Escherichia coli* Nissle, *Enterococcus faecalis*, *S. boulardii* and *L. rhamnosus* GG are most common microorganisms found in medicine products used for human (Wassenaar and Klein 2008). Which bacteria can be used as probiotic was defined by FDA (Food and Drug Admiration) as GRAS (generally recognized as safe). However, the sensitivity to or resistance against the antibiotics is an important selection criterion for selecting the probiotic bacteria (Gismondo et al. 1999; Saarela et al. 2000; Yuksekdag and Aslim 2010). Wide and unconscious use of antibiotics caused the emergence of antibiotic-resistant microorganisms. Moreover, the potential of transferring the genes of antibiotic resistance from different microorganisms to especially the pathogenic bacteria is a very important health problem of today and the antibiotic resistance genes became an important risk factor. Besides that, the increasing use of antibiotics caused the development of resistance to the antibiotics used for the human and the erythromycin and tetracycline resistances were observed among the lactobacillus bacteria (Özteber 2013). It was reported that the antibiotic resistance of probiotic bacteria and the propagation of antibiotic resistance genes is an significant point that must be investigated for the reliability of these bacteria. As well as it can be a natural characteristic of the bacteria, the antibiotic resistance can also develop as a result of mutations or gene transfer. The possibility of the transfer of natural or mutant genes of antibiotic resistance is very low. Besides that, despite the high possibility of the resistance acquired due to a genetic mutation or a DNA transfer from the other bacteria and despite that these transferred genes are not solely a cause of disease, there also are risks such as the increase in the disease rates, the prolongation of the processes, and the increase in mortality rates together with the increasing antibiotic resistance of pathogen bacteria (Ammor

et al. 2007). In many studies, it was reported that the significant increase in the consumption of probiotic products and traditional fermented foods brought the risk of the antibiotic resistance gene transfer from the probiotic bacteria to the pathogenic ones and it may cause health problems among the people (Saarela et al. 2000).

In other words, according to the criteria set by FAO/WHO (2002), the resistance gained against the antibiotics and the propagation of these antibiotic resistance genes pose an important problem in terms of the reliability of these bacteria (Herreros et al. 2005; Yuksekdag and Aslim 2010; Muñoz-Atienza et al. 2013). For this reason, the transferrable genes of antibiotic resistance must be controlled from the aspect of the resistance of antibiotics due to the potential of transfer from probiotic bacteria to pathogenic ones (Ammor et al. 2007; Dewan and Tamang 2007; Ouoba et al. 2008). Metabolic activity (Biogenic amine production, bile salt 7-hydroxylased D- vs. L-Lactate production, mucin degradation, cholyl glycine hydrolase, bile salt 7-hydroxylased azoreductase, nitro reductase, β-glucuronidase, β-glucosidase, N-nitrosation), infectivity (Platelet aggregation, adhesion, haemolysis, aggregation of erythrocytes, epidemiology, lethally irradiated mice), gene transfer (Plasmid transfer) and immune functions (Phagocytosis, congenital immune deficient mice) of probiotic bacteria are the possible safety assessment criteria (O'Brien et al. 1999).

According to a Report of 2002 published by Food and Agriculture Organization (FAO) and World Health Organization (WHO) of United Nations, "the probiotics can be held responsible theoretically for four types of adverse effects:

1. Systemic infections.
2. Harmful activities of metabolism.
3. Over stimulation of immunity among the sensitive individuals.
4. Gene transportation"

Moreover, the small gastrointestinal syndromes were also documented.

WHO/FAO study group mentioned that the toxin production, antibiotic resistance, and metabolic activities such as hemolytic potential, synthesis of D-lactate and deconjugation of bile salts should be tested, that the human studies should be maintained in order to evaluate the adverse effects and the surveillance of commercial consumers, that the contagiousness should be examined by using the probiotic organism on the animals with ideally suppressed immune system, and that the novel probiotic microorganisms should be analyzed from the aspect of reliability.

Besides the antibiotic resistance, there also are theoretical concerns about the safety and adverse effects of probiotics. The systemic infections caused by the probiotic microorganisms, the harmful metabolic activity risks, adjuvant adverse effect risk, immunomodulation risk, and gene transfer risk are some of the subjects that are theoretically discussed. The importance of these negative probiotic effects would be better understood when the mechanisms of the interaction between host and colonizing microorganisms are better understood. In this chapter, by making use of the epidemiological data and contagious cases, it was aimed to evaluation of the safety of the probiotic product consumption.

Theoretical Adverse Effects of the Use of Probiotics

There are some negative theoretical concerns about the use of probiotics by the humans (Salminen et al. 1998; Ishibashi and Yamazaki 2001; Reid 2002; Clancy 2003; Henriksson et al. 2005; Senok et al. 2005; Boyle et al. 2006). They have negative effects on the translocation potential, colonization of probiotics, metabolic/physiologic effects, and gastrointestinal physiology and function (Saarela et al. 2000; Henriksson et al. 2005; Senok et al. 2005). Moreover, the local and propagating undesired immunologic effects, the possibility of the transfer of antibiotic-resistance gene from the commensal/probiotic bacteria to the other ones and potential pathogens within the gastrointestinal system, and the potential of this antibiotic-resistance gene to the bacteria and potential pathogens are also among these theoretical adverse effects (Saarela et al. 2000; Ishibashi and Yamazaki 2001; Salyers et al. 2004; Senok et al. 2005).

Translocation Potential

The indigenous bacteria normally do not exist in the spleen, mesenteric lymph nodes or the blood of healthful animals. The immune system of the host annihilates these indigenous bacteria, which translocate through the mucosal epithelial tissue. For this reason, most of the studies, in which the probiotics were applied to the healthy subjects at high doses, showed that the probiotic translocation did not occur. In fact, even while translocating from the gastrointestinal system, the probiotics infrequently promote severe diseases among the healthful subjects. The observational reports presented in Lactic Acid Bacteria Industries Platform (LABIP) organized by the European Union showed that, except for the enterococcus, the risk of general infection arising from the lactic acid bacteria (LAB) is quite low (Adams and Marteau 1995). This observation was supported with various safety assessments performed for the probiotics consumed as food supplements. Making use of a rat model, Huang et al. (2003) studied the reliability of the application of *Propionibacterium jensenii* 702, which is a new probiotic, for a healthy human. The researchers determined that, at the doses as high as 10^{10} CFU/rat/day, live cells of *P. jensenii* 702 was not acquired from the blood, mesenteric lymph nodes, spleen or liver of the rats, hence no disease- or treatment-related mortality was observed. These results were supported by another study reporting that, when the dose of 10^{11} CFU/rat/day corresponding to 50 g/kg/day for rats, the translocation of the LAB did not occur. These lactic acid amounts are much higher than the amounts normally consumed by the people, and this suggests that the strains examined here are safe for human health (Zhou et al. 2000a, b). Likewise, Shu et al. (1999) applied 5×10^{10} CFU/rat/day dose and reported that there was no *Lactobacillus* or bifidobacteria in the spleen and kidneys of the healthy rats. This

dose is much higher than the regular daily intake and it corresponds to the dose 10^9–10^{11} CFU/day recommended for a human weighing 75 kg. Under the light of data they obtained, the researchers claimed that the use of probiotics among the humans might be safe.

Zhou et al. carried out a comprehensive study on the probiotic translocation (Zhou et al. 2000a, b). In that study, the researchers applied *Lactobacillus* and bifidobacteria at a dose as high as 10^{12} CFU/kg body weight/day to the healthy mice. *B. lactis*, *L. acidophilus* and *L. rhamnosus* strains are given to the mice together with their meal during 4 consecutive weeks. According to the study results, it was determined that these probiotics had no negative effect on the hematology and blood chemistry of those mice and on the intestinal mucosal histology. In a different study made by Asahara et al. (2003), probiotics are evaluated like commensal microorganisms. They transferred seven different probiotic microorganisms to Japanese rabbits (male) having no specific pathogen infection. The researchers determined that one strain of *L. casei* and four strains of the *L. rhamnosus* colonized and developed in liver and spleen at 10^2–10^4 CFU/g and 10^8–10^9 CFU/g concentrations, respectively. Besides that, no rabbit observed to have colonization even after 14 days did die. Moreover, the authors did not report the *L. gasseri* DSM 20243, *L. casei* Shirota and *L. acidophilus* ATCC 4356 infectivity.

Despite the reports stating that there was no probiotic translocation in healthful subjects, the enterococci or lactobacilli or were defined as the most common species settling in the mesenteric lymph nodes of the healthful mice having no pathogenic infection (Berg and Garlington 1979). The reason for frequent translocations of lactobacilli and enterococci is that they normally show colonization at high population levels in the gastrointestinal system. Rodriguez et al. (2001) reported that, in case of the healthful mice were given 2×10^9 living *L. rhamnosus* suspension orally on daily basis. Seven days after, the living bacteria were detected in spleen and liver of the mice. In that study, isolated bacterium colonies from liver, biochemically characterized. Then they were exposed to casually reproduced polymorphic DNA, the amplification patterns of five strains showed similar characteristics with *L. rhamnosus* ATCC 7469. Similarly, Perdigon et al. (1997) determined the *L. rhamnosus* translocation after giving to the healthful mice. The researchers reported that the translocation was induced 2 days after the application of the dose of 10^{11} CFU/day/mouse.

Despite these findings, in many of the studies carried out on the healthy subjects, no strict infection induced by the probiotic microorganism was reported even if they translocated from the intestinal system. The reason is still unknown but there are several theories on this subject. One of these theories is that the probiotic microorganisms are much more sensitive to intracellular killing by the macrophages after the translocation because the phagocytes are known as effect protectively after the initiation of infective endocarditis induced by the Gram-positive bacterium (Duffy 2000; Veltrop et al. 2000).

Bacteremia and Endocarditis Potential

It is known that the LAB containing the *Bifidobacterium* spp. are isolated as the promoters of endocarditis and bacteremia (Kalima et al. 1996; Oggioni et al. 1998; Kunz et al. 2004; Cannon et al. 2005; De Groote et al. 2005; Henriksson et al. 2005). Some of the endocarditis- or bacteremia-related organisms are *L. plantarum*, *L. rhamnosus*, *L. casei*, *L. paracasei*, *L. acidophilus*, *L. salivarius* and many other *Lactobacillus* species (Cannon et al. 2005). Moreover, in addition to the *Leuconostoc* and *L. lactis*, also the *Pediococcus* spp. were found to cause endocarditis and bacteremia. Some of *Bifidobacterium* spp. were also isolated from the blood and from the endocarditis patients (Spinosa et al. 2000). *Enterococcus* species were well-known to cause the bacteremia and endocarditis (Vergis et al. 2001).

Given the sepsis cases related with probiotics, it was determined that there were three children with short bowel syndrome having bacteremia due to *Lactobacillus* GG, one central endocarditis case, two endocarditis cases, and one liver abscess case with bacteremia related with *Lactobacillus* GG (Kalima et al. 1996; Oggioni et al. 1998; Rautio et al. 1999; De Groote et al. 2005; Salminen 2006). Moreover, there also was an endocarditis case caused by the *L. rhamnosus* strain, subcharacteristics of which have not been exactly determined. Five bacteremia cases were reported to be related with *Bacillus subtilis* (Richard et al. 1988). Moreover, in a patient with Hodgkin disease and HIV infection, the *L. acidophilus* bacteremia and the *Lactobacillus* infection after the bone marrow transplantation were detected (Kalima et al. 1996).

Lactobacillus GG bacteremia cases were detected also in patients with short gout disease (Kunz et al. 2004; De Groote et al. 2005; Land et al. 2005). All the cases are defined with the central venous catheters and the bowel feeding tube. Two out of four isolated strains are proven to be *Lactobacillus* GG by using PFGE and one of four strains was proven to be *Lactobacillus* GG by using both of PFGE and PCR. Only one isolate was not proven as *Lactobacillus* GG specifically. Central venous catheter infections were detected in two of four cases and the positive catheter culture results were obtained. These studies emphasize the *Lactobacillus* GG bacteremia risk related with the short bowel syndrome. The origin of these organisms is thought to be the central venous catheters contamination throughout the handling, particularly along feeding.

The surveillance information of Finland did not show expand in *Lactobacillus* bacteremia between 1990 and 2000 (Salminen et al. 2002). In this period, the *Lactobacillus* species constituted 0.02% of all the positive blood cultures. In another study carried out in National Public Health Laboratory, it was determined that the *Lactobacillus* was found in 0.24% of all the positive blood cultures referred to the laboratory (Salminen et al. 2004). Although these cultures were reported to be *Lactobacillus*, only 27% of them were proven. In these analyses, it was determined that *Lactobacillus* GG constituted 11 of 26 *L. rhamnosus* strains isolated from blood. *L. rhamnosus* constituted 54% of all the isolated *Lactobacillus*. Given the fact that *Lactobacillus* GG intake in Finland during the working period increased

from 1 to 6 L/person/year, it is attention-grabbing that there was no change in the prevalence of *Lactobacillus* bacteremia, especially in the prevalence of *Lactobacillus* GG, in the last decade (Salminen et al. 2002).

The *Lactobacillus* bacteremia in Sweden was examined for 6 years and it was determined that three probiotic microorganisms were entered into clinical use in this period (Sullivan and Nord 2006). The probiotics that were examined were *Lactobacillus* GG, *L. paracasei* and *L. acidophilus* NCFB 1478. The researchers defined that most of the lactic acid bacteremia cases were polymicrobial in reality.

Besides that, there also are probiotic-related sepsis cases. The most significant ones were related to *S. boulardii* (Bassetti et al. 1998; Hennequin et al. 2000; Perapoch et al. 2000; Lherm et al. 2002; Cassone et al. 2003). In their study carried out on 23 patients, 16 candidemia cases were detected. Some of these patients experienced septic shock. The molecular diagnosis and the confirmation of probiotic strain could be performed to a certain level in many of the cases (Fredenucci et al. 1998; Cesaro et al. 2000).

Gastrointestinal Toxicity Studies

When the possible effect of the probiotic microorganism use on the intestinal physiology is examined, it can be seen that the reproduction of undesired metabolites is possible especially for the patients with the small intestine syndrome (Marteau et al. 1990). It is claimed that the probiotic bacteria are theoretically risky because the deconjugation of bile salts cause the malabsorption and, thus, they might extend the colon cancer risk. Besides that, there is no clinic or epidemiological proof supporting that hypothesis. There also are theoretical information showing that the probiotics have an inhibiting effect on the colon cancer in animals (Snydman 2008).

Among the additional toxicity potency, it is also theoretically possible that the development of lactic acidosis might cause D-lactate secretion. The studies were carried out on the healthy people with ileostomy and it was determined in these studies that the *L. acidophilus* and *Bifidobacterium* species transformed the conjugated bile acid to non-toxic secondary salts (Connolly et al. 2005). Among the patients with the small intestine syndrome, the bile acid metabolites may accumulate in the bowels and cause malabsorption (Bongaerts et al. 2000). This may cause the lactate accumulation and it poses risk for the colon cancer. Moreover, it is theoretically possible that the deteriorations might occur in the intestinal mucosa (Ruseler-van Embden et al. 1995). Moreover, in both *in vitro* studies and in those carried out on gnotobiotic rats, no evidence suggesting that the probiotics would deteriorate the intestinal mucus could be found (Ishibashi and Yamazaki 2001; Snydman 2008).

The studies showed that the probiotics could alter the immune responses of the individuals, increase their responses to vaccination or change the natural history of the allergic response. The probiotic bacterium can alter the non-specific immune, cellular and humoral responses. In addition, they can also affect the local immune

response, in addition to the local secretion of cytokines. Some of these responses are thought to be specific to the strain and host (Senok et al. 2005). The gastrointestinal microbiota role in the growth of intestines recommended that the manipulations caused by the probiotics might theoretically have a negative immune-modulator effect. Another population, in which an adverse immunologic response may theoretically occur, is the pregnant women. Besides that, the probiotic microorganism use in pregnancy and in children and newborn has not been related to any immunological effect (Snydman 2008).

Antibiotic Resistance Transfer

The most important concern about the theoretical risks of the probiotics is the potential transfer of antibiotic resistance between probiotics and pathogen bacterium in the intestinal canal (Salyers et al. 2004; Mathur and Singh 2005). Given the antibiotic resistance transfer in LAB, the plasmid existence having antibiotic-resistant genes containing the genes coding the macrolide-lincosamide streptogramin, tetracycline and chloramphenicol resistance was reported (Lin et al. 1996). These plasmids of resistance were found in *L. reuteri*, *L. acidophilus*, *L. plantarum* and *L. fermentum* isolated from animal feces, raw meat and silage (Gevers et al. 2003). Streptomycin, tetracycline, and chloramphenicol resistances and 214 plasmids were detected in *L. lactis* isolated from soft cheese and raw milk. The resistance of tetracycline was detected in *L. plantarum* 5057 (Snydman 2008).

The natural *Lactobacillus* plasmids transfer is rarely seen. Fermentation of lactose plasmids were transferred to *L. casei* (Ahn et al. 1992) and the bacteriocin reproduction was transferred to *L. johnsonii*. There are several evidences suggesting that the *Leuconostoc* and *Pediococcus* species can be accepted as the wide-interval antibiotic-resistant plasmids when compared to the *Lactococcus* species (Snydman 2008). The conjugation from enterococci to *Lactococcus* and lactobacilli may take place in animal bowels, and also it occurs *in vitro*. Besides that, the lactobacilli transfer is very rare (Dessart and Steenson 1991; Mathur and Singh 2005).

Moreover, the attempts were made in order to molecularly identify the genes with resistance to vancomycin in lactobacilli. For this purpose, five *L. reuteri* strains and one *L. rhamnosus* strain were examined in terms of vanA, vanB and vanC genes and none of these genes was found in any of the strains (Klein et al. 2000). *Lactobacillus* GG was specifically investigated and no evidence related with vanA, vanB, vanH, vanS, vanX, vanY and vanZ genes was found by using PCR (Tynkkynen et al. 1998).

The Infection Risks of Probiotics Among the Healthy Individuals and the Individuals with Suppressed Immune System

Lactobacillus *and* Bifidobacterium *Safety*

Some of the probiotic foodstuffs (such as cheese, yogurt, cabbage pickle, and other fermented herbs and olives) have had the history of safe use for a long time (Shortt 1999). Among the healthy persons, the normal concentrations of lactobacilli are 10^3–10^4 CFU/g in the oral cavity, 10^3–10^7 CFU/g in ileum, and 10^4–10^8 CFU/g in the colon. In addition these microorganisms are also the dominant microorganisms of the vagina (Borriello et al. 2003).

Most of the rarely seen *Lactobacillus* infection cases develop in patients having rigorous underlying conditions (Gasser 1994; Saxelin et al. 1996; Husni et al. 1997); most of these patients die within 1 year after the development of infection (Husni et al. 1997). Lactobacillemia is a frequently seen indicator of an underlying lethal or severe disease (Gasser 1994; Saxelin et al. 1996; Husni et al. 1997). The patients who has the suppressed immune system are usually more sensitive to the pathogenic microorganism infection and the prevalence of opportunistic infection is very high. Besides that, there is no study proving that the intake of probiotics including *Lactobacillus* or bifidobacteria increased the risk of opportunistic infection among these persons. Furthermore, two clinical studies were carried out on small groups of patients with a suppressed immune system (i.e., the patients with HIV infection) to evaluation of the reliableness of probiotics and the data obtained from the study supports the reliability of the probiotic microorganisms consumed by patients (Wolf et al. 1998; Cunningham-Rundles et al. 2000).

Many attempts have been made in order to assess the factors that might predispose the persons, who have severe diseases, to the *Lactobacillus* or bifidobacteria infections (Patel et al. 1994; Husni et al. 1997). In some cases, together with the chronic immune-suppressive and antibiotic treatment, the invasive procedures involving the gastrointestinal system (the *Lactobacillus* and bifidobacteria have large communal populations) and the other organs contributed the increasing risk (Antony et al. 1996). Besides that, the statistical analyses were generally not used and, when used, the studies were carried out on very few cases in order to enable the general suggestions. As far as we know, there is no medical guideline submitted on the inpatient patients' consumption of probiotic or the other products including applicable lactobacilli or bifidobacteria. Although there are guidelines on the probiotic yeast preparations, the current evidences do not guarantee the probiotic lactobacilli and bifidobacteria in the nutrients.

Safety of Probiotic Enterococci

The enterococci safety is an important point but the enterococci were reported to have healthy and important benefits. Some probiotic enterococcus species have long-term reliable usage history. On the other hand, it is also known that the enterococci are opportunist pathogens and they play an important role in hospital infections. Moreover, the antibiotic resistance (they may have multiple antibiotic resistances) characteristic is generally coded by the transferrable elements. Because of this harmful characteristic, there are concerns regarding their use in probiotic foods (Franz et al. 2001).

The *Enterococcus* spp. are significant nosocomial pathogenic bacteria causing bacteremia, urinary tract infection, endocarditis and other infections (Murray 1990; Morrison et al. 1997). The *Enterococcus* spp. generally known as the opportunist pathogens causing infections among the individuals having a rigorous underlying diseases or immune deficiency (Morrison et al. 1997). One of the factors contributing to pathogenicity and causing worldwide concerns is the resistance to a large scale of antibiotics, that hinders the number of treatment options (Murray 1990; Landman and Quale 1997; Leclercq 1997). The enterococci, which are especially resistant to vancomycin, induced global hospital crises (Willems et al. 2001; Ruiz-Garbajosa et al. 2006; Werner et al. 2008).

Besides that, the enterococci virulence cannot be defined solely with the resistance of antibiotic. The enterococci virulence factors are playing role in the strain pathogenicity include the circumstances related with the colonization and the host tissue invasions, such as the resistance mechanisms to non-specific and specific host protection mechanisms. Moreover, these virulent species should cause pathologic alterations directly via production of toxin or implicitly via inflammation (Johnson 1994). The enterococcus virulence factors have been intensively investigated in recent years and some of the virulence factors have been well defined. The other "more detailed" virulence determinants are still being investigated. The virulence factors that have been defined to date are related with the colonization, the invasion, and the pathologic changes.

The various studies on the food-origin enterococci's virulence factors showed that the personal virulence factors' existence and prevalence are specific to the strain (Eaton and Gasson 2001; Franz et al. 2001; Yousif et al. 2005; Lepage et al. 2006; Pérez-Pulido et al. 2006; Aakra et al. 2007; Serio et al. 2007; Abriouel et al. 2008; McGowan-Spicer et al. 2008; Valenzuela et al. 2008; Martin-Platero et al. 2009). It is reported that the virulence is not occur from the specific virulence determinant presence though it is a more complex procedure. Interestingly, the virulence strains, inversion sequence elements, phages, transposons, and mobile genetic elements like a pathogenic island are seen to acquire specific virulence-related genes or the antibiotic resistance genes (Lepage et al. 2006; Aakra et al. 2007; McBride et al. 2007; Solheim et al. 2011). Thus, the virulence strains developed as a result of acquiring the genetic materials allowing the increase of their vitality when they adapt themselves to the host (Lepage et al. 2006). As a result, it was determined that

the new sorts of antibiotic resistance entered into the species and the different genetic strains of *E. faecalis*, in which the virulence properties are different, emerged (McBride et al. 2007). The clonal clusters (CCs), which are some strains called CC9, CC8 and CC2 have antibiotic resistance and pathogenic island genes more than the other strains and they spread over the entire world (McBride et al. 2007). Solheim et al. (2011) defined a series of genes enhanced in CC2 strains and related with mobile elements such as *faj03*, Tn*916*, and efaB5.

Several probiotic strains have been well-investigated from safety and functional aspects. Two of the best defined in terms of safety are *E. faecalis* Symbioflor 1 (produced by SymbioPharm, Herborn, Germany) and *E. faecium* SF68 (NCIMB 10415 produced by Cerbios Pharma SA, Barbengo, Switzerland). These probiotics have safe and long usage histories (longer than 20 years each) without any harmful effect. Kayser (2003) reported the safety of *E. faecium* SF68 produced by Cerbios Pharma and investigated the absence of virulence determinants. According to the research results, *E. faecium* SF68 does not contain a plasmid that is sensitive to the sex pheromone. *E. faecalis* strain is Symbioflor 1 (produced by SymbioPharm) CC25 strain (Solheim et al. 2011). The whole genome of this strain was arranged in a raw and then compared to *E. faecalis* V583, a pathogenic strain. Although there is a general synteny between the sequences of both strains, the detailed analyses showed the absence of a large genomic zone, which indicates the loss of a gene, in the chromosome of the probiotic strain. The genes that do not exist in *E. faecalis* Symbioflor 1 are cytolysin, Esp, gelatinase, hyaluronidase, and peptide antibiotic AS-48. Besides the reproduction of AS and the collagen-adhesive proteins, the different determinants such as reactive oxygen anions and resistance to capsule formation were detected. All of these characteristics are thought to be the colonization factors that provide a competitive advantage to the probiotic strain and, thus, supporting the probiotic character and activity (Domann et al. 2007). Although these probiotics have been commonly used at the highest doses to date, no infection related with these two probiotic enterococci was reported.

Safety of Probiotic Bacillus *Species*

Bacillus clausii, *B. subtilis*, *B. pumilus*, *B. coagulans* (generally mislabeled as '*Lactobacillus sporogenes*') and *B. cereus*, which are probiotics from spore-forming *Bacillus* species, are the less-known ones among the lactobacilli and bifidobacteria (Sanders et al. 2003; Hong et al. 2005). The addition of *B. subtilis* as a food additive was approved in minimum one European country (Italy). However, it is not the case for the other species, except for *B. clausii* which is licensed in that country as "Enterogermina" product, which is a prophylactic medication (produced by Sanofi-Aventis, Milan, Italy). The use of *Bacillus* spp. rises many safety questions since it is known that many species including *B. cereus* and *B. anthracis*, *B. pseudomycoides*, *B. thuringiensis*, *B. pseudomycoides* and *B. weihenstephanesis* are known as pathogenic. *B. cereus* is a factor playing role in food poisoning, which is

well-documented to arise from the one or more enterotoxins production (Granum and Lund 1997; Granum 2002; Guinebretiere et al. 2002). Besides that, the pathogenicity is specific to the strain since there are some *B. cereus* species producing no enterotoxin and, as specified before, they are used as probiotic for humans and animals. Although the diseases caused by *B. weihenstephanensis* and *B. thuringiensis* strains are not understood well, they possibly arise from the production of enterotoxins similar to those produced by *B. cereus*.

Few things are known about the other *Bacillus* species but there are few reports related with *Bacillus* spp. under clinic conditions (De Boer and Diderichsen 1991; Osipova et al. 1998; Salminen et al. 1998; Sanders et al. 2003; Logan 2004). The prevalence of the diseases related with *Bacillus* is rare but, in most cases, they may be misdiagnosed by recycling the "spores" from the clinical samples. The opportunist infections were reported (for instance) in the patients with a suppressed immune system. However, these infections may frequently exist together with the members of other "non-pathogenic" species. Besides that, in some reports, it is stated that the isolates of *Bacillus* species have toxigenic characteristics (Rowan et al. 2001; Phelps and McKillip 2002; From et al. 2005). In conclusion, the dose of consumed bacteria is a significant factor playing role in the growth of the disease (Kramer et al. 1982; Duc et al. 2005).

De Boer and Diderichsen (1991) investigated the safety of *B. subtilis* and *B. amyloliquefaciens*. In this review, the published cases of *Bacillus* infections were specifically investigated. These infections do not arise directly from the digestion of *Bacillus* but the other sources. The results showed that the infections were mainly observed in the persons having immune-suppressed endocarditis history or recently undergone surgical operation. Although it is stated in the report that the prevalence of the food poisoning cases arising from the *B. subtilis* is very low, it is also emphasized that it is very difficult to obtain exact and reliable numbers. This is because the hospitals do not make an absolute distinction between *B. cereus* and other *Bacillus* species as food poisoning agents. The *Bacillus* species were related to the nosocomial bacteremia (Richard et al. 1988).

Some infection cases arising from the consumption of the *Bacillus* probiotic were reported. Oggioni et al. (1998) reported a septicemia case caused by the *B. subtilis* strains from a probiotic preparation used by a patient with a suppressed immune system. Spinosa et al. (2000) examined two samples of *Bacillus* infections that might be related with a commercial probiotic preparation. A contiguous agent, which has been found in a cholangitis case in France in 1996 and in a recurrent septicemia case in Italy in 1998, could not be distinguished from a *Bacillus* spp. found in an Italian probiotic foodstuff (*B. clausii*). Besides that, the authors could not confirm the causative role of Italian probiotic in the infections. Both of the infections developed in the patients with a suppressed immune system (French patient has undergone kidney transplantation and Italian patient has received chemotherapy). Attempts were made in order to define the antibiotic resistance of *Bacillus* spp. used as probiotic foodstuff. Both Ciffo (1984) and Mazza et al. (1992) investigated four *B. clausii* strains including Enterogermina, which is a commercial product, from the aspect of the resistance to therapeutic antibiotics. Mazza

et al. (1992) continued testing the transferability and stability of these resistance characteristics. The resistance to quinolones, cephalosporins and macrolides was observed to be stable, but it was also determined that these resistance phenotypes were not transferred to the other bacteria *in vitro* or *in vivo*.

A significant aspect of ensuring the safety of probiotics is the taxonomical characterization of the bacteria included in the foodstuff. Green et al. (1999) analyzed two commercial products (Enterogermina and Biosubtyl) and they showed that none of them consisted of *B. subtilis* as the manufacturers claim. This result was based on various important phenotypic characteristics (alkaline formation and amylase activity) and totally 16S rDNA sequences. Enterogermina strain, which is at closest alignment with *Sperolactobacillus* group (a subgroup of *B. alcalophilus*), and Biosubtyl strain were found to be in relation with *B. pumilus*.

It is necessary to accurately represent the probiotic product, which is capable of forming spore, to the customers. One way of this is to improve and use objective and scientific guidelines for the commercial products by the industry. As an alternative, the governments may realize the necessity of making stronger regulations on specific microorganisms. The list of suggestions presented below is based on the current guidelines (Donohue and Salminen 1996; Przyrembel 2001) and global organizations (SCAN 2002a, b; FAO/WHO 2001). It is recommended for the companies to consider the minimal safety information, which is provided below, for introducing the spore-forming bacteria to the market in form of probiotic products.

1. Each bacteria strain in the product must be isolated, named, and taxonomically identified. The most current and valid method must be used in order to ensure the exact speciation. In general, the combination of the gene-based and phenotypic methods is necessary. In comparing all the strains, the best available method for most of the microorganisms is 16 rRNA genes identified from the well-known culture collections such as ATCC, European culture collections (DSMZ, LMG, CIP, NCIMB) or Japanese collection (IAM). If the identity of a strain in a product is suspicious, then no decision can be made about the safety of that product.
2. The identification of the bacteria must be in harmony with the scientifically known names. The use of old or misleading names for a long time in the product tags is unacceptable. A "Validation List Declaration" confirming the names of bacteria must be prepared. The Validation List must be published in *Intl J of Syst* and *Evolutionary Microbiol*.
3. The in-vitro characterization of each strain including the antibiotic resistance profile, emetic or enterotoxin production, gastric acid or bile salt resistance must be enough. The bacterial strains exhibiting transferrable resistance of antibiotic must not be used. A elaborated schema for testing the production of toxin is already provided by the Scientific Committee of Animal Nourishment (SCAN 2002b). The strains that are capable of producing toxin must not be used as probiotic. The bacteria must be defined by the existence of plasmids or transferrable DNA vectors. It was determined that the *Bacillus* plasmids mediate the transfer of antibiotic resistance between the zones (Koehler and Thorne 1987) and one must avoid this risk.

4. The safety assessment must be performed by a professional, who has experience in this field. Depending on the species and genus being used, the dose being aimed, and the target audience, the safety characterization might be made as follows; the capability of remaining attached to appropriate human cell lines and invading and modulating capabilities must be tested. The strongly adhesive or invader bacteria species, which are in vegetative or spore forms, must not be used as probiotic. The acute and embryonic toxicity studies would contribute to the safety approach. Each strain must be tested in concentrated form (in both vegetative and spore forms) and in final product on minimum one mammalian species. The dosage chronic toxicity studies, which must be continued for a minimum 9 months, must be performed for each strain in concentrated form (in both vegetative and spore forms) and in final product on minimum two mammalian species (preferably a rodent and a larger species; i.e., a rodent and pig, rabbit or cat).
5. On the tag and marketing literature, the contraindications for the *Bacillus* species or spore-forming bacteria must be listed, including the specific references for the patients or customers with suppressed immune system because of HIV infection, chemotherapy, or allograft treatment.
6. The marketing literature or product tag must provide the information below:
   (a) Usage indications supported by the clinical evidence.
   (b) Net definitions of species, genus, strain, and concentration of each element of the bacterium.
   (c) A phone number for negative feedbacks must be provided on the product tag.

Mortality Related with Probiotic Infection (Mortality Rate)

The deaths related with the probiotic infections, especially those involving healthy individuals, are very rare. Although they cause low-grade infection and endocarditis among the individuals with the suppressed immune system, the rate of mortality related with *Lactobacillus* spp. is very low (Olano et al. 2001).

In some studies, it is emphasized that the correlativity between probiotic infections and death is very weak. Cannon et al. (2005) reviewed 92 research papers involving 241 *Lactobacillus* spp. infection cases [localized infection (39 cases), bacteremia (129 cases) and endocarditis (73 cases)] and reported that none of these infections was related with the mortality. Antony et al. (1996) analyzed 53 probiotic infection cases and the researchers reported that only three of the deaths could possibly be explained with the *Lactobacillus* spp. infection. In another study involving 45 patients, Husni et al. (1997) reported that the *Lactobacillus* spp. might possibly have contributed to the bacteremia, which caused only one death. The researchers emphasized that *Lactobacillus* spp. bacteremia rarely threatens the life and it is an indicator of a severer underlying disease.

One of the enhanced studies on probiotic bacterium was carried out by Salminen et al. (2004) and it involves 89 cases. The researchers reported that the mortality-related *Lactobacillus* bacteremia cases are generally accompanied by additional underlying severe diseases, that there were severe underlying diseases in 82% of all the cases. Despite that, at the end of research, the mortality rate was found to be 26% at the end of 1 month and 48% within 1 year after the onset of disease. It was determined that the mean survival time of *L. rhamnosus* GG, which is widely used in probiotic preparations, in *L. rhamnosus* bacteremia patients is approx. 2.5 months. This duration is statistically significantly shorter than the patients with bacteremia induced by other specific *Lactobacillus* species (8.9 months) or mean survival time of the patients with bacteremia caused by uncharacterized lactobacilli (34 months) ($p < 0.0425$). Although *L. rhamnosus* is realized as the most lethal *Lactobacillus*, there are evidences asserting the contrary. It was reported that the mortalities related with *L. casei* (32.6%), *L. plantarum* (30.8%) and *L. acidophilus* (28.6%) were much higher than the mortality related with *L. rhamnosus* (13.6%) (Cannon et al. 2005).

McNaught et al. (2002) carried out a randomized research on 129 selected surgical patients in order to examine the effects of *L. plantarum* 299v on the barrier function of bowels (probiotic group n = 64). The researchers determined that *L. plantarum* 299v application did not influence the bacterial translocation, stomach colonization or postoperative septic morbidity incidence. Besides that, the mortality rate was found to be slightly higher in the probiotic group when compared to the control group, and the difference was not statistically significant.

Since the relationship between death and probiotic infection is difficult to reveal in human, although the use of animal models would be a better option to obtain more data. For instance; Wheeler et al. (2003) used a mutant probiotic yeast (*S. cerevisiae*) with a demolishing SSD1 gene altering the joint of cellular surfaces and the architecture of cellular wall, and they revealed that the yeast became more virulent and less sensitive to being destroyed by the macrophages. It was determined that the deceased mice have a high amount of living yeast ($>10^6$ yeasts) in kidneys. These results suggest that the yeasts cause death because of their development and the interaction with the host.

Difficulties in Identifying the Probiotic Infections

It has always been hard to verify the ineffectiveness of probiotic bacterium because of their anaerobic structure. Because of their low contagiousness, the probiotics are seldom suspected of infection among healthy people. For this reason, it is desired to detect the pathogenic potential of a microorganism before the application. One of the most reliable methods is the use of chronic and acute toxicity tests that may yield information also about the toxicity. Zhou et al. (2000a, b) asserted that the feed consumption, activity status and rate of growth in an animal model are the most susceptible parameters that can be used in examining the acute toxicity of the strains being tested. Moreover, the lethal doses of strains being tested can also be used as

an toxicity indicator. The reports showed that *B. longum* BB536 is safe and, even given at a dose higher than the maximum oral dose of 5×10^{13}/kg, exhibited no toxicity (Ishibashi and Yamazaki 2001; Liong and Shah 2005). *B. longum* BB536's safety was proven after applying the dose of 2.5×10^{11} kg/day for a year and detecting no toxicity.

The preliminary detection of the toxicity is very complicated because the procedure is very long. When the probiotic strains cause infection, it generally cannot be determined in the laboratory because their long-term safety history eliminated the suspicions on them. Moreover, it is difficult to selectively define the probiotic microorganisms in laboratories since most of them are anaerobic. In order to maintain the anaerobic structure in elective counting, the redox decreasing compounds such as L-cysteine · HCl (Liong and Shah 2005) were used and, because of their adaptation to the laboratory environment, the sub-enculturation was minimized in order to prevent any change. The exact identification of the rod-shaped anaerobic probiotic microorganisms such as *Lactobacillus* is very hard since most of the commercially available identification systems are inadequate for identifying *Lactobacillus* (Murray et al. 2003). While examining the field of effect of *Lactobacillus* bacteremia in Central Hospital of Helsinki University, Salminen et al. (2002) isolated 66 cultures, which were characterized at the beginning, as *Lactobacillus*. Besides that, when further analyses were performed for identifying the species, 18 of 66 isolates were found to be other microorganisms (four *Actinomyces*, four *Clostridium*, three *Bifidobacterium*, one *Weissella confusa*, and one *Carnobacterium*). It was observed that microscopic identification is very difficult. The morphology of *Lactobacillus* is similar to the other members of species including *Corynebacterium*, *Clostridium*, *Nocardia* and *Streptococcus* (McNaught et al. 2002; Cannon et al. 2005). Based on the Gram-staining identification, the probiotic microorganisms such as *Lactobacillus* were perplexed with diphtheroid (Gallemore et al. 1995). Similarly, since both of two are aminopeptidase-positive and they may have resemble morphologies based on the Gram staining from blood agar discs, *Enterococcus* spp. was confused with *W. confusa* (Olano et al. 2001). Thus, a detailed identification such as those performed by using a specific polymerase chain reaction and pulsed-field gel electrophoresis (Ouwehand et al. 2004) is needed.

Conclusion

Although they have a safe usage history and several health benefits, the probiotic microorganisms may have harmful effects especially on the individuals with a suppressed immune system. It may be misleading to identify a probiotic or product as GRAS by making use of the safety history. Thus, the GRAS status must be determined in accordance with the purpose of use. For regulatory purposes, the probiotic strains' translocation, antibiotic resistance, and capability of causing infection must be taken into consideration.

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Beneficial Biofilm Applications in Food and Agricultural Industry

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Abstract Biofilm is defined as a community in which microorganisms adhere to a living or inanimate surface, embedded in a gelatinous layer in a self-produced matrix of extra polymeric substances, adhered to each other, to a solid surface or to an interface. Adverse environmental conditions caused biofilm formation by inducing transition of microorganisms from planktonic cell form to sessile cell form and altered metabolism of bacteria in biofilms. Bacteria in biofilm matrix produce the specific secondary metabolites and gain robustness. Although biofilms are often accepted as potentially destructive for clinical and other industrial fields, many biofilms are beneficial and there are several reports related to the positive use of these biofilms. Beneficial biofilms could be used for wide applications (antibacterial, food fermentation, biofertilizer, filtration, biofouling, prevention of corrosion, antimicrobial agents, wastewater treatment, bioremediation and microbial fuel cells) in food, agricultural, medical, environment and other fields. According to previous reports, certain strains including *Bacillus* spp. (*B. subtilis*, *B. thuringiensis*, *B. brevis*, *B. licheniformis*, *Bacillus polymyxa*, *Bacillus amyloliquefaciens*) *Lactobacillus* spp. (*L. casei*, *L. paracasei*, *L. acidophilus*, *L. plantarum*, *L. reuteri*) *Enterococcus* spp. (*E. casseliflavus*, *E. faecalis*, *E. faecium*), *Pseudomonas* spp. (*P. fluorescens*, *P. putida* and *P. chlororaphis*), *Acetobacter aceti*, some fungi and *Pseudoalteromonas* sp., etc. led to beneficial biofilm formation. Food and agricultural industry may mostly benefit from biofilms in terms of their biochemical, fermentative, antimicrobial and biotechnological characteristics. Microorganisms in biofilm matrix could positively affect quality characteristics of food products such as texture, biochemical composition and sensorial properties *via* the production of specific secondary metabolites. Additionally, biofilms have an importance in water and soil safety of

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agricultural land. The present chapter highlights beneficial biofilm applications in food and agriculture industry.

Keywords Biofilms · Beneficial microorganisms · Probiotics · Food and agriculture industry

Introduction

Biofilm is multicellular and cooperative communities of microorganisms attached to biotic or abiotic surfaces and frequently embedded under the extracellular polymeric substances (EPS) (Todhanakasem 2013). Microbial adhesion to a surface is the first stage of biofilm formation and is affected by several factors including hydrophobic and electrostatic interactions, substratum surface roughness, surface charges and cell surface structures (Sarjit et al. 2015). Three-dimensional structure of adherent cells in biofilm matrix contains networks of channels for supplying of nutritional compounds and a wide range of microbial cell-to-cell communication (quorum sensing) synchronizing the activities of microbial consortium (Ayala et al. 2017). Signaling molecules provided cell to cell interactions are the main mechanism that explain colonization or adhesion of bacteria or fungi based strains on various surface. Microorganisms exhibit desirable or undesirable effects in industrial fields by using this mechanism (Velmourougane et al. 2017).

Biofilms are found everywhere, ranging from the environment to the human body. Biofilms mostly have clinical scientific interest due to pathogen infection with regard to human health (Jefferson 2004). On the other hand, biofilm formations originated from food-processing equipment, environment and staff in food industry may threat the microbiological quality and safety of food products due to cross contamination and lead to foodborne outbreaks and economic losses. Therefore, food industry prioritized food safety policies to prevent and control biofilm formation (Lindsay and Holy 2006; Houdt and Michiels 2010; Wingender and Flemming 2011). There are different types of bacteria concerning biofilm formation. Biofilm formation can be enhanced through synergistic interactions among multispecies biofilms, along with some other nutritional and environmental conditions (Berlanga and Guerrero 2016). Although biofilms were mostly considered in a negative sense, beneficial biofilms with their positive characteristics were also stated in various industrial fields (Ercan and Demirci 2015). In terms of beneficial aspects of biofilm formation, food and agricultural industry utilized biofilms in food fermentation, bioremediation, wastewater treatment, biotechnological applications and as probiotics, biofertilizers, biocontrol agents and microbial fuel cells, etc. (Qureshi et al. 2004; Qureshi 2009; Shah 2018). The high biomass density in biofilms result in quite more biochemical activities than that of planktonic cell (Marapatla 2014). Biofilm applications in these fields led to higher productivity in comparison to con-

ventional fermentation. While industry and researchers try to degrade detrimental biofilm formation, they support the utilization of beneficial biofilms in various industrial fields with the improvement of novel biotechnological applications (Winkelströter et al. 2014).

In brief, while there are innumerable studies based on negative aspects of biofilms, there are also various favorable properties of biofilms. Especially, in recent times, researchers focused on potential strategies for the enhancement of beneficial biofilms. This chapter described several examples of beneficial biofilms in food and agricultural industry and encouraged further research in this area.

Biofilm Formation: Good or Bad

Biofilms were first observed and defined in 1684 by Anthony van Leewenhoek, however called as a term centuries afterwards. The first observation about microbial biofilms which adhere to tooth surfaces and form sessile communities were detected by using his primitive light microscope (Vos 2015; Shi and Zhu 2009). The Royal Society of London reported that the vast accumulation of microorganisms were observed in dental plaque (Jefferson 2004; Lens 2011). Although the relation between cell surface structures (mostly pili and capsules) and adhesion was detected earlier (Robertson and McLean 2015). As known, biofilms could be formed by both bacteria and fungi on natural and artificial surfaces. The flagella, fimbriae, pili, lipopolysaccharides and membrane proteins are responsible for biofilm formation (Velmourougane et al. 2017).

Biofilms are formed with aggregation of microbial strains that are enclosed in self-produced extracellular polymeric substances. Biofilm formation can be observed everywhere and most microorganisms are presently considered to be capable of biofilm formation on earth (Toyofuku et al. 2016). Microbial populations in biofilms generally differ from their planktonic counterparts in terms of nutrient uptake, nutrient cycling, respiration and overall growth and also display differences in terms of their molecular structures (Blenkinsop and Costerton 1991; Dunne 2002). Many microorganisms have ability of attachment to surfaces and thus could form biofilms in various industrial fields (Houdt and Michiels 2010). Biofilms may attach on a wide variety surfaces including plastics, metal, glass, soil particles, wood, medical implant materials, tissue, food products, food processing equipment, devices and machines, etc. Microbial adhesion is supported by fimbriae, pili, flagella and EPS that play an important role to found a bridge between microorganisms and the conditioning film (Kokare et al. 2009).

For agricultural and food industry, microbial biofilms can be detrimental or beneficial (Ercan and Demirci 2015). It is essential to aware of the mechanisms of biofilm formation in order to control deleterious biofilm formation and/or to promote beneficial biofilm formation (Sarjit et al. 2015). The formation of biofilm occurs in five stage including initial attachment, irreversible microbial attachment, early development of biofilm structure (micro colony formation), maturation and

dispersion (Jara et al. 2016). Several environmental factors are effective on each stage regulating the biofilm formation process. Microbial attachment to a surface is the main process due to transformation from planktonic cell form to the biofilm form. This stage called as primary adhesion, the bacteria may embed to the biofilm or leave the surface and return to the planktonic form (Hall et al. 2004; Petrova and Sauer 2012). After reversible attachment, microbial cells undergo irreversible attachment. Here, surface proteins and EPS contribute the attachment between the cell and surface. During the transition to irreversible adhesion, an intracellular secondary messenger produced by microbial cells provide the regulation of EPS production and motility (Hinsa et al. 2003; Toyofuku et al. 2016).

The formation of biofilm is challenging for food industries such as dairy, fish processing, poultry, meat and ready-to-eat foods industries due to their resistant to antimicrobial agents. The properties of the surfaces to which microorganisms attach and species are the most important factors affecting biofilm formation (Srey et al. 2013). Biofilms formed by spoilage and pathogenic microorganisms on food processing surfaces could result in food spoilage and outbreaks in view of inappropriate and inadequate cleaning with regard to sanitation (Todhanakasem 2013). The prevention of biofilm formation is essential in order to ensure food safety and protect public health. In agricultural industry, biofilm formation has negative sense because microbial colonization in plant by biofilm could cause infections (Laranjo et al. 2017). As detrimental biofilms of pathogenic and spoilage microorganisms are robust to antimicrobial substance used in sanitation, their presence endangers human health and quality properties of food (Berlanga and Guerrero 2016). For instance, contamination of nonstarter lactic acid bacteria (*Lactobacillus curvatus*, *Lactobacillus fermentum*, etc.) into equipment surfaces used in dairy industry led to adverse biofilm formations in cheeses (Somers et al. 2001; Agarwal et al. 2006). In agriculture industry, the development of pathogen biofilm in plants causes economic losses due to damage and disease of the plants (Velmourougane et al. 2017).

In addition to detrimental aspects of biofilm, there are positive aspects related to the formation of biofilm. Beneficial biofilms could be utilized for wide applications (biocontrol agents, food fermentation, biofertilizer, filtration, biofouling, prevention of corrosion, wastewater treatment, bioremediation, nutrient mobilizer, plant growth promoter and microbial fuel cells, etc.) in food, medical, agriculture, environment and other industrial fields (Stepanovic et al. 2007; Wood et al. 2016; Velmourougane et al. 2017). Biofilm formation in the food processing industry could be beneficial, especially in fermented and probiotic food products such as vinegar and cheese (Zottola and Sasahara 1999). In agriculture industry, biofilm-based biofertilizers provide use of sustainable soil because of the utilization of less or none chemical fertilizers in farming areas. In bioremediation and waste water treatment, biofilms are used for the removal of various industrial pollutants from sewage, industrial waste streams or contaminated ground water (Sarjit et al. 2015; Vilamakis 2011; Zottola and Sasahara 1999). Another good aspect, biofilms formed mostly by *Bacillus* species were utilized in the prevention of infection from plant pathogens in plant growth promotion (Winkelströter et al. 2014). With regard to positive aspects in electrical energy, biofilms are involved in microbial fuel cells which could use microbial metabolism to produce an electrical current from organic substrates

(Sarjit et al. 2015; Vilamakis 2011). Another desirable biofilms are anticorrosive biofilms with regard to their industrial importance. These biofilms can prevent the corrosion on metal surface of equipment or devices in food and agricultural industry (Wood et al. 2010).

Beneficial Biofilm Applications

Bacteria are often referred to as harmful microorganisms; however, the human body, despite having some amounts of detrimental bacteria, is replete with beneficial bacteria that thrive and decrease the number of harmful bacteria (Rajpal et al. 2017). The economic, medical and industrial importance of biofilms required detailed and meticulous studies for the controlling of microbial attachment and subsequent biofilm formation. Similar to microbial free cell, microorganisms in biofilm matrix compete with each other. Dominant microbial cell having higher cell density in population encourage biofilm formation (Robertson and McLean 2015). Cell-to-cell communication provide the organization and differentiation of microbial strains in biofilms. Signal molecules called as quorum sensing were used in microbial cell to cell communication. As microorganisms produce signal compounds, cell density increase in biofilm matrix. As high cell density led to the great accumulation of signal molecules, biofilms produce increasing amounts of metabolites and secretes (organic acids such as lactic acid and acetic acid, hydrogen peroxide and bacteriocins) (Toyofuku et al. 2016). In recent studies, instead of focusing on killing the harmful bacteria that cause an infection, this study focuses on ways to maximize the growth of good or helpful bacteria that will thrive and destroy the detrimental bacteria naturally. Quorum sensing explains the reason of biofilm formation. The relationship between quorum sensing and biofilm formation explains pathogen inhibition by beneficial biofilms (Rajpal et al. 2017). Quorum sensing behavior in biofilm matrix is thought to offer significant benefits to bacteria in terms of biofilm community structure, defense against competitors, adaptation to environmental changes and overall host colonization (Engevik and Versalovic 2017). Biofilm specific cell signaling are generally known with the providing of communication among microbial cells and information exchange. On the other hand, cell signaling in biofilms could achieve cross-kingdom interactions defined as interactions between organisms of different kingdoms (plants, algae, fungi, human, etc.) and mediate other biofilm based formations (Lens 2011; Stavridou and Forzi 2011).

Biofilm structure as microbial community or consortia has a wide range of advantages compared to a planktonic form. Biofilms generated by beneficial microorganisms are utilized in many industrial fields due to their advantageous structure. The most widely used beneficial biofilm applications in food and agricultural industry are listed as probiotic and bacteriocin producer strain biofilms, microbial biofilms specific to certain fermented food process, bioremediation, wastewater treatment, biofilms as biofertilizer or biocontrol agent, anticorrosive biofilms and biofilm reactor.

Probiotic and Bacteriocin Producing Biofilms

Human gastrointestinal tract consists of densely colonized microbial ecosystem. Microbiota in this system allows for beneficial and complex interactions with its host (Kalkan et al. 2018). Biofilms or biofilm-like forms in the human digestive tract may influence the function of the intestinal microbiota and its interactions with the host (Vos 2015). Adhesion potential of microorganisms into gut epithelial mucosal surface to form biofilm is used as a criterion in the selection of probiotic cultures (Benarjee and Ray 2017). Probiotics are viable microorganisms intended to provide health benefits due to their contributions in prevention and treatment of various diseases when consumed (Galgano et al. 2015; Kerry et al. 2018). Previous reports proved that the beneficial effects of probiotic strains were associated with their biofilm formation which pose increased robustness to temperature, low pH, osmotic stress and mechanical forces to that of their planktonic counterparts. The biofilms originated from *Lactobacillus* strains (especially *L. rhamnosus*, *L. plantarum*, *L. reuteri*, and *L. fermentum*) are used as probiotics in food and agricultural production due to various health benefits (Jara et al. 2016). Additionally, probiotic biofilms provide a variety of technological advantages in food and agricultural industry. For example, these biofilms can prevent the growth of undesirable microorganisms such as pathogenic and spoilage microorganisms and also improve the quality properties of food products (Gomez et al. 2016). Although there were a great number of probiotic applications in food and medical field, agricultural applications of probiotic have raised gradually (Song et al. 2012).

Similar to probiotic free cell, probiotic biofilms could be applied for several purposes such as inhibition of pathogen and spoilage microorganisms in foods (—fruits and vegetables, —meat and meat products, —food processing plant and surfaces), the production of some fermented foods and animal feeding (for the prevention of disease from zoonotic and other enteric pathogens), poultry, pigs, cattle and goat (for the prevention of pathogen infections) and lastly in aquaculture as biocontrol agents (Hossain et al. 2017).

Biofilms generate intimate relationship between the human gastrointestinal system and its inhabitant microorganisms. Thus, probiotic strains develop their mechanisms associated with biofilm formation (Farahmand et al. 2013). Commensal probiotic strains inactivate enteric pathogen microorganisms and regulate host immune responses in the intestinal system, however researches evaluating specific functions of biofilms from beneficial microorganisms have been inadequate. Many treatments for bacterial infections have been focused on getting rid of the pathogenic bacteria, but recent works focuses on multiplying the nonpathogenic bacteria in the body (increasing biofilm formation) under the most favorable conditions, so these beneficial bacteria can thrive in the body and reduce the number of detrimental bacteria. *Lactobacillus acidophilus*, *Streptococcus thermophilus*, *Lactococcus lactis*, and *Leuconostoc mesenteroides* subsp. *mesenteroides*, are known as lactic acid bacteria, are gram-positive, are beneficial for the body, and are found in food products (Rajpal et al. 2017).

Lactobacillus reuteri as probiotic bacterium exhibits positive effects on human health similar to other probiotics and produce the antimicrobial agent referred as reuterin. Similarly, biofilms of *L. reuteri* present beneficial effects on human health. For instance, reuterin produced by biofilm of *L. reuteri* could inhibit foodborne pathogens which cause disease in humans (Jones and Versalovic 2009).

Bacteriocins produced by microorganisms in biofilm matrix influence microbial competition in this matrix. Researchers detected that bacteriocins had inhibitory effect on pathogenic bacteria such as *Listeria monocytogenes*, *E. coli*, *Salmonella* spp. and *Staphylococcus aureus*. As a matter of fact some bacteriocins (for example nisin) were commercially used in industrial and medical aims (Engevik and Versalovic 2017).

Similar to planktonic forms of lactic acid bacteria, lactic acid bacteria biofilms as bacteriocin producers have a potential as antibacterial agents in food industry (for instance the field of food packaging). For example, biofilms formed by *Lactobacillus plantarum* and *Enterococcus casseliflavus* as bacteriocin producers exhibited antibacterial effect on *Listeria monocytogenes*. Food industry could utilize these bacteriocin producer biofilms as an antilisterial agent (Guerrieri et al. 2009).

B. subtilis is used in food and beverage production due to beneficial properties. When *B. subtilis* is regularly consumed through foods, it notably prolongs human life expectancy and assist to eliminate the detection of age-related diseases. Especially, probiotic *B. subtilis* biofilms result in the improved lifespan and healthy longevity of *Caenorhabditis elegans*. This dual microbial-worm interaction between *B. subtilis* and *C. elegans* provide colonization and a multicellular biofilm formation in the friendly environment of the worm gut mucosa (Ayala et al. 2017).

Bacillus subtilis has recently an increasing interest due to its probiotic properties. Biofilms of probiotic *Bacillus subtilis* have more favorable properties than its planktonic form. The use of biofilms of these bacteria is beneficial approach, especially as probiotic bacteria must enter the gastric system without losing their survival. Probiotic biofilms of any beneficial bacterium protect not only its own viability but also the viability of other beneficial strain by coating this strain. For example, EPS matrix in biofilm of *B. subtilis* protect other probiotic strains (e.g. *L. plantarum*) against adverse factors throughout gastrointestinal tract (Yahav et al. 2018).

Beneficial Biofilms Specific to Certain Food Process

Biofilm formation with regard to beneficial aspects in food industry could improve biochemical quality, tastes, flavors and textural properties in food products (Jahid and Ha 2014). The microbial interactions in biofilms induced certain food process. In particular, fermented beverages production and cheese ripening or production are carried out by these microbial interactions (Qureshi 2009). Various fermented food products such as fermented dairy products (yogurt, cheese, kefir), meat products (sausages, salami) and vegetable products (vinegar, pickle) in worldwide have their own microflora. Microbial diversity in fermented food arisen from production

methods and microbial ecosystems in production environment. In worldwide, various biofilmed form in fermented foods were listed as mixed biofilms associated with surfaces (cheese rinds), suspended biofilms in liquid (kombucha, kefir, and vinegar), dispersed growth in liquid (lambic beers, natural wines, and yogurt), or in semi-solid substrates (kimchi and miso) (Wolfe and Dutton 2015). The wine production has been leaded by mixture of fungi, yeast and bacteria species from ripening of grapes in vineyards to wine bottling. The cell to cell communication has a key role to produce approvable last product of the wine. On the other hand, during the cheese ripening period mixed communities have been found in the fermentation medium as well (Gulgor and Korukluoglu 2016).

In the Sicilian Protected Denomination of Origin (PDO) Ragusano cheese production, raw milk is put in a wooden vat referred as a Tina without adding starter culture. Fermentative microflora (*Streptococcus thermophilus*, *Lactobacillus lactis*, *Lactobacillus delbrueckii*, and *Enterococcus faecium*) in this cheese production arise from raw milk and Tina biofilm (Licitra et al. 2007). The Tina biofilm consists of lactic and non-lactic species and generate various flavour compounds due to proteolysis and lipolysis during the cheese ripening by these bacteria. In addition to this, lactic acid bacteria in beneficial biofilms from Tina wooden vat accelerate the processing of acidification (Lortal et al. 2009).

In the production of traditional stretched cheeses, desirable biofilms form as a result of adhesion of nonstarter lactic acid bacteria to wooden vat or wooden plank. The ripening of cheese in wooden utensil provided due to the presence of beneficial biofilms both an increasing sensorial quality and biocontrol against pathogen bacteria (*L. monocytogenes*, *Salmonella* spp.) (Scatassa et al. 2015).

Traditional Vastedda cheeses were produced in virgin wooden vat. The surface of this vat subjected to microbial colonization and formed biofilms which are responsible for fermentation and ripening process of cheeses. Biofilms microbial flora caused biodiversity in Vastedda cheeses and ensured favorable sensorial profile for consumers (Gaglio et al. 2015).

In olive production, product specific quality properties stem from biofilmed microbial flora, mostly lactic acid bacteria and yeast. Biofilms mostly form on olive skin or vessels used in production and compose the most microbial communities during olive processing (Heperkan 2013).

Another food process benefited from biofilms is black olive production. During the production of black olives by submerged fermentation, *L. pentosus* and *P. membranifaciens* attach to the surface of black olive and generate biofilm in the stomatal apertures and on the epidermis of olive. As biofilm formation enhanced quality properties of black oil with regard to organoleptic and biochemical, recent olive fermentations were based on beneficial biofilms (Grounta and Panagou 2014).

After harvesting, olive is unfavorable for consumption as a fruit due to its oleuropein content (bitter component). Fermentation process is required for olives to be suitable for consumption and thus table olive is obtained with favorable sensorial and biochemical properties. Biofilms formed by microbial flora on the skin of the olives are responsible for the fermentation in table olive production. During the fermentation of “Spanish-style” green olives, microorganisms consisting of

Enterococcus, *Pediococcus*, *Leuconostoc*, *Lactococcus*, *Candida*, *Pichia*, and *Saccharomyces* are responsible for biofilm formation on surface of green olive. These biofilms act in the preservation against undesirable microbial flora (spoilage and pathogen) and ensure textural and aromatic quality as in table olive (Berlanga and Guerrero 2016).

The nonstarter lactic acid bacteria form dominant microflora which have an impact on quality in most cheese varieties during ripening. Nonstarter lactic acid bacteria in Cheddar are responsible for typical flavor development and notes. However in some cases, these nonstarter bacteria could lead to aroma defects or losses in sensorial quality (Banks and Williams 2004).

Biofilms additionally act in conventional fermented food production ranging from solid biomaterials and proceeding to the moromi-mush, semisolid state. Koji molds, yeasts, lactic acid bacteria and acetic acid bacteria contribute to many traditional fermentations (sourdough, kefir, cacao processing, sausage, tofuyo) and brewing processes (sake brewing, wine and beer brewing, Shochu, awamori, and whiskey, vinegar brewing, soy sauce brewing). Fallen crops (for instance rice), dropped ripe fruits and vegetables are available media for the growth of such microorganisms (Furukawa et al. 2013).

The traditional Minas cheese is manufactured from raw cow's milk by employing wooden utensils which are the source of fermentative or ripening biofilm (*Lactobacillus* and *Lactococcus*) in manufacture. Such biofilms stemmed from wooden utensils synthesized enzymes, organic acids and other antimicrobial substances (peptides and bacteriocins) and thus ensure the product safety. With regard to product quality, they also improve the organoleptic and textural quality properties of the cheese (Galinari et al. 2014).

Traditional smear cheeses produced by using wood utensils were preferred because of their specific sensorial quality. These wood utensils contain biofilms formed by innumerous microorganisms which support formation of flavor compounds and otherwise affect hygienic quality in cheese (Mariani et al. 2007). As a result of cross contamination with pathogen, wood materials could subject to contamination of *Listeria monocytogenes* which is the most important risk factor during ripening of smear cheeses (Aziza et al. 2006). The spontaneous microflora stemmed from biofilms on wooden shelves were utilized in the ripening process of a soft and smear cheese and so had a potential to inactivate *Listeria monocytogenes* (Guillier et al. 2008).

During Spanish style green table olive production, microflora consisting of *Lactobacillus pentosus* and yeast led to mixed biofilms formation on both abiotic (glass slide or vessels which olives placed on) and biotic (olive skin) surfaces as a result of fermentation process. Green olive skin act as a convenient surface for the adhesion of microorganisms and induced formation of complex biofilms during controlled or spontaneous table olive manufacturing (Manzano et al. 2012). Microorganisms responsible for biofilm formation on vessels as abiotic surface were mainly *Candida* spp., *W. anomalus*, *D. hansenii, P. guilliermondii* and *L. pentosus* (Grounta et al. 2015).

'Gerles' (wooden vats utilized the production of Protected Denomination of Origin Salers cheese) provides an appropriate environment for biofilm formation. These beneficial biofilm formation inhibit the growth of pathogen bacteria and as well as contribute the improvement of favorable organoleptic characteristics (Didienne et al. 2012).

Various researchers reported that colonization of food or food contact surfaces by starter cultures was a favorable situation because such colonization by beneficial microflora prevented pathogenic or spoilage bacteria growth. As known, there is competition between beneficial and undesirable microorganisms. Starter cultures exhibits antagonistic characteristic against pathogens and spoilage bacteria. Lactic acid bacteria biofilms may be used to control the formation of biofilms by the food-borne pathogens *Listeria monocytogenes*, *Salmonella Typhimurium*, and *Escherichia coli* O157:H7. Biofilms formed by lactic acid bacteria have been stated to be a stress response and survival strategy in adverse conditions. These defensive mechanisms of bacteria were related quorum sensing, adhesion, and biofilm formation (Laranjo et al. 2017).

Nonstarter lactic acid bacteria biofilms had a potential to inhibit the growth of *Listeria monocytogenes* in soft cheeses. These antilisterial biofilms were formed by *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus curvatus* and *Lactobacillus paracasei*. In future, the detection of novel nonstarter lactic acid bacteria biofilms could also ensure biocontrol against various undesirable microbial strains in various food products (Speranza et al. 2009).

In aquacultures, biofilms were used as food source and thus enhance the fish production. Microalgae and probiotic bacterial products have bioavailability in terms of their beneficial dietary composition. The utilization of probiotic biofilms in shrimp juvenile stimulated its nutrient quality as biofilms had high protein contents (Pandey et al. 2014).

Beneficial Biofilm Based Practices for Sustainable Food and Agriculture

Nowadays, there are increasing interest on the subject of sustainable food and agriculture as a result of biosecurity concern (Bhardwaj et al. 2014). According to FAO, biosecurity is associated with the sustainability of agriculture, food safety and the protection of the environment. On this sense, beneficial biofilm based approaches were offered for sustainable food and agriculture. For example, in organic farming, beneficial biofilms were practiced as biofertilizer and biocontrol agents. Additionally, for waste management, bioremediation through biofilms are seen as environment friendly method and provide degradation of environmental in water soil and other related fields (Baht et al. 2018).

Bioremediation

Bioremediation is defined as a contaminant or pollution treatment technique. In this technique, a variety of pollutants are biologically destructed or converted into less deleterious forms. Microorganisms in bioremediation process are used for degradation of the environmental contaminants (toxic heavy metals, other toxic compounds, plastic wastes and synthetic dyes) or transformation into less toxic forms of these pollutants. Bioremediation process is essential for health and environmental protection (Vidali 2001). Bioremediation could be applied in water, soil and other related fields. Great applications were commercially based on bioremediation, as the microbial bioremediation of contaminated soil or water is economic and reliable (Mueller et al. 1996; Edwards and Kjellerup 2013). Beneficial physical and physiological interactions among organisms in biofilms could be used to help degradation or transformation of environmental contaminants (Horemans et al. 2016). Biofilms are particularly applied for bioremediation of recalcitrant pollutant. High microbial biomass of biofilms facilitates the immobilization of this pollutant. The enhancement of gene transfer among microorganisms in biofilm promote the bioremediation and induce bacterial chemotaxis for degradation of bioavailable contaminants (Singh et al. 2006). Biofilm-based bioremediation is a proficient and safer alternative to bioremediation with planktonic microorganisms because biofilms are more resistant to toxic conditions and xenobiotics as well as increase bioavailability of contaminants to microbial cells for degradation. Additionally, the lipopolysaccharides and EPS in biofilm structure could act as a chelating agent facilitating the bioremediation of toxic pollutant (chlorinated organics). Therefore, microbial colony with great density on surfaces and materials are utilized in the processing of bioremediation (Sarjit et al. 2015). Biofilms mostly use substrates including straw, saw dust, or corn cobs as carbon sources to enhance degradation (Vidali 2001). Biofilms due to their great cell density and stress robustness could effectively metabolize hydrophobic and toxic substances. In general, biofilm formation is explained by quorum sensing mechanism based on a population density from cell–cell communication through signaling molecules. Biofilm signaling molecules are involved in the degradation and detoxification of pollutants. As the degradation of pollutants depend on quorum sensing signal of biofilms, biofilms with high cell signaling charge are favored for bioremediation (Mangwani et al. 2015).

Mostly aerobic microorganisms as well as fungi and anaerobic microorganisms are used in bioremediation. Aerobic microorganisms including *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium* have a potential for degradation of pesticides, hydrocarbons, alkanes and polyaromatic substances. These aerobic bacteria utilize these pollutants for the requirement of carbon and energy. Anaerobic bacteria are utilized in order to degrade polychlorinated biphenyls in river sediments and dechlorinate the solvent trichloroethylene and chloroform (Vidali 2001). Bioremediation by fungi are called as mycoremediation. Fungi (*Phanaerochaete chrysosporium*, *Aspergillus terreus*, *Aspergillus niger*, *Rhizopus nigricans* and *Cunninghamella*) provide degradation of wide variety toxic

contaminants by secreting their extracellular enzymes (Bennett and Faison 1997). Contaminant-specific fungi are used in mycoremediation (Kshirsagar 2013).

Biofilms have been known as available for the remediation of various contaminants due to their great microbial density and ability with regard to immobilization of contaminants. Biofilm researches about ecology of soil, sand, sediments and wetland vegetation have found out that biofilms are also useful for wastewater treatment. Biofilms may be successfully applied for the bioremediation of waste waters (Das et al. 2017). In wastewater treatment, the addition of a certain chosen strains to a complex environment is referred bioaugmentation (Morikawa 2006). Some microorganisms which form beneficial biofilm in the process of wastewater purification are reported as *Enterobacter agglomerans*, *Cronobacter sakazakii*, and *Pantoea agglomerans* (Turki et al. 2017). Microbiological and chemical contamination of water from industrial areas endangers safety of drinking water. Additionally, contaminated water causes toxic effect to marine life and humans (Sarjit et al. 2015). In waste water treatment colonization of beneficial biofilms occur firstly on suspended solid particles within wastewater. Applications related to wastewater treatment were often observed in food and agricultural (mostly animal husbandry) industries. For examples, beneficial biofilms by *Pseudomonas* sp. and *P. diminuta* were applied in bioremediation of polluted wastewater with vegetable oil and grease. A biofilm sand filter system designed for removing of these pollutants exhibited high degradation activity (Masry et al. 2004).

The most commonly used wastewater treatment is a trickling filter since 1880 (Robertson and McLean 2015; Qureshi 2009). Beneficial microbial cells in the biofilm degrade various compounds including phosphorous and nitrogen-containing substances, carbonaceous materials, and trapped pathogens from the wastewater. After the removal of contaminants or mess, treated water of a biofilter is either released to the nature or utilized for agricultural and other recreational targets. The removing of the contaminants from wastewater are performed through biofilm on various filter media (Sehar and Naz 2016).

Food industry is one of the most important industrial fields with regard to the accumulation of waste water production and other pollutants. Bioremediation is recent approach to food waste management. Olive oil industry, fruit and vegetable processing industry, fermentation industry, dairy industry and meat and poultry industry produce a variety of wastes at different rate and composition, mostly solid suspensions, product specific-liquids, biological oxygen demand and chemical oxygen demand (Thassitou and Arvanitoyannis 2001; Alimoradi et al. 2018). For instance, cheese whey wastewater treated to aerobic and anaerobic biodegradation for the aim of bioremediation (Carvalho et al. 2013). Additionally, phenols as agro-industrial effluents could pose a risk in drinking water and irrigation water or in cultivated land. Removal or transformation of phenols is possible with bioremediation (Chiacchierini et al. 2004).

Agriculture industry generates wastes including pollutants (chemical fertilizer, pesticide and others) and water used in farming fields. Bioremediation process can be applied for degradation of these wastes in agriculture industry (Das et al. 2017). Contamination of the veterinary antibiotic sulfamethazine to agricultural soils

through manure applications pose a risk for the ecology and human health. For this reason, researchers offered the removal of this pollutant with bioremediation. Here, beneficial biofilm formed by *Microbacterium* spp. induced the degradation of pollutants (Hirth et al. 2016).

As some of marine bacteria have ability of biofilm formation and extracellular polymeric substances production, they could be used in bioremediation process of heavy metals, hydrocarbon and many other recalcitrant compounds and xenobiotics (Dash et al. 2013).

Biofertilizers and Biocontrol Agents for Plants

For sustainable agriculture and ensuring of food safety, another approach is to apply composition of organic fertilizers and biofilm-based biofertilizer and biocontrol agents in soil. Adhesion of various microbial cells to the plant roots generates antagonism between plant and biofilms and provide robustness to detrimental conditions (Ramey et al. 2004; Hettiarachchi et al. 2014). Microbial colonization on diverse plant part surfaces of different plant species induces beneficial effects such as biocontrol and symbiosis (Rudrappa et al. 2008). Some colonized bacteria for desirable properties are as follows:

- *Bacillus subtilis*, *Pseudomonas fluorescens*, *Pseudomonas putida* and *Pseudomonas chlororaphis* based biofilms around the root of crop plants for biocontrol,
- *Microsphaeropsis* sp. based biofilms around the root of onion for biocontrol,
- *Bacillus polymyxa* based biofilms around the root of cucumber for biocontrol,
- *Rhizobium* and *Sinorhizobium* based biofilms around the root of legumes for symbiosis,
- *Azorhizobium caulinodans* and *R. leguminosarum* around the root of rice for beneficial effect,
- *Azospirillum brasilense* and *Klebsiella pneumoniae* based biofilms around the root of wheat for beneficial effect
- *Gluconacetobacter diazotrophicus* based biofilms around the endophytic of sugar cane for beneficial effect (Rudrappa et al. 2008).

There are increasing demands on beneficial biofilm practices as biofertilizers or biocontrol agents (Garcia et al. 2011). Biofilm based products as biofertilizer and biocontrol agent in plants were increasingly improved for novel applications. The regulations supporting organic farming or sustainable agriculture production will be induced the use of beneficial biofilms (as biofertilizer and biocontrol agents) and detection of a variety microbial strains forming biofilm (Seneviratne et al. 2008). Until now, beneficial biofilm applications have been performed on various crops such as soybean, chickpea, tomato, bitter gourd, radish, okra, chilli, Hungarian wax pepper, aubergine, cabbage, mungbean, wheat, maize, corn, rice, lettuce, onion, strawberry and tea. However, as a novel strategy, the discovery of more resistant biofilms to adverse environmental factors (drought, salinity, inorganic, and organic

pollutant, etc.) is the purpose to improve the food quality (Malusa et al. 2012; Marapatla 2014; Seneviratne et al. 2016; Velmourougane et al. 2017; Singhalage et al. 2019).

Industrial farming used synthetic chemical fertilizers, pesticides, herbicides and other continual inputs endangers sustainable agriculture. These synthetic chemical substances could eliminate beneficial microorganisms which causes a drop in soil fertility and product yield (Seneviratne et al. 2008). Recently, as a result of boosting interest in sustainable agriculture applications, beneficial microbial strains have been utilized as biofertilizer. Biofertilizers are natural substances with microbial origin (bacteria, algae and fungi) which increase bioavailability and uptake of essential nutrients for crop plants such as rhizobia and mycorrhizal fungi. They could apply to seed, plant surfaces, soil by colonizing the rhizosphere or the interior of the plant (Gupta and Anand 2018). Biofertilizers were often called as plant growth promoting microorganisms (Rafique et al. 2015; Seneviratne et al. 2016). Microbial strains such as *Bacillus*, *Enterobacter*, *Burkholderia*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Beijerinckia*, *Erwinia*, *Flavobacterium*, *Rhizobium* and *Serratia* are the main plant growth promoting bacteria (Kasim et al. 2016). On the other hand, plant growth promoting microorganisms are mostly divided into three class including arbuscular mycorrhizal fungi, plant growth-promoting rhizobacteria and nitrogen fixing rhizobia (Malusa et al. 2012). These microorganisms enhanced the bioavailability of phosphorus (P) and nitrogen (N) and other essential trace elements by plant (Basu et al. 2017). Nitrogen fixing and P solubilizing bacteria called as "a Plant Growth Promoting Rhizobacteria" increase the N and P uptake (essential nutrients) of the plants. Beneficial biofilms exhibited great proportions of biological nitrogen fixation and organic acid production (Babu et al. 2017). Nitrogen fixing bacteria enhances the growth and resistance of effective microbial communities in the soil by supplying N through biological nitrogen fixation. On the other hand, N fertilizers having negative impact on N2 fixers reduced soil fertility and crop yield. Beneficial biofilms with N2 fixers restored soils deteriorated by common agricultural practices in tea cultivation. Nitrogen fixing bacteria including *Acetobacter* spp., *Azotobacter* spp., *Rhizobium* spp., *Bradyrhizobium* spp. and *Colletotrichum* spp. could be used as biofertilizers in sustainable agriculture (Seneviratne and Wijepala 2011).

Plant growth promoting microorganisms are able to produce exopolysaccharides and volatile organic compounds. Interactions between plant and microorganisms were established through signal molecules from quorum sensing that induces biofilm formation, competence, sporulation, and antibiotic production (Basu et al. 2017). Rhizosphere is a region around the plant root which acts as habitat of various microorganisms such as bacteria, fungi, actinomycetes, protozoa and algae. Some of these microorganisms promote the growth of plants. Plant growth promoting bacteria consist of planktonic cell or microbial endophytes colonizing part of the interior tissues of the plant (Santoyo et al. 2016; Basu et al. 2017).

Beneficial biofilms formed by fungi and bacteria were successfully utilized as biofertilizers to increase productivity in nonlegume crops. For instance, inoculation of biofilm forms of fungal rhizobia caused more N_2 fixation in soybean than

inoculation of traditional rhizobium. Wheat seedlings subjected to bacterial biofilm showed higher productivity in moderate saline soils. Additionally biofilm forms acquired robustness to microbial cells for the survival stability against adverse environments. For example, the survival of rhizobia in biofilm matrix are 105-fold more than rhizobial monoculture at great salty conditions (Malusa et al. 2012).

In maize, wheat and cereals, *Azospirillum brasilense* and other *Azospirillum* sp. (plant growth promoting bacteria) exhibit the formation of biofilm on the surface of the root. Various Agrobacterium sp. and symbiotic rhizobia strains have the ability of adhesion on root and form microbial colony or biofilms. *Agrobacterium tumefaciens* cause thick and complex biofilm formation on the surface of root (on mostly epidermis and root hairs) (Rafique et al. 2015).

Saline soils in agricultural fields have a negative effect on productivity of crop. *Rhizobacteria* ensuring plant growth under stress conditions regulate nutritional and hormonal balance. For example, biofilms from *Bacillus amyloliquefaciens* as plant growth promoting *Rhizobacteria* were also useful for ensuring of salinity tolerance in barley (Kasim et al. 2016).

In organic farming or sustainable agriculture, animal manure as source of nutrients could enhance crop productivity by improving the biochemical composition of the soil. However, the bioavailability of manure by plants depends on recover and deposition of nutrients in manure. On this sense, microbial biofilms (For example biofilms from Chlorella vulgaris, green microalga as a biofertilizer) were applied and provided the conversion of organic and inorganic components in dung into more available substances (cellular components) (Rajendran et al. 2018).

As known, plant growth in corn are promoted by biofilms. Biofilms of *Pseudomonas* spp., *Bacillus* spp. and *Aspergillus* spp. caused increased productivity in maize (*Zea mays*) (Babu et al. 2017). Similarly, biofilms formed as a result of interaction and colonization between *Azorhizobium caulinodans* and *Aspergillus* spp. around rice root acted successfully as biofertilizers (Trimanne et al. 2018). In some cases, biofertilizers are used in combination with chemical fertilizers or other natural fertilizers for higher productivity. For instance, the treatment of biofilm biofertilizer obtained from *P. fluorescens* and *R. leguminosarum* with bentonite and chemical fertilizer increased growth and productivity of wheat (Ratha and Jasim 2018). In another study, a fertilizer consisting of biofilm fertilizer (*Aspergillus* sp. and *Enterobacter* sp.) and chemical fertilizer resulted in increased strawberry yield (Singhalage et al. 2019). The practices of organic fertilizer composted with fluid biofilm biofertilizer on dry land enhanced uptake of soil nutrients and spinach productivity (Sudadi and Triharyanto 2018). As a result of all these literatures, in next studies, different biofilm combinations should be tested for more effective biofilm practices.

In addition to biofertilizer properties of biofilms, biofilms formed by microbial strains as biocontrol agents were successfully practiced in crop or plants. For example, fungal–bacterial biofilms (*Pleurotus ostreatus–Pseudomonas fluorescens*) acted as biocontrol agent in the edible mushroom and also enhanced the protein amounts of mushroom with their positive effect on nutritional product quality (Seneviratne et al. 2008). *Bacillus* spp. was mostly used as biocontrol agent in

agriculture industry. Also, *Bacillus*-based pesticides, fungicides and fertilizers are commercially available (Garcia et al. 2011). *Bacillus* species such as *B. subtilis*, *B. thuringiensis* and *B. amyloliquefaciens* mostly placed on plant rhizosphere could produce beneficial biofilms prevent the infection from plant pathogens (Ayala et al. 2017; Beauregard et al. 2013). In the rhizosphere which is the area of soil surrounding a plant root system, *B. subtilis* promotes plant growth and inhibits undesirable microorganisms. Its biofilms are commercially used as biocontrol agents to overcome fungal infections in plants. Surfactin producer *B. subtilis* biofilms could prevent or restrict the colonization of other microbial free cells. Similarly, virulence of plant pathogen *Erwinia carotovora* are restricted by *Bacillus thuringiensis* with signaling molecules (Morikawa 2006). *Pseudoalteromonas tunicata* called as marine specific-endophytic bacterium is colonized through stimulation of the green macroalga (*Ulva lactuca*) and thus generates antifouling substances to prevent adhesion of harmful microorganisms. Another marine macroalgae *Delisea pulchra* with great antifouling activities restricts the colonization of detrimental bacteria. In marine aquaculture, coating of fish egg by beneficial biofilms hinders infection from harmful microorganisms and additionally provide the protection of the water quality and health of adult fish (Wesselin 2015). *Pseudomonas* sp. have also high performance as biocontrol agents because this strain forms biofilms with great mass on the surface of root. High biomass in biofilm matrix from *Pseudomonas* sp. cause too extensive biofilm network channel and signaling molecules supporting inhibitory activity (Ratha and Jasim 2018). Another beneficial biofilm practice is adhesion of *Pseudomonas chlororaphis* to wheat rhizosphere against fungal diseases. Favorable microorganisms tend to nutrients or metabolites from biotic surfaces (root, rhizosphere, leave and other surfaces of crop or plant) through chemotaxis. Accumulation of beneficial microbial colonies on the surface of crop or plant ensures the protection against pathogenic microorganisms. As a result, there is a competition among microbial communities (beneficial or detrimental) on the surface of plant and new biotechnological applications are to render beneficial microorganisms or biofilms dominant (Wesselin 2015).

Biofilm Reactors (Bioreactor)

Bioreactor are often used for innumerous productions in various industrial field. Production in bioreactors are performed economically with high yield (Qureshi 2009). Recently, researchers focused on the term of biofilm based bioreactor. Because, biofilm formation in reactor lead to higher productivity through wide surface area of biofilm structure (Ercan and Demirci 2015). The use of biofilms in reactors provides many advantages such as higher biomass density, improved productivity and stability (Cheng et al. 2010). Biofilm reactors are used for a wide range of purposes including wastewater treatment, biofuels (ethanol, buthanol), organic acid production (citric acid, lactic acid, acetic acid, succinic acid and fumaric acid, polysaccharide, alcohol production (ethanol, buthanol), enzyme

production, vinegar production (Qureshi et al. 2004; Qureshi 2009; Cheng et al. 2010; Todhanakasem 2013; Ercan and Demirci 2015). Particularly, biofilms in the manufacture of vinegar have been successfully performed for a long time. Vinegar were produced by *Acetobacter* or *Gluconobacter* on free-floating wood chips with higher yield. Wide surface area of wood chips boosts the growth and activity of vinegar bacteria which convert substrate into product (Zottola and Sasahara 1999). Additionally, biofilm reactor causes more yield in the production of antimicrobial substances and pigments (Morikawa 2006).

Some of products obtained in bioreactors were presented in Table 1.

Microorganisms on the surface of marine algae compete with each other for the limited amounts of nutrients. The production of inhibitory metabolites by marine epibiotic microorganisms are more than by planktonic cell. Additionally, under in vitro conditions, microbial cells in shaking flask cease the production of antibacterial metabolites. However, Bioreactor produce more antimicrobial substances by *B. subtilis* and *Bacillus pumilus* and a red pigment by *B. licheniformis*. As a result, cell to cell communication or signal molecules in biofilms regulates the expression of pigments and antimicrobial metabolites (Morikawa 2006).

Biofilm reactors have a potential for wastewater treatment in food and agricultural industry (Sarjit et al. 2015). Decomposition of food waste could be performed

Table 1 Beneficial biofilm based productions in bioreactor (Rosche et al. 2009; Qureshi 2009; Cheng et al. 2010; Ercan and Demirci 2015)

Biofilmed microorganisms used in bioreactor	Products
Zymomonas mobilis *S. cerevisiae*	Ethanol
Acetobacter aceti	Acetic acid or vinegar
Gluconobacter oxydans	Dihydroxyacetone
Lactococcus lactic, *L. lactis*	Nisin
Acetobacter xylinum	Pyruvic acid, Bacterial cellulose
Aspergillus niger	Citric acid, cellulose, xylanase
R. oryzae	Fumaric acid
L. amylophilus, *L. casei*, *L. delbrueckii*, *Pseudomonas fragi*, *Streptomyces viridosporus*, *Thermoactinomyces vulgaris*, *Rhizopus oryzae*, *Lactobacillus plantarum*, *L. brevis*, and *L. fructivorans*	Lactic acid
A. succinogenes	Succunic acid
C. acremonium	Cephalosporin C
E. coli	Amylase
A. terreus, *Trichoderma viride*	Cellulase
P. chryosporium	Lignin peroxidase
Rhizopus chinensis	Intracellular lipase
Phanerochaete chrysosporium	Lignin peroxidase
A. pullulans	Pullulan
X. campestris	Xantan

by anaerobic microorganism in bioreactor (Khan et al. 2018). Olive mill wastes form the most important sources of antimicrobial phenolic compounds (Carraro et al. 2014). Bioremediation of olive mill wastewater is quite hard because of its high chemical oxygen demand, high phenolic content and dark color. Anaerobic reactors with high performance as well as aerobic reactors are successfully used in degradations or removal of olive mill effluents (Mcnamara et al. 2008). In terms of waste, cheese industry also generates a great amount of whey and milk permeate and this situation causes economic loss originated from waste. For the benefit of agricultural and food industry, cheese factory effluents or wastes should be converted into another valued product (Wang et al. 2009). As a matter of fact, in a previous study, cheese whey was fermented by *Clostridium acetobutylicum* to produce buthanol in biofilm reactor (Raganati et al. 2013).

Anticorrosive Biofilms

In food and agricultural industry, corrosion is undesirable situation because of both economic and processing loss (Gupta and Anand 2018). When metal surfaces in various devices, equipment, etc. subjected to any substances from processing conditions or environment, deterioration on metal surfaces could be observed. Factors led to corrosion were divided into chemical (oxygen, ammonia or hydrogen sulphide, organic and inorganic acids) and biological (enzymes, microorganisms—mostly sulfate-reducing bacteria) (Beech and Sunner 2004; Ivanova and Ivanov 2013).

Industry applies various methods to prevent corrosion and recently have preferred biological methods such as the use of microorganisms due to environmental concern. In particular, oxygen-consuming and iron-reducing bacteria (*Shewanella oneidensis*) were reported as anticorrosive (Lee et al. 2006). Although anticorrosive strategies with microbial cells are commonly used, the prevention of corrosion through beneficial biofilms is a new technique. Beneficial biofilms successfully prevent and decrease the metal corrosion. Methods used in repress of corrosion with biofilms are divided into three. In first method, bacteria removes corrosive substances (oxygen and others) with aerobic respiration. In another method, corrosive microorganisms (sulfate reducing bacteria) are inactivated by inhibitory compounds from biofilms. As third method, biofilm coats the surface of industrial equipment and act as defensing cover (Zuo 2007).

As mentioned above, sulfate reducing bacteria cause the biological corrosion of metal including iron, copper, aluminum carbon steel, stainless steel and some alloys. *Bacillus brevis* biofilms inhibit *D. orientis* known as the sulfate-reducing bacterium and *L. discophora* as the iron-oxidizing bacterium and thus achieve the reduction in mild steel corrosion (Morikawa 2006). Secretion of polyanionic chemical substances (inhibitory proteins) by microbial cell in biofilm matrix (antimicrobial proteins) destroy corrosive sulfate reducing bacteria. Aerobic bacteria, mostly *Bacillus* species such as *Bacillus Subtilis* or *Bacillus licheniformis* were utilized to suppress metal corrosion (Örnek et al. 2002; Wood et al. 2002; Narekumar et al. 2017).

Additionally, EPS synthesis by *Lactobacillus delbrueckii* and *L. fermentum* as well as biofilms from this lactic acid bacteria assist to the prevention of metal corrosion. This mean that lactic acid bacteria could have anticorrosive potential (Ivanova et al. 2009; Ivanova and Ivanov 2014; Tsveteslava and Ivanov 2014). In another report, *Acetobacter aceti* biofilms exhibited anticorrosive properties by protecting or coating carbon steel surfaces. This finding is highly interesting and novel because acid-producing bacteria have been known as corrosive until now. Briefly, biofilm structure of microbial strains protects steel surfaces against corrosion unlike planktonic cells of microbial strains (France 2016).

Conclusion

Biofilm formation is a significant problem in health, food, agriculture and other industrial fields as biofilms are commonly regarded as detrimental. On the other hand, biofilms in food and agriculture industry have recently gained attention because of their beneficial properties such as food fermentation, probiotic potential, the promoting of plant growth, prevention of corrosion, inactivation of undesirable microorganism growth, wastewater treatment, destruction of industrial pollutants, etc. In particular, production with higher yield could be possible with biofilms because biofilms as microbiota embedded in extracellular polymeric substances matrix have high biomass due to a wide diversity of microbial life and metabolic potential. Although, desirable activities of biofilms are mostly associated with their quorum sensing mechanisms that provide cell to cell interactions through signaling molecules, their function are not yet completely clear. Until now, researchers have mostly focused on detrimental biofilms, biofilm formation stages, persistence of biofilms and biofilm architecture. This chapter pointed out the importance of beneficial biofilm applications in food and agricultural industry and prompts future studies based on beneficial biofilms. Especially, in future, the discovery of beneficial biofilm formation by a variety of microorganisms will bring about novel biotechnological applications in different industrial fields.

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Next-Generation Probiotics Their Molecular Taxonomy and Health Benefits

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Abstract The concept of probiotics although perceived as new is more than a century old. Since the early studies of the Elie Metchnikoff in 1903, a number of commercial products containing probiotics are in the market. The recent success of converting probiotic products into commercial reality was achieved by the scientists like Minoru Shirota and Kellog. Minoru Shirota is a Japanese scientist who successfully demonstrated the health benefits of probiotics and commercialized the globally known probiotic drink Yakult. This renewed interest in probiotics is spurred by the recent advances made in understanding the human microbiome and its role in human health. The link between the gut microbiome and human health is becoming increasingly clear and is well described. Nevertheless, the gut microbiome is continuously influenced by a number of factors like diet, lifestyle and consumption of antibiotics. A healthy gut microbiome can be retained and maintained by using various probiotics. Moreover, the probiotic microorganisms are no more limited to a few conventionally used bacteria and are being currently represented by more phylogenetically diverse microorganisms than previously thought. These probiotic microorganisms include conventionally used Lactic acid bacteria, like *Lactobacillus* and recently identified probiotic bacteria like *Akkermansia muciniphila*, *Bifidobacterium infantis*, *Bacteroides fragilis*, *Clostridium butyricum*, *Faecalibacterium prausnitzii* and *Streptococcus thermophiles* etc. Many of these probiotic strains have a shared mechanism of action, while strain specific, species-specific or genus-specific probiotic effects have also been documented. Probiotics are administered as live cultures or as spores, directly or through fermented dairy products, food, and drinks. Probiotics based therapies like fecal microbiota transplant are also being used successfully for treating medical conditions and diseases like diarrhea, constipation, vaginitis, necrotizing enterocolitis, inflammatory bowel disease, *Clostridium difficile* infection, and others. Reports showing a clear role of probiotics in immunomodulation, prevention of cardiovascular diseases and even cancer are also emerging. Yet, a number of microorganisms in the gut remain uncultured and many candidate probiotic

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microorganisms remain poorly identified, requiring correct identification and a rigorous evaluation as probiotics. Probiotics may be a century old but require fresh attention keeping in view the recent advances made in understanding the gut microbiome and the role of these microorganisms in human health.

Keywords Probiotics · Health benefits · Molecular taxonomy · Microorganisms

Introduction

The name Probiotics originated from the Latin word "*pro*" meaning "for" and the Greek "*βιοσ*" meaning "Life". Werner Kollath (1953) used the term for active substances that are essential for healthy development of life (Gasbarrini et al. 2016). History of the use of probiotics is as old as 10,000 years, but the more recent work of the Russian scientist Elie Metchnikoff (1900s) is considered as pioneer work on probiotics (Ozen and Dinleyici 2015). Though Louis Pasteur did the pioneering work on fermentation but the effect of fermented products on human health was studied by Elie Metchnikoff. He associated the long-life expectancy of Bulgarian people with the consumption of yogurt containing the bacterium referred to as Bulgarian Bacillus (Mackowiak 2013). His conclusions that the intestinal microbes depend on the food, and the type of food may help in modifying the flora in our bodies and to replace the harmful microbes by useful microbes, clearly laid the foundation of the probiotic concept (Burki 2018). Metchnikoff actually used the term "Orthobiosis" in his publications entitled, "The nature of man: Studies in Optimistic Philosophy (1903)" and "The Prolongation of Life: Optimistic Studies (1907)" for natural ways for delaying senility and for health (Podolsky 2012). Although a number of products containing probiotic bacteria were in use traditionally, the commercialization of Yakult in 1930 is undoubtedly a global success story of probiotic commercialization.

Since then the research on probiotics was largely ignored till the revolutionization of DNA sequencing techniques in the late 1990s. These techniques greatly improved our understanding of human microbiome and its role in health and disease (Cho and Blaser 2012). The role of microorganisms in many diseases is becoming increasingly clear (Fong 2014; Neish 2009; Macpherson and Harris 2004). Even the problems of obesity, some cancers, and metabolic syndrome are being associated with the unhealthy microbiome (Vrieze et al. 2012; Boulangé et al. 2016; Chang and Parsonnet 2010). Therefore, from the current understanding of the human microbiome and their role in controlling various diseases new evidences have been gathered in support of the theories of Metchnikoff. Furthermore, many more new microorganisms are being identified as probiotic, and the mechanism of their probiotic activities are now better understood. These probiotics are often referred to as next-generation probiotics and are discussed in this chapter.

Prebiotics, Postbiotics/Parabiotics, and Synbiotics

Since, many terms including prebiotics, postbiotics, and synbiotics are in use it is necessary to discuss these terms briefly before a detailed discussion on probiotics. Prebiotics can be defined as nutrients that favor the growth of probiotic bacteria in the gut (Delgado-Fernández et al. 2019). Some of the well-known prebiotics include fructan and nonfructan oligosaccharides (Anadón et al. 2016). While, the postbiotics/parabiotics can be defined as functional foods that contain probiotic effector molecules in the form of nonviable probiotic organisms, or cell lysates resulting in the required health benefits of probiotics. Generally, these postbiotics include molecules like acetaldehydes, bacteriocins, organic acids, and hydrogen peroxide. Recent studies suggest that effector molecules present in the diet even in the absence of producer probiotic organism show the probiotic activity. For example, it has been demonstrated that muramyl dipeptide-based postbiotics curtails liver insulin resistance and fat inflammation via NOD2 (Cavallari et al. 2017). Synbiotics are functional foods or formulations that contain both probiotics and prebiotics. Since, synbiotics are a combination of both probiotics and prebiotics, these formulations enhance the establishment and selection of probiotic bacteria in the gut (Mohanty et al. 2018). An overview of the four terms is shown in Fig. 1.

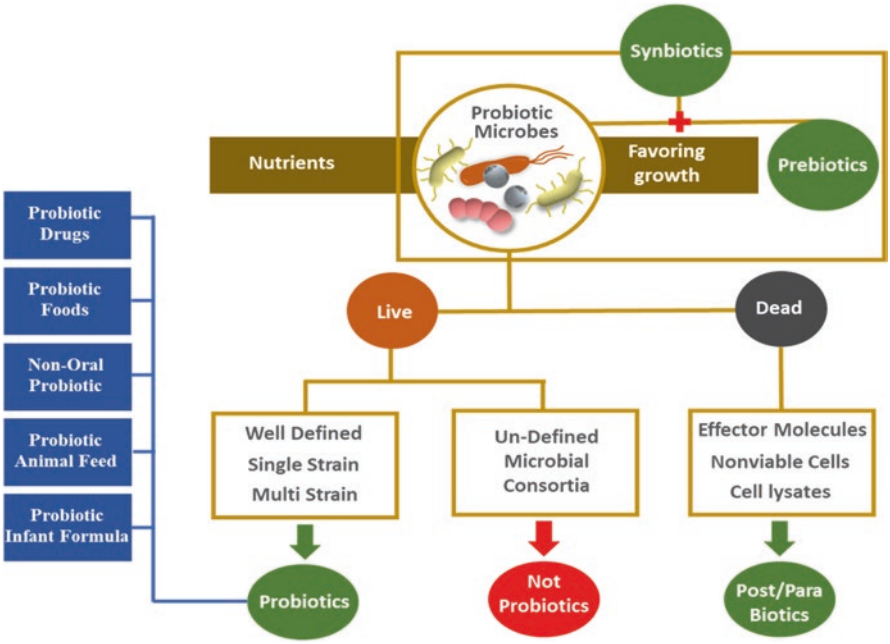

Fig. 1 The concept of probiotics, prebiotics, post/parabiotics and synbiotics

Definition of Next-Generation Probiotics

Though there is no legal definition of Probiotics for regulatory purposes in the United States. Various definitions for probiotics have been proposed earlier (Sanders 2008). For example, Fuller described probiotics as "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance" (Fuller 1989). Havenaar and Huis In't Veld defined probiotics as follows "a viable mono or mixed culture of bacteria which, when applied to animal or man, beneficially affects the host by improving the properties of the indigenous flora" (Havenaar and Huis In't Veld 1992). A more recent definition of probiotics is "live microorganisms, which when consumed in adequate amounts, confer a health effect on the host" (Guarner and Schaafsma 1998). This definition has been slightly grammatically modified by the International Scientific Association on Probiotics and Prebiotics (ISAPP). ISAPP has redefined probiotics as "*live microorganisms that, when administered in adequate amounts, confer a health benefit on the host*" (Hill et al. 2014). This definition is widely accepted, which may also change based on the new knowledge. However, as of now, the next generation probiotics can be defined as above. Other terms are also used for probiotics such as live therapeutic products.

Guidelines for the Use of Term Probiotics

Widespread commercialization of probiotics requires clear guidelines for identifying probiotic strains, their safety evaluation, and regulation. This has also renewed the interest of Scientific community in probiotics and as of 11 Jan 2019, there were more than 20,000 and 30,000 documents in PubMed and ScienceDirect on probiotics, respectively. Many commercial products claim unsubstantiated health benefits of the probiotic products. Furthermore, although the use of undefined gut microbial communities such as fecal microbiota transplants as probiotics is also becoming increasingly acceptable. But the use of such undefined microbial communities has its own potential risks. These developments should be properly regulated based on controlled studies. World health organization and Food and Agriculture Organization (FAO) organized expert meetings in 2001 and 2002 to issue a consensus statement for using the term probiotics and other guidelines related to probiotics. It was agreed in the meeting that the term probiotic should only be used for microbial species exhibiting clear health benefits in properly controlled studies. Commercial products should only use the term "contains probiotics" and any other claim regarding the health benefit should be substantiated. The use of live microbial species without any known health benefit should be discouraged and the term probiotic should not be used for such microbial species. Similarly, undefined microbial communities should not be defined as Probiotics. While, defined microbial consortia of human origin with known health benefits and safety can be defined as Probiotics (Table 1) (Hill et al. 2014).

Table 1 Some of widely used probiotic strains

Genus	Species and strain	Reference
Lactobacillus	*L. rhamnosus* GG, *L. acidophilus* NCFM, *L. casei* Shirota, *L. reuteri* MM53, *L. rhamnosus* GR-1, *L. fermentum* RC-14	Reid (1999)
Bifidobacterium	*B. lactis* HN019, *B. longum* CECT 7210, *B. catenulatum*, *B. breve* Yakult, *Bifidobacterium bifidum* NCFB 1454, *B. animalis*	Vlasova et al. (2016)
Bacteroides	*B. uniformis* CECT 7771, *B. fragilis*	El Hage et al. (2017)
Bacillus	*B. coagulans* 15B, *B. subtilis* CU1, *B. licheniformis* CH200	Elshaghabee et al. (2017)
Streptococcus	*S. thermophilus* FP4	Jäger et al. (2016)
Clostridium	*C. butyricum* MIYAIRI 588	SEKI et al. (2003)
Enterococcus	*E. faecium* K77D	Hanchi et al. (2018)
Akkermansia	*A. muciniphila*	Cani and de Vos (2017)
Faecalibacterium	*Faecalibacterium prausnitzii*	Martín et al. (2017)

Phylogenetic Diversity of Next-Generation Probiotics

Understanding the taxonomy of probiotic bacteria is key to their commercialization, since one strain of the same species may exhibit probiotic properties while other strain may not (Campana et al. 2017). It is recommended that the strains should be identified using International Code of Nomenclature and should be deposited in an Internationally recognized microbial culture collection (Morelli and Capurso 2012). Species-level identification should be based on DNA-DNA hybridization and 16S rRNA gene sequences. While, the strain level identification should be based on pulse field gel electrophoresis and randomly amplified polymorphic DNA or RAPD (Hill et al. 2014; Morelli and Capurso 2012). With the discovery of improved sequencing technologies more sophisticated approaches like whole genome sequencing, average nucleotide identity and multilocus sequence analysis is also being used to better understand the taxonomy of the closely related probiotic strains (Diancourt et al. 2007; Huang et al. 2018). The whole genome of hundreds of the probiotic strains including the members of the genus *Bacillus*, *Bifidobacterium*, *E. coli*, and *Lactobacillus* has been published (Siezen and Wilson 2010; Lukjancenko et al. 2012; Kang et al. 2017). However, even with the genome sequences of many strains available it remains difficult to have clear guidelines to delineate different strains as the genetic diversity of strains within a species varies greatly (Truong et al. 2017).

One of the oldest and well documented probiotic bacteria identified by Metchnikoff is *Lactobacillus bulgaricus* referred by him as Bulgarian Bacillus. Since then various species of *Lactobacillus* have been traditionally used as probiotics (Gasbarrini et al. 2016). Currently, the probiotics are not only limited to *Lactobacillus* and a number of different bacteria have been identified as probiotic bacteria. Which also necessitates a clear definition of probiotics, better regulatory guidelines and distinct guidelines for identifying the probiotics. For oral and dietary formulations bacteria belonging to different genera are being used in various commercial products. Some of the most widely used next-generation probiotics bacteria belong to the species of *Akkermansia*, *Bacteroides*, *Bifidobacterium*, *Clostridium*, and *Faecalibacterium* (Hill et al. 2014; Saarela 2019). It is to be noted that most of the genera listed above are Gram-positive bacteria but contrary to popular belief probiotic bacteria may also belong to Gram-negative bacteria such as *Akkermansia* and *Escherichia coli* Nissle 1917 (EcN). The latter being a well-studied probiotic bacterium (Behnsen et al. 2011). Other studies have claimed that the members of the genera *Pediococcus*, *Streptococcus*, *Propionibacterium*, and *Saccharomyces* also exhibit probiotic properties. Studies on gut microbiome have revealed that some genera though not the part of conventional probiotic food are associated with a healthy and robust gut microbiome. Strains of *Akkermansia muciniphila*, *Eubacterium hallii*, *Faecalibacterium prausnitzii*, and *Roseburia* spp. are not only associated with robust gut microbiome but also demonstrate clear health benefits in studies on animals. The suitability of these probiotics for their use in food is yet to be evaluated. Various species and strains of these genera identified as probiotics are shown in Fig. 2, which shows a phylogenetic tree of these bacteria based on 16S rRNA gene sequences.

Two of the most widely used genera as probiotics are *Lactobacillus* and *Bifidobacterium* belonging to phylum Firmicutes and Actinobacteria, respectively. The genus *Bifidobacterium* was originally described by Orla Jensen in 1924 and currently contains seventy species and ten subspecies (Orla-Jensen 1924). The current status of the Genus *Bifidobacterium* based on the genome sequences of 233 strains including the genomes of type strains is discussed in details by Lugli and colleagues (Lugli et al. 2018). Some of the species of Genus Bifidobacterium that are commonly used as probiotic include *B. adolescentis*, *B. animalis*, *B. bifidum*, *B. breve* and *B. longum*. The genus *Lactobacillus*, *however*, is a traditionally known probiotic bacteria which was officially published by Beijerinck in 1901. The genus currently contains 237 species and 29 subspecies. It is interesting to note that close to 200 species from the genus are used as probiotics (Salvetti et al. 2018). Some of the notable species of Lactobacillus used as probiotic include *L. acidophilus*, *L. casei*, *L. fermentum*, *L. gasseri*, *L. johnsonii*, *L. paracasei*, *L. plantarum*, *L. rhamnosus*, and *L. salivarius*. Salvetti and colleagues have discussed the recent advances in the taxonomy of the genus *Lactobacillus* and have suggested the presence of ten subclades within the genus (Salvetti et al. 2018). *Bacteroides* spp. are also used as probiotics and are found in high numbers in gut. *Bacteroides thetaiotaomicron*, *Bacteroides fragilis* and other species of Bacteroides can efficiently metabolise complex polysaccharides, produce short chain fatty acids and are known to modulate host immune

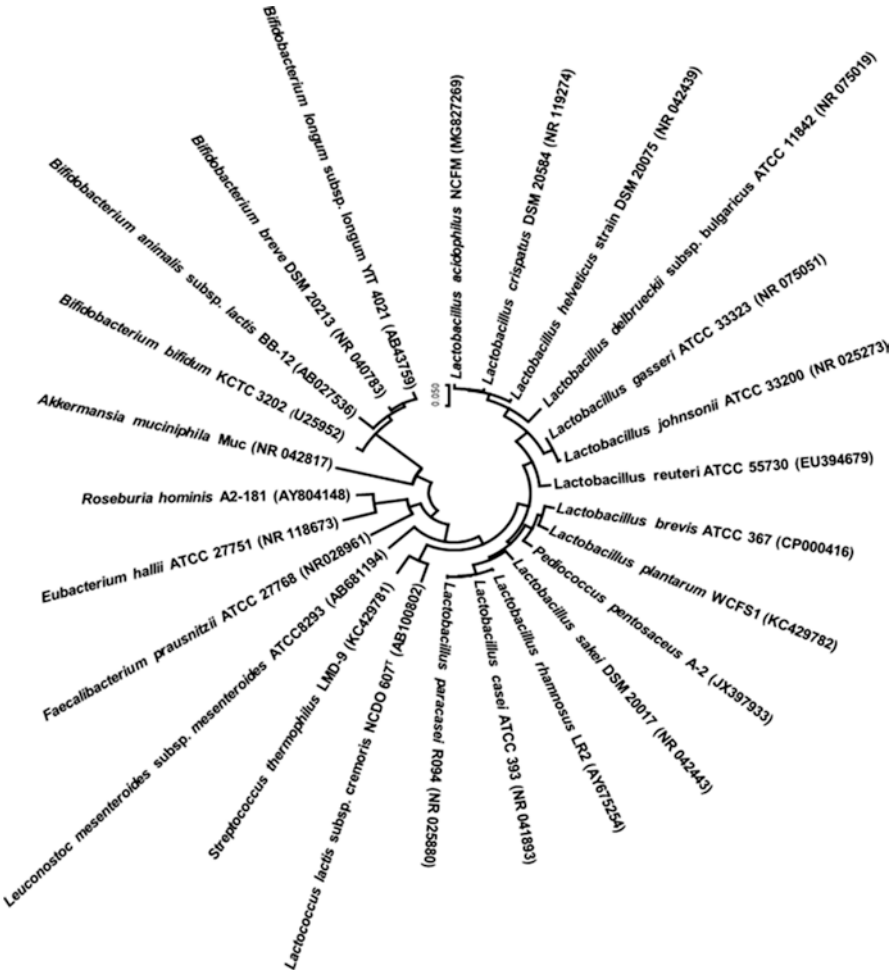

Fig. 2 Phylogenetic tree based on the 16S rRNA gene sequence showing the phylogenetic positions of some commonly used probiotic bacteria

system. Another potential probiotic that has been studied in details is *Clostridium butyricum* MIYAIRI 588. This strain is already available commercially as supplements. The role of the strain to provide protection against the infections of *E. coli* O157:H7 in gnotobiotic mice has been documented (Hayashi et al. 2013; Murayama et al. 1995). The use of *Clostridium butyricum* MIYAIRI 588 for animal feed is already authorized by the European Union (Saarela 2019). *Faecalibacterium prausnitzii* is a Gram-negative butyrate producing bacterium commonly found in the gut. In addition to the production of butyrate the bacteria also have immunomodulatory effect on the host. The ability of the bacterium to contain diarrhea in dairy calves has also been documented (Foditsch et al. 2016).

Next-Generation Probiotics: How to Select

Since the market for the probiotics is growing rapidly and a number of new probiotic candidates are being isolated and identified for their intended use by humans. A candidate strain must be thoroughly screened for a number of characteristics before their use as probiotics is finally approved, the first and the most important being the correct taxonomic identification of an organism to strain level. Identification of the health benefits associated with a candidate organism especially the core health benefits associated with probiotics (Hill et al. 2014). Furthermore, it is important to ensure that a candidate strain must not have properties that in any way may harm the consumer host such as infectivity and pathogenicity. A candidate strain must also meet the criteria described by regulatory authorities and should also be suitable for industrial production. For example, it should have adequate growth rates etc. This makes selection of probiotic microorganisms a systematic approach wherein a step by step screening of a candidate strain is involved (de Melo Pereira et al. 2018). Some of the important traits are discussed below and a brief outline is given in Fig. 3.

Correct Taxonomic Identification

Correct identification of a candidate strain is key to its success as it has been observed that many strains have strain-specific health benefits. It is recommended by the regulatory authorities like FAO that a combination of phenotypic and modern genotypic techniques should be employed for the identification of the organism at genus, species and strain levels. The use of modern techniques like 16S rRNA gene

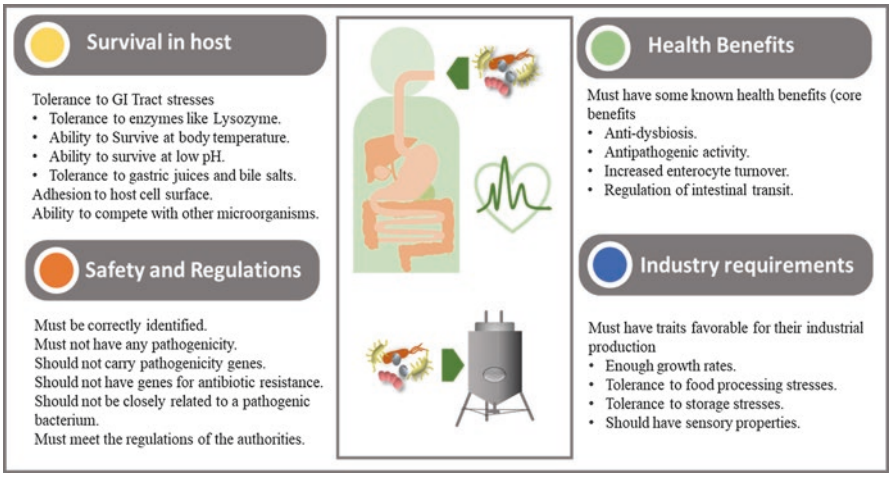

Fig. 3 A summary of parameters used for the selection of a candidate probiotic strains

sequence analysis, Fatty acid analysis, Pulse field gel electrophoresis, and even the use of whole genome sequence is also recommended. Furthermore, the old names should be replaced with new names if required. WHO also recommends that the probiotic strains should also be deposited in internationally recognized microbial culture collections (Huys et al. 2013).

Tolerance to GI Tract Related Stress

It is important for a candidate probiotic strain that it should tolerate the stresses posed by the human body Fig. 3. Once a candidate strain is given orally it should be resistant or tolerant to enzymes present in the oral cavity especially lysozyme. As most of the Gram-positive bacteria are sensitive to lysozyme, while some Lactobacillus bacteria (LAB) are resistant enough to be the part of human oral microbiome (Kõll et al. 2008). During the further passage of bacteria into the GI tract, the bacterium will be exposed to gastric juices and pepsin in the stomach and to the bile juices secreted by the liver. Therefore, a candidate probiotic strain should be tolerant to bile juices. It has been demonstrated that *Lactobacillus*, *Bifidobacteria* and many other probiotic bacteria contain Bile Salt Hydrolase (BSH) activity (Begley et al. 2006). Although the tolerance or resistance to various stresses varies with the probiotic strain. But it is recommended that the probiotic strains should be tolerant to a pH range of 2–5 and to a bile salt concentration range of 0.3–2% (Ogunremi et al. 2015). Mechanisms used by the probiotic bacteria to tolerate the GI tract stresses are reviewed in details by Bustos and colleagues (Bustos et al. 2018).

Potential to Colonize and Adhere to GI Tract

Another important property of a candidate probiotic bacterium is their ability to adhere to GI tract and to consequently colonize the GI tract. Various studies have demonstrated the ability of probiotic bacteria in-vitro to adhere to the surfaces coated with intestinal mucin or to GI tract cell lines such as HT29 (Nishiyama et al. 2016; Turpin et al. 2012). The adhesion of a candidate probiotic strain depends on the surface properties of the host epithelial cells and the biochemical composition of the probiotic strain's cell surface. The extracellular material secreted by bacteria is also known to influence the adhesion significantly (Boonaert and Rouxhet 2000; do Carmo et al. 2018a). Auto aggregation of probiotic bacteria helps the bacterium to achieve a high cell density which consequently helps the bacterium to adhere to the intestinal surfaces. The hydrophobicity of Bacterial cell surface is another property that affects its binding to epithelial cells. In an interesting study, about 163 strains of *Lactobacillaceace* were isolated and were screened for the presence of 14 genes potentially involved in the binding. It was observed that some of these genes (*ef-Tu*, *gap*, *groEl*, and *srtA*) were housekeeping genes and were therefore present in all the

strains (Turpin et al. 2012). While, other genes (*apf*, *cnb*, *fpbA*, *mapA*, *mub1*, and *mub2*) were present only in 86–100% LAB bacteria tested. This study provided the genetic evidence for the binding properties of the probiotic bacteria.

Health Benefits/Activity Against Pathogenic Bacteria

Probiotic candidates must have some distinct health benefits one of the most important being the antimicrobial activity against pathogenic bacteria. The antimicrobial activity of these probiotic bacteria maybe due to the chemical exclusion or through competitive exclusion. Chemical exclusion refers to the production of antimicrobial compounds like bacteriocins, enzymes, hydrogen peroxide and organic acids (Neal-McKinney et al. 2012; Dobson et al. 2012; Cotter et al. 2012). While, the competitive exclusion refers to antagonism through competition for nutrients and space for attachment (Lebeer et al. 2018; Callaway et al. 2008). There are many other health benefits associated with probiotic microorganism discussed below in details. For the screening of these traits, different assays are used (Papadimitriou et al. 2015).

Safety Assessment

Although, the probiotics are generally recognized as safe (GRAS) but there are many reasons that make it important to assess the safety of the probiotics. One is the ever-increasing list of microorganisms that are being identified as probiotics. The presence of antibiotic resistance genes and the possible presence of genes for pathogenicity (Zheng et al. 2017; Kochan et al. 2011; Doron and Snydman 2015). Therefore, regulatory authorities like USFDA, WHO, EFSA of European Commission and NHPR of Canada are making new regulations for the safety assessment of probiotics. These new regulations require information on history of isolation, correct taxonomic identification of the candidate strain, and absence of harmful traits such as infectivity, virulence, toxicity and the presence of transferable antibiotic resistance genes (Sanders 2008; Venugopalan et al. 2010; Wright 2005).

Strain Stability, Viability, and Commercial Production Related Properties

A number of other properties make a candidate strain suitable for its industrial production such as the ability to survive in the food, the ability to survive during storage without losing the viability and the ability to grow quickly during the fermentation to reach a desired optimal population.

Source of Probiotic Isolation

Probiotic microorganisms can be isolated from a number of sources. Since probiotics have been used traditionally in food such as dairy products and fermented foods globally. These products are good source of probiotics. Dairy products include milk from different dairy animals, yogurt, cheese, and fermented milk etc. Human milk has also been used for the isolation of probiotic microorganism (de Melo Pereira et al. 2018). While other probiotic based products include fermented meat, fish, pickles, cereals, vegetables, miso, tempeh and others (Rezac et al. 2018). These products can also be used for the isolation of probiotic bacteria. Bacteria from the gut of healthy individuals are also good source of probiotic bacteria. Lactic acid bacteria (LAB) have been isolated from feces samples of children and adults for their potential use as probiotics (Rubio et al. 2014). One of the well-known probiotic *Escherichia coli* Nissle 1917 was isolated from the feces of a soldier (Behnsen et al. 2011). A detailed list of various sources of probiotics is given in the review of de Melo Pereira (de Melo Pereira et al. 2018).

The Health Benefits and Mechanism of Next-Generation Probiotics

Plenty of literature is available demonstrating various health benefits of probiotic microorganisms (Behnsen et al. 2011). Probiotic microorganisms are not only known to help in the maintenance of healthy gut microbiome and treating gastrointestinal problems but are also known to treat and provide protection against many other microbial and non-microbial diseases including obesity and cancer (George Kerry et al. 2018; Song et al. 2018). Although the health benefits associated with various probiotic strains vary greatly, majority of the strains share some core health benefits. For example, majority of the probiotic bacteria produce β-galactosidase which helps in the digestion of lactose. The two most common benefits associated with probiotics are the maintenance of a healthy digestive tract and a strong immune system (Hill et al. 2014). The mechanisms through which these microorganisms exhibit probiotic activities have also been categorized into three classes. The first category which is widespread and is found in most of the probiotic microorganisms include resistance to colonization, production of short chain fatty acids, normalization of perturbed gut microbiota, increased turnover of enterocytes, competitive exclusion of pathogens and regulation of intestinal transit Fig. 4 (Hill et al. 2014). The second group of mechanisms that are less commonly found and are associated at species level include the production of vitamins, enzymatic activity, neutralization of carcinogens, bile salt metabolism and direct antagonism. While the third category of mechanisms are rare are found only in a few strains or they are strain-specific effects. These mechanisms include neurological effects, immunological effects, endocrinological effect and the production of specific bioactive compounds

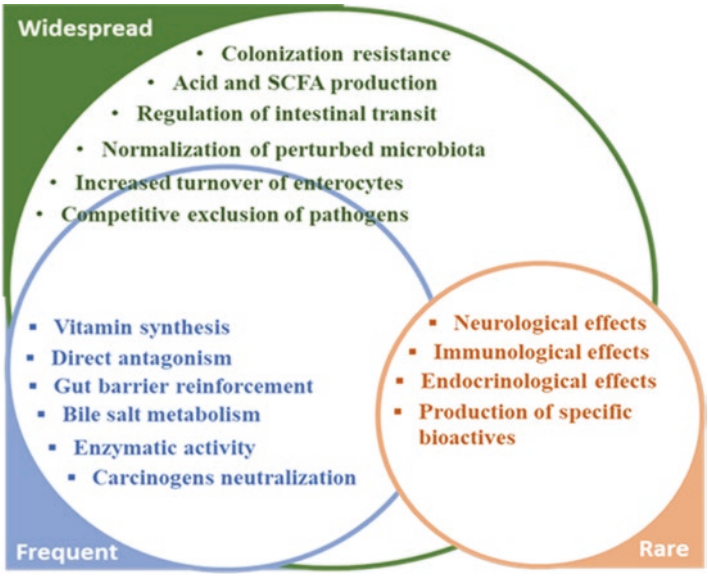

Fig. 4 Categorization of different health benefits associated with different probiotic strains according to the frequency of their prevalence in different probiotic strains

(Hill et al. 2014). The main health benefits of probiotics discussed below, therefore, are the modulation of activity and composition of host microbiota, enhancement of epithelial barrier function, modulation of the immune system, modulation of systemic metabolic response and modulation of central nervous system signaling (Lebeer et al. 2018). For the delivery of the proper health benefits, it is also required that the strains should be present in enough numbers. It is required by Health Canada that the viable population should be in the range of 1×10^9 cells per serving (Hill et al. 2014). Regulations requiring similar viable count per serving are also in place in Italy. Some of the mechanisms through which probiotic bacteria exhibit their activity are shown in Figs. 4 and 5 and are listed in Table 2.

Role of Probiotics in the Treatment of Gastrointestinal Disorders

One of the most important health benefits of probiotics is the maintenance of a healthy digestive tract microbiome. The human gut hosts around 10^{13}–10^{14} phylogenetically diverse microorganisms which are often perturbed by various factors such as food and the use of antibiotics (Kau et al. 2011; Gill et al. 2006). The microbiome present in the gut is mainly subdivided as the core, transient and variable microbiome (Derrien and van Hylckama Vlieg 2015). As the names suggest the core micro-

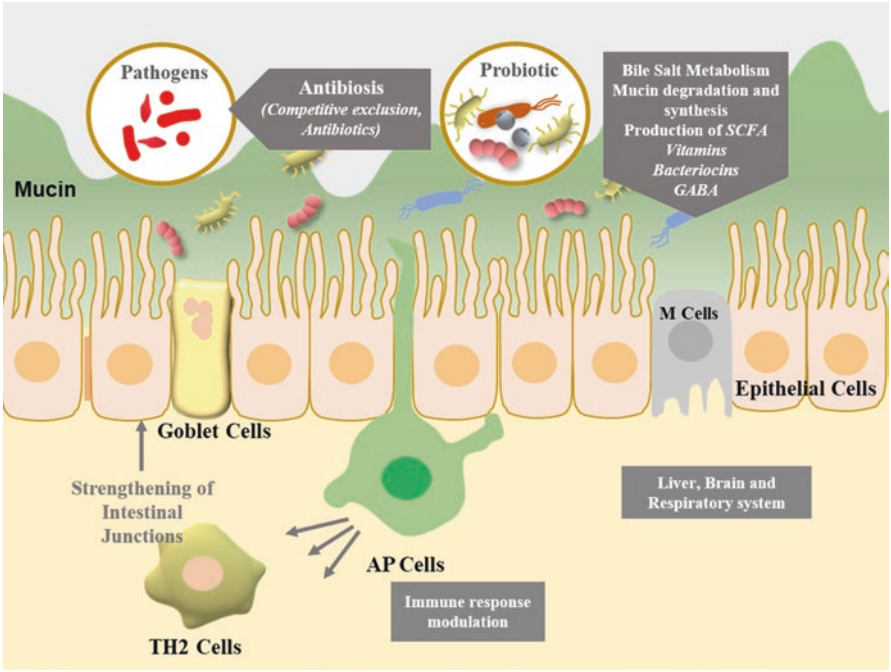

Fig. 5 A schematic presentation of various mechanisms through which the bacteria exhibit their probiotic activity

Table 2 Health benefits associated with some of the probiotic strains

Bacterial strain	Health benefit	Reference
Akkermansia muciniphila	Ameliorates HFD-induced obesity and insulin resistance	Plovier et al. (2016), Zhou (2017)
Clostridia (clusters IV and XIVa)	Increased intestinal barrier function	Kelly et al. (2015), Kelly et al. (2005)
B. fragilis	Modulation of host metabolism	Chimerel et al. (2014), van Baarlen et al. (2013)
Lactobacillus spp.	Immunomodulation, Maintenance of mucosal homeostasis and intestinal barrier function	van Baarlen et al. (2013)
Bifidobacterium spp.	Reduced adiposity	Segovia et al. (2017)
F. prausnitzii	Improved insulin sensitivity	Villanueva et al. (2015)
L. acidophilus La5, *B. lactis* Bb12	Inhibitory effect against *Helicobacter pylori*	Iannitti and Palmieri (2010)
L. plantarum, *L. reuteri*	treatment of IBS	Iannitti and Palmieri (2010)
Bifidobacterium animalis subsp. *lactis* CNCM I-2494	Increased no of species producing butyrate, decrease the infection of *Bilophila*	Amoretti et al. (2002)
L. reuteri NCIMB 30242	Increased Firmicutes/Bacteroidetes ratio	Martoni et al. (2015)
Bacteroides fragilis	Anti-inflammatory	Dasgupta et al. (2014)

biome is made up of microorganisms that have been found in the human gut across the globe. The variable gut microbiome is made up of microbial species that are found in some humans but are absent from the gut of other humans. This variable microbiome doesn't change with diet perturbation etc. While the transient microbiome is the microbiome which is influenced by various factors like diet. The composition of the microbiome also changes with the part of the intestine (Derrien and van Hylckama Vlieg 2015) and age. The age influences the gut microbiome mainly due to the change in the type of diet and the change in immune system fortitude (Durack and Lynch 2019). Gut microbiome has been extensively studied and the relation with various diseases has also been reviewed extensively. Probiotic bacteria are now well known to effectively treat various intestinal ailments such as Inflammatory bowel disease (IBD), Diarrhea, Crohn's disease, Colitis, liver conditions and other intestinal disorders (Parker et al. 2018; Bermudez-Brito et al. 2012).

The two most commonly treated intestinal ailments using probiotics are Diarrhea and necrotizing enterocolitis (Kleerebezem et al. 2019). Various types of Diarrhea have been treated using probiotics including antibiotic-associated diarrhea (AAD), acute infectious diarrhea, and *Clostridium difficile*-associated diarrhea (CAD). The mechanisms used by probiotics to fight diarrhea include (a) competitive exclusion of pathogen causing diarrhea (b) Production of acids (like lactic acid and short chain fatty acids) consequently lowering luminal pH (c) production of bacteriocins, (d) promotion of mucus production enhancing epithelial barrier (Isolauri 2003) (e) production of β-galactosidase to help lactose digestion and (f) promoting the production of antimicrobial proteins cathelicidins and defensins by gut epithelium (Schlee et al. 2008). The strains of probiotic bacteria that are known to treat the diarrhea and details of the mechanisms have been summarized in reviews published earlier (do Carmo et al. 2018b). Enterocolitis especially the necrotizing enterocolitis is an inflammatory bowel disease (IBD), mainly affecting premature infants. The disease may result in bloody diarrhea, bloating and sensitive abdomen. Probiotic bacteria are known to treat the disease, *Bifidobacterium* is found to be more effective than any other probiotic bacteria (Kleerebezem et al. 2019). The mechanism involves the ability of the bacterium to utilize human milk oligosaccharides. While *Bifidobacterium longum* subsp. *infantis* is known to downregulate TLR4 by secreting a small glycan consequently preventing epithelial inflammatory response (Meng et al. 2016). Another probiotic bacterium *Lactobacillus rhamnosus* GG suppresses the inflammatory response in the intestine and the expression of TLR3 and TLR4. Crohn's disease (CD) is another prominent IBD which has been associated with the increase in the population of Enterobacteriaceae. While the anti-inflammatory role of certain symbiotic taxa like *Faecalibacterium prausnitzii* has been demonstrated (Sokol et al. 2017). *Lactobacillus lactis* which expressed the anti-inflammatory molecule from *F. prausnitzii* reduced the intestinal inflammation in mice (Quévrain et al. 2016).

Probiotics for the Prevention of Obesity and Type 2 Diabetes Mellitus (T2DM)

Obesity is one of the major global health problems and a disturbed gut microbiome is one of the important reasons of obesity (Thaiss 2018). Even the microbiome distinctly associated with obesity has been identified (Turnbaugh et al. 2006). This obesity-related microbiome is often characterized by a reduction in the population of *Bacteroides* spp. and is capable of degrading the flavonoids in the diet (Durack and Lynch 2019). Which results in harnessing of much higher energy from the diet (Thaiss 2018). Another mucin-degrading gut bacteria is *Akkermansia muciniphila* which is often referred to as anti-obesity bacterium (Dao et al. 2016). This bacterium is also known to prevent other diseases such as IBD, hypertension and liver diseases (Cani and de Vos 2017). It has been documented that the polyphenols from the plants in the diet enrich *A. muciniphila*. The outer membrane protein of the bacterium interacts with TLR2 of the host helping in the restoration of gut barrier function (Anhê et al. 2015; Plovier et al. 2016). Furthermore, *A. muciniphila* can enhance the population of *Bacteroides* spp. which is the part of healthy gut microbiome (Gibson and Roberfroid 1995). The gut microbiome undergoes a transient change in its composition and function with the time in a day and these changes are referred to as circadian oscillations. It is reported that in obese persons these circadian oscillations are disturbed. In addition to the microbial interventions, an inulin-type fructans based diet can also improve metabolic disorders associated with obesity, such as a decreased fat mass, insulin resistance, lower liver steatosis and fortification of the gut barrier (Cani and de Vos 2017).

The Role of Probiotics in Immunity

A number of probiotic microorganisms are known to modulate adaptive and innate immunity through well-understood mechanisms (Yan and Polk 2011). These mechanisms involve the change in gene expression, protein synthesis, and modulation of signaling pathways in immune and intestinal epithelial cells (Yan and Polk 2011). Probiotic microorganisms modulate the functions of dendritic cells, macrophages, and T and B lymphocytes. It has been found that bacteria like *Bifidobacterium adolescentis* modulates the GI T helper 17 cells (Ivanov et al. 2009). These bacteria play a key role in the maintenance of barrier function and provide protection against pathogenic microbes through the production of antimicrobial peptides (Pandiyan et al., 2011; Wang et al., 2014). Short chain fatty acids produced by *Clostridium* species induce +CD4+Foxp3+ T reg cells regulate T cell-mediated host immune responses. Surface polysaccharide of *Bacteroides fragilis* has been shown to bind to Toll-like receptor 2 on dendritic cells (DCs), which subsequently induces the production of the anti-inflammatory cytokine IL-10 by T reg cells and promotes immune tolerance (Dasgupta et al. 2014). It also has been observed that variation in

microbiome lipopolysaccharide (LPS) immunogenicity results in autoimmunity in humans (Vatanen et al. 2016). In this study it was found that the LPS from genus *Bacteroides dorei* inhibits the immunostimulatory activity of *Escherichia coli* LPS.

Treatment of Atopic asthma

Asthma is a serious disease affecting 300 million individuals worldwide. It is an inflammatory disease of the airway, leading to hyper-production of mucus in the airway hyperresponsiveness and obstruction of the airway (Kudo et al. 2013). Asthma is a well-known T helper cell's (Th2) disease, resulting in increased levels of IgE and eosinophilic inflammation of the airway. Asthma-like changes of the airways and lung parenchyma include eosinophilia, alternative macrophage activation, pulmonary lymphocytosis, mastocytosis, and epithelial cell proliferation with goblet cell hyperplasia and these are induced by Th2 cytokines (IL-4, IL-5, IL-9, and IL-13). The inhalation of allergens is known to activate T cells to produce a Th2 responses through stimulation of the innate immunity (Saenz et al. 2008; Otani et al. 2013). The increase in the levels of metalloproteinases in inflammatory condition has been documented. It has been reported that the levels of metallopeptidase 9 (MMP9) increase significantly in asthma (Okada et al. 1997). Probiotic bacteria including *Bifidobacterium bifidum*, *B. lactis*, and *L. lactis* are shown to inhibit Th2-related cytokines IL-5 and IL-13 and induces IL-10 significantly (Gorissen et al. 2014). In another review it has been shown that the probiotics consumption by expecting or nursing mothers or by infants reduces the risk of eczema in infants (Forsberg et al. 2016). Furthermore, short chain fatty acids generally produced by probiotic microorganism was also found to ameliorate airway inflammation in mice. The amelioration activity was dueto the decreased activity of T cells and dendritic cells, decreased numbers of CD4+ T cells producingIL-4, and reduced levels of circulating IgE (Cait et al. 2017). Convincing evidences are available in the literature to prove the role of gut microbiome dysbiosis in childhood Asthma. Depletion of symbiotic bacterial population such as *Akkermansia*, *Faecalibacterium*, and *Lachnospira* and an increase in certain fungi (*Candida* and *Rhodotorula*) in infants was found to be associated with the risk of developing atopy or asthma (Durack et al. 2018; Arrieta et al. 2015; Stokholm et al. 2018; Fujimura et al. 2016). The soluble proinflammatory products from the microbiome were shown to induce Th2 cells.

Probiotics and Central Nervous System (CNS)

In addition to various health benefits of the probiotics clinical studies also suggest the role of gut microbiota on human brain development function (Tillisch 2014). As the brain and gut have a strong, two-way communication system which is often

referred to as the gut–brain axis. Probiotic dietary intervention of children with autism spectrum disorder (ASD) have shown improved performance of these children in schools (Umbrello and Esposito 2016). ASD children were shown to have some signature dysbiosis characterized by higher population of Bacteroidetes and Proteobacteria, and a lower abundance of Actinobacteria (especially *Bifidobacterium*) and Firmicutes (Finegold et al. 2010). This altered microbiome produced metabolites like significantly higher concentrations of ammonia that are considered neurotoxic and may further promote the adverse neurological effects associated with ASD (Wang et al. 2012; Morland et al. 2018). Furthermore, strains of *L. brevis* DPC6108 and *Bifidobacterium dentium* produced large amounts of the neurotransmitter γ-aminobutyric acid (GABA), which helps to suppress anxiety and depression (Barrett et al. 2012). Significant effect of probiotic intervention was observed in human subjects when single-strain probiotic including subspecies of *L. casei* (*rhamnosus*, Shirota), *L. plantarum*, and *B. infantis* were used for study (Rao et al. 2009).

Fecal Microbiota Transplant (FMT): The Concept of Repoopulation

Many diseases especially those associated with GI tract, and conditions like irritable bowel syndrome (IBS) and inflammatory bowel diseases (IBD) are due to the dysbiosis or the change in the gut microbiome. One of the recent approaches to treat these GI tract diseases is through Fecal microbiota transplant (FMT). The FMT is found to be especially effective in treating (>90%) recurring infections of antibiotic-resistant *C. difficile* infection (Smits et al. 2013). Based on such studies now FDA's guideline recommend the use of FMT for treating recurring *C. difficile* infection. As the name indicates FMT simply means the transfer of Fecal microbiota from a healthy donor to a diseased persons GI tract. The methods of transfer include nasogastric tube, nasojejunal tube, upper tract endoscopy and colonoscopy (Gough et al. 2011). Although it appears to be a new concept but it has been in use traditionally as it is reported that the Bedouins used to eat fresh camel feces to treat bacterial dysentery (Smits et al. 2013). The concept of FMT is gaining popularity as it is a very effective way of transferring a seed microbiome into a diseased person. Moreover, many microorganisms cannot be cultured under laboratory condition which makes it difficult to independently isolate these bacterial strains and then supplement these cultures as probiotic bacteria. The unknown composition of the Fecal microbiota is a matter of concern as the number and type of microorganisms in the samples to be transferred cannot be controlled and involves the risk of unexpected consequences. Although, the standard procedures involve precautionary measures such as screening of the donor's Fecal microbiota for transmittable diseases and fecal pathogens.

Safety Assessment and Regulations of Next Generation Probiotic

Although probiotic bacteria are generally recognized as safe (GRAS), but there are genuine reasons to believe that probiotic strains should be evaluated carefully for their safety. One simple reason is the rapid industrialization of probiotic market and the increasing diversity of microorganisms that are used as probiotics. Many strains related to these probiotic bacteria such as the members of the genus *Clostridium* are pathogenic (Saarela 2019). Another property of probiotic bacteria which is often ignored is the fact that the genes for antibiotic resistance if present in probiotic strains may be horizontally transferred to the gut microbiome. Main risks involved with probiotics are possible infection and production of toxins by a contaminant strain or a misidentified probiotic strain. There are reports in the literature suggesting that the amendment of the diet with probiotics sometimes may result in disturb metabolism and in systemic infection (Doron and Snydman 2015). Therefore, it is necessary to ensure the safety and suitability of new probiotics. Some of the important properties that should be evaluated have been described by Saarela (Saarela 2019). The strain must be correctly identified using standard and modern molecular identification methods. A given probiotic preferably should not be related to any well-known pathogenic species. The whole genome sequencing should be carried out to check whether the strain contains antibiotic resistance genes and the strain should not have virulence genes. A candidate strain should not have enzymes involved in pathogenesis like collagenase, hyaluronidase and neuraminidase. The strains should also meet other requirements such as growth parameters and tolerance to gastrointestinal stresses. The strain should also exhibit technological feasibility for industrial production such as required growth in pilot scale and industrial scale fermenters. Candidate strains should also be evaluated in human studies for dose and other parameters.

Regulatory aspects involved in the industrial production of probiotic products such as efficacy, safety, and quality control as recommended by regulatory authorities must be followed. These regulations vary from country to country and so far, there are no universally agreed framework (de Simone 2019). Although, efforts have been made to develop such common guidelines and framework (Hill et al. 2014). European Union, regulate the probiotics under the Food Products Directive and Regulations. The EFSA (European food safety authority) has also allowed the use of some well identified strains as probiotics (Ricci et al. 2017). Under the European regulations any health claim for probiotics and probiotic based product has to be authorized by the EFSA. It is interesting to note that EFSA has rejected all submitted health claims for probiotics so far. In the USA also, the probiotic products are classified as foods or food supplements. And these products are required to comply with Good Manufacturing Practice (GMP) guidelines. It is allowed to make functional claims about the probiotic products in the United States. Nevertheless, the claims must not be misleading, and must be substantiated by scientific evidence (de Simone 2019). But the research on probiotics is regulated differently in

USA. Where, probiotics are categorized as drugs making it difficult to do much needed research on the probiotics (Hill et al. 2014). It is also important that the public health officers and medical professionals continue a post market surveillance of the products as recommended by FAO/WHO.

Commercial Success of Probiotics

The recent commercial success of probiotics cannot be ignored as evident from the availability of various probiotic based products in the market. Some of these commercial dairy and non-dairy products include Yogurt, Kefir, Sauerkraut, Tempeh, Kimchi, Miso, etc. Web grab of some of these products are shown in Fig. 6. Especially the market of dairy products containing probiotic has grown tremendously (Stanton et al. 2001). According to the Journal of functional food the global market for probiotics has been estimated at US$33.19 billion in 2015. While, the value of functional food market in Europe alone in 1997 was at US$889 million. According to International Probiotic Association Europe (IPA, Europe) the sales of probiotic yogurt was valued at 5 billion Euro in 2016. It was found that UK and Italy were the largest consumer countries in Europe. On the contrary the market for probiotics was comparatively underdeveloped in USA by European standards. It has been pointed out in the report of IPA, Europe that the sales of the probiotic products have recently increased tremendously in North America and China. However, various new regulations on probiotics may adversely affect the market prospects. For example, it has been pointed out by the expert panel that new regulations of USFDA in this regard for probiotics will discourage much needed research on probiotics (Hill et al. 2014).

Conclusions

The probiotic market is expanding rapidly throughout the globe and is a multibillion-dollar industry. This expansion is spurred by the better understanding of gut microbiome and its health benefits. With the advancement of science and technology, intervention trials, meta-analyses and systematic reviews convincing evidence of the health benefits associated with probiotics have been gathered. It has been proved that probiotics can effectively treat various GI tract associated diseases and disorders the most common being different types of diarrhea, enterocolitis and IBS. The new FDA guidelines to treat recurring infection of *C. difficile* recommend the use of Fecal microbiota transplant. It is also claimed that probiotics can help in controlling diabetes, cancer and obesity. For many of such claims molecular markers have been identified. Though it is difficult to understand the molecular basis of some of these claims especially of mixed strain probiotics or undefined community such as Fecal microbiota. While the mechanism of others is much simpler to under-

Fig. 6 Variety of the commercial products available in the market. These include dairy products like milk, yogurt, ice cream, soft drinks, fermented foods like pickles and health supplements

stand such as the production of lactase which helps in lactose digestion. Notably, regulatory authorities have not approved any health benefit claims of probiotics so far. Expert committee has also recommended that robust evidences must be provided to claim any health benefit associated with a candidate probiotic. Although it is accepted by the expert committee that these microorganisms have some core health benefits.

The commercial success of the probiotics has also led to an increased interest of the scientists in probiotics resulting in the discovery of many new and nonconventional probiotic microorganisms referred to as next generation probiotics in this book chapter. These microorganisms have been isolated from a variety of sources such as dairy products, conventionally used fermented foods, fecal matter, human milk etc. The discovery of these microorganisms and many more in near future would require clear guidelines to correctly identify a candidate strain. Since, the direct consumption of live cultures is involved any misidentified strain may result in infection and disease. Therefore, stringent regulations for probiotics are required to protect the interests of producers as well as of other stake holders. These regulations should be aimed at controlling the misuse of the term probiotics and protecting the consumers from any potential harm of misidentified products. For example, the dispersal of antibiotic resistance genes through probiotics should be checked carefully. The unfounded claims should be discouraged. The new candidate strains must be correctly identified using modern and conventional techniques and must be evaluated for the presence of the core health benefits. It is also recommended that these strains should be submitted to at least two international culture collections. The use of undefined microbial communities such as the Fecal microbiota from healthy individual though have clear benefits but technically cannot be categorized as probiotics. The use of such undefined microbial communities is always risky and require proper screening and control measures to minimize the risks involved.

However, on the other hand framed regulations should not discourage research in the field which may deprive the consumers from any future benefit of the probiotics. As the expert panel have pointed out that the classification of probiotics under the "drug" category for research in the USA may discourage the much-needed research in this area. Therefore, regulations on probiotics must be balanced and in the interest of a sustainable development of the probiotic market. In fact, this will require a close communication and collaboration between academics, health professionals, industry, regulatory agencies, and policy makers. Furthermore, the regulations for the probiotic products vary from country to country resulting in confusion. A consensus is required for the smooth and global implementation of these regulations. It is also equally important to have regulations for post-production follow up to ensure the safety and effectiveness of the products by an industry. Post/parabiotics which are the metabolic by-products, dead microorganisms, or other microbial-based products are comparatively safer but cannot have long term effects that are obtained through the establishment of probiotic bacteria in the gut.

Probiotics hold great health benefits and in future can play a bigger role in treating and preventing various diseases especially those caused by dysbiosis. Since, much progress is already made in understanding the role of these microorganisms

in treating various diseases. These microorganisms can also be customized to deal with specific health issues. For example, the role of *Akkermansia muciniphila* in controlling obesity, Fecal microbiota transplant in treating *C. difficile* infections, *Lactobacillus* in treating different types of diarrhea and role of *Bifidobacterium* spp. in prevention of type 2 diabetes and obesity is very clear. It is also suggested that various probiotic strains can be genetically modified for improving their potential as probiotics (Ahmed 2003). Scientists are even checking the survival of these probiotics in international space stations (Sakai et al. 2018). Although, the understanding of probiotics and the associated health benefits has improved significantly a lot still needs to be done. Especially through successful isolation of various strains in monocultures that will also make it easier to understand the potential health benefits of these strains and the mechanism involved.

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Continuing Controversies Regarding Human Health Concerns from Nitrite and Nitrate Consumption in the Diet

Hakan Benli

Abstract Nitrite, salt, seasonings and other ingredients are used for the curing to give unique color, flavor and texture to the meat products. Sodium or potassium nitrite is incorporated into the processed meats to provide desirable meaty flavor, prevent warmed-over flavor, develop a bright reddish pink color and inhibit the microbial growth, particularly for out-growth of *Clostridium botulinum* spores. Sodium or potassium nitrate can also be used to cure the processed meats. However, nitrate has to be reduced to nitrite by the microorganisms to be effective for curing and mostly used for the slow-cured products including some fermented sausages and country style hams. The safety of nitrite and nitrate used for meat curing was questioned in the 1970s due to their potential to form carcinogenic nitrosamines in the stomach following the ingestion. Conversely, some potential health benefits were also attributed to both nitrite and nitrate in the recent studies since both compounds contribute to nitric oxide production in human body. Nitric oxide produced directly from nitrite has a significant effect on cardiovascular health by controlling blood flow in the cardiac muscle. Thus, the continuing controversy regarding human health concerns from nitrite and nitrate consumption in the diet are evaluated and discussed in this chapter.

Keywords Nitrate · Nitrite · Nitric oxide · Meat curing · Human health

Introduction

Curing is one of the basic processing procedures used in meat processing. Application of nitrite, salt, seasoning and other ingredients to meat is defined as meat curing in today's technology. Curing is important to give unique color, flavor and texture to meat products and for preventing microbial growth. The salt was originally used at high concentrations for curing and preserving the meat in ancient

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times. The preservative effect of salt is related with the water activity. Salt reduces the water activity of the product and limits the microbial growth. However using only salt produces an unattractive brownish-gray color and often produces too salty meat products. Early meat processors discovered that use of potassium nitrate (saltpeter) found in salt as an impurity fixes and preserves the pink color of cured meat. Although meat curing used to have a sole purpose of preserving meat, today, effects of curing on color development, flavor and textural properties of meat products are equally as important as preservation due to the availability of refrigerators (Aberle et al. 2001; Pearson and Gillett 1996).

Use of nitrite as a sodium or potassium salt in meat curing has several benefits including stabilizing the bright reddish pink color of the lean tissues, contributing desirable flavor of cured meat, preventing development of rancidity and warmed-over flavor, effectively inhibiting the growth of the *Clostridium botulinum* spores. The most important reason for adding nitrite was defined as the effect of the nitrite for preventing the growth of the *Cl. botulinum* in cured meat products. Although sodium or potassium nitrate were originally used for meat curing, microorganisms firstly converts nitrate to nitrite during the processing (Aberle et al. 2001; Pearson and Gillett 1996).

Although use of nitrate has a similar functionality as nitrite, nitrate acts slower and consequently it is used less often. The bacterial reduction is required as an additional step for conversion of nitrate to nitrite. Natural bacterial flora in the meat or added starter cultures that have nitrate reducing capabilities are responsible to reduce nitrate. Nitrate reductases expressed by the bacteria that use nitrate as a substrate for anaerobic respiration include following strains; *Lactobacillus plantarum*, *Lactobacillus sakei*, *Leuconostoc strains*, *Staphylococcus xylosus*, *Staphylococcus carnosum*, *Staphylococcus aureus*, *Bacillus subtilis* etc. (Bonomo et al. 2008; Hammes 2012; Heaselgrave et al. 2010; Majou and Christieans 2018; Talon et al. 1999).

Thus only nitrite is mostly used for obtaining desirable flavor and color and microbial safety in modern meat curing. Limited use of nitrate is still available for some traditional and slow cured products including country cured ham and fermented sausage. Nitrite directly added or converted from nitrate produces nitric oxide which reacts with myoglobin to produce raw cured meat color. This pigment is called nitric oxide myoglobin which has an attractive, bright red color before heat processing. The heat treatment denatures the protein portion of myoglobin and yield a pigment called nitrosylhemochromogen which is responsible for the stable characteristic pink color of cured meats (Aberle et al. 2001; Pearson and Gillett 1996).

Two pathways were described for nitrite reduction to nitric oxide (NO·). The first pathway includes periplasmic (gram negative bacteria) or cytoplasmic bacterial nitrite reductases and second pathway includes nitrite reductase activity of deoxymyoglobin of meat. Myoglobin exists in three different forms in meat including reduced form called deoxymyoglobin, oxygenated ferrous myoglobin called oxymyoglobin or oxidized form called metmyoglobin. The existence of three different forms of myoglobin depends mostly on the partial pressure of oxygen. (Aberle et al.

2001; Gladwin and Kim-Shapiro 2008; Majou and Christieans 2018; Moller and Skibsted 2006; Pearson and Gillett 1996).

In the United States, addition of nitrite is limited to 200 ppm for most cured meat products, 156 ppm for comminuted meats and 120 ppm for bacon depending on the amount of meat and meat by product in the formulation. However, in the European Union the amount of in going nitrite is restricted to 150 ppm for cured products. Addition of reducing agents including the sodium salts of ascorbic acid (sodium ascorbate) or erythorbic acid (sodium erythorbate) to the curing mixture greatly accelerates nitric oxide and further nitric oxide myoglobin formation while the natural reduction is a slow process and is only beneficial for manufacturing of some traditional or slow cured products. Thus use of reductants decreases the time required to develop cured meat color to several hours from several days which is essential step for continuous or accelerated productions (Aberle et al. 2001; Santarelli et al. 2008).

Curing ingredients can easily be incorporated into the comminuted meat products during mixing stage. Dry curing and pickle curing are two fundamental methods used for curing primal or subprimal cuts of meat although there are a number of modified or combined methods available. Dry curing takes longer to cure the meat cuts since curing ingredients including mostly salt, sugar, and nitrite and/or nitrate are added and rubbed on the meat cuts without additional water. Efficacy of this method is determined by the diffusion of salt into the meat. In pickle curing, a brine is prepared by dissolving the same curing ingredients in water. A container is used to submerge the meat cuts into the pickle to allow the curing ingredients penetrate the meat completely. Since pickle curing may require time as much as the dry curing to complete some other methods were developed to increase the rate of curing like artery pumping, single-needle stitch pumping and multiple-needle stitch pumping (Pearson and Gillett 1996).

Concerns Related to Nitrate and Nitrite in the Diet

A continuing debate regarding human health concerns has been going on due to the consumption of nitrite and nitrate in the diet for more than 40 years. Scientific studies have been conducted to determine the safety of nitrite or nitrate in foods and their interactions in the human body after ingestion. High levels of nitrate found in drinking water were associated with infant methemoglobinemia in 1960s (Bryan et al. 2012). In addition, nitrite and nitrogen oxides react with secondary amines and N-alkylamides to produces N-Nitroso compounds which are alkylating agents and they can react with DNA. N-Nitroso compounds include nitrosamines and nitrosamides which have been shown to be carcinogenic to animals in laboratory. N-nitrosamines can be found in tobacco smoke and some foods including certain processed meats (e.g. grilled bacon), smoked fish, cheeses and beers which raised questions about the potential human health concerns (Santarelli et al. 2008).

Nitrite intake from cured meats has been reported to contribute 4.8% of daily intake (Archer 2002). In 1970s cured meats were recognized for their potential for nitrosamine formation under special conditions including presence of secondary amines, available nitrite for reacting, neutral pH values, and high end cooking temperatures of the products (>130 °C) such as fried bacon. In 1978, a final rule was published after discussions of several purposed regulations, stating that use of nitrite was limited to 120 and 550 ppm and sodium ascorbate or sodium erythorbate should be added in bacon. Use of nitrate was banned for bacon production. After implementation of nitrosamine monitoring program all bacon producers were in compliance with specified limits in the regulations (Sindelar and Milkowski 2012).

During the 1970s and 1980s the reactivity of nitrite with nitrosatable amines was investigated to determine their toxicity using animal models. At the same time, manufacturers evaluated the presence of N-nitrosamines in processed foods and beverages and developed new processes and ingredient changes to eliminate or minimize the formation of N-nitrosamines. Thus some modification in brewing methods for alcoholic beverages and use of reductants in cured and processed meats were introduced for the industry. In addition, many countries changed their regulations related to use of nitrite and nitrate in cured meat products to balance toxicological risk with the benefits of using nitrite and nitrate for food preservation and food safety purposes (Bryan et al. 2012).

Daily dietary intake of nitrate is estimated about 43–131 mg by the World Health Organization. Plant derived foods are the primarily exogenous sources (87% of dietary nitrate intake) for human intake of nitrate. Drinking water is also reported as a significant contributor in the total nitrate intake. Beets, spinach, celery, radishes, cabbage, lettuce, and collard greens are among the vegetables contain naturally occurring nitrates in higher concentrations (Archer 2002; National Academy of Sciences 1981; Sindelar and Milkowski 2012; World Health Organization 2007).

Fertilizers cause a greater uptake of nitrogen in vegetables resulting in higher nitrate content. Nitrate content of vegetables is affected by nitrate reductase activity, nitrate uptake, growth conditions and growth rate. In addition some processing methods including heat treatments and storage conditions can reduce the nitrate. For instance, the nitrate content of vegetables decreases with increased storage temperatures due to the reduction of nitrate to nitrite with increased bacterial activity (Chung et al. 2004; Hord et al. 2009; Wolff and Wasserman 1972).

The large portion of total daily ingestion of nitrite (93.0%) was estimated that comes from saliva while only a small portion of the daily nitrite intake comes from foods. The total daily nitrite intake of a person is approximately 1.2–3.0 mg of nitrite per day. Salivary nitrate is reduced to nitrite by the bacteria activity in the oral cavity (Sindelar and Milkowski 2012; World Health Organization 2007).

Most of the gastrointestinal cancers in individuals are known to arise with environmental risk factors rather than hereditary risk factors. Diet is among the strongest contributors related to the environmental factors which increase the risk of gastrointestinal cancer development. Typical Western diets are rich in fat and meat but are poor in fiber content which are related to an increased risk of colorectal cancers. Bile acid secretion increases with a high-fat diet and microflora in colons

transforms the bile acid into secondary bile acid which has genotoxic properties to DNA due to nitrogen species and reactive oxygen (Kobayashi 2018; Watson and Collins 2011). Contrary, anaerobic microflora in lower intestine ferments undigested carbohydrate residue consumed in a high fiber diet. These bacteria provide a beneficial environment in the intestine to protect against inflammatory responses and cancer development. However, microbial fermentation of undigested protein residues due to the consumption of protein rich diets is the source of inflammatory and toxic nitrogenous metabolites including amines, phenols, ammonia and indoles. Furthermore, formation of potential carcinogens such as nitrosamine and nitrosamide due to the reaction of nitrosating agents including nitrite and secondary amines and amides are a major risk factor for gastrointestinal cancers (Hamer et al. 2009; Hughes et al. 2000; Lee and Hase 2014). Nitrate is reported as a relatively non-toxic compound below the maximum levels in relations to carcinogenicity. In addition, epidemiological studies indicated that no consistently increased risk of cancer was related with increased nitrate consumption (Alexander et al. 2008; Kobayashi 2018). Colon cancer risk were reported to increasing with chronic exposure to nitrate due to drinking water and food consumption although the risk was associated with low vitamin C consumption while consuming high amount of meat (De Roos et al. 2003; DellaValle et al. 2014). Although fruits and vegetables are the main source of dietary nitrate and nitrite intake, epidemiological and experimental studies are suggesting that the nitrosation inhibitors in the fruits and vegetables have protective effects against cancer development (Bradbury et al. 2014). Furthermore, swallowing saliva with foods has been shown that the major contributor of dietary nitrite intake since 25% of the nitrate ingested in diet is converted to nitrite by oral bacteria after entering the enterosalivary route (Archer 2002; Bryan et al. 2012; Pannala et al. 2003).

Nitric Oxide and Human Health Benefits

Nitrite shown since 1980s is an important molecule for human health. Nitric oxide (NO) is a product of nitrite has so many benefits in human body including controlling blood pressure, wound repair, immune response, neurological functions and blood flow in cardiac muscle as well as preventing some cardiovascular disease like hypertension, atherosclerosis and stroke (Hunault et al. 2009; Sindelar and Milkowski 2012).

An essential role is played by the salivary glands and the oral bacteria to convert nitrate (NO_3^-) and nitrite (NO_2^-) to nitric oxide (NO). NO has important vascular and metabolic functions in the body and is recognized as a messenger molecule. Endogenous production of NO catalyzed by complex NO synthases through the L-arginine pathway. However Nitrate ingested from foods contributes the main extrinsic NO production through NO_3^-–NO_2^-–NO pathway. The salivary glands actively take up to 25% of the nitrate in circulation. Furthermore, nitrate is reduced to nitrite by the oral bacterial species present at the posterior part of the tongue.

Nitrate and nitrite is used by the oral bacteria in their respiration as final electron acceptors (Qu et al. 2016). Since human beings lack of the enzymatic system to convert nitrate to nitrite, bacteria are essential for reducing nitrate to nitrite (Sindelar and Milkowski 2012).

In humans, nitric oxide involves in several physiologic and pathologic processes and identified as an important cellular signaling molecule (Hou et al. 1999; Moncada and Higgs 1993). Nitrate and nitrite ingested by adequate consumption of some fruits and vegetables including beetroot, chervil, lettuce, celery and radish have been shown several potential health benefits for myocardial infarction, acute stress response, hypertension and exercise capacity (Bryan and Ivy 2015; Kobayashi et al. 2015; Machha and Schechter 2012).

Nitrate and nitrite have allowable daily intakes (ADI) of 3.7 and 0.06 mg/kg or 220 and 8 mg/person/day, respectively. The upper small intestine (duodenum and jejunum) absorbs nitrate and nitrite completely and rapidly. Although a small portion of ingested nitrite is converted to NO in the stomach most of the nitrite is absorbed and entered to the circulation. The NO produced in stomach contributes to inactivation of pathogens, mucosal blood flow regulation and formation of mucus. In Addition, enterosalivary circulation of ingested nitrate provides continuous production of NO in the gastric lumen (Bahadoran et al. 2015; Carlstrom et al. 2010; Chow and Hong 2002; Ghasemi and Jeddi 2017; Kevil et al. 2011; Lundberg and Weitzberg 2013; Pannala et al. 2003; Weitzberg and Lundberg 1998).

Nitric oxide (NO) has been reported to have significant roles in mitochondrial respiration, vasodilation, glucose and calcium homeostasis, fatigue development and skeletal muscle contractility**.** The continuous production of NO is important since the half-life of NO is short and ranging from millisecond to seconds. Two pathways have been defined in the human body to generate NO including NO synthase dependent pathway and NO_3^-–NO_2^-–NO pathway which is a NO synthase independent pathway. Three different isoforms of NO synthase have been determined and identified as neuronal, endothelial and inducible NO synthases. L-arginine and oxygen are utilized by these enzymes to generate NO and L-citrulline is also used in a reaction requiring several cofactors comprising nicotinamide adenine dinucleotide phosphate, tetrahydrobiopterin, flavin mononucleotide, flavin adenine dinucleotide, and calcium-calmodulin. Although increased NO production has been reported following the oral supplementation of L-arginine, the bioavailability of NO does not increase in healthy humans who have been consuming pure L-arginine. The end products of NO synthase dependent pathway are nitrite and nitrate since NO is quickly oxidized to form these two anions (Bredt 1999; Dejam et al. 2005; Jones et al. 2018; Moncada and Higgs 1993).

A recently defined NO production pathway involves NO_3^-–NO_2^-–NO pathway with reduction of NO_3^- to NO_2^- and to NO. Green leafy vegetables including beetroot, chervil, lettuce, celery, radish, rocket, and spinach are rich in nitrate containing over 250 mg/100 g of fresh produces that are also exogenous source of inorganic nitrate in the diet. Ingested nitrate reaches the upper gastrointestinal tract where it is absorbed and enters the systemic circulation. After 60 min following the consumption of nitrate, the peak plasma concentrations are achieved. The salivary gland

absorbs 25% of the nitrate through sialin which is an active transporter and then nitrate is concentrated up to 20 fold in saliva. Most of the remaining nitrate is either extracted by kidney or excreted in the urine. Facultative anaerobic bacteria in the oral cavity which are positioned on the dorsal side of the tongue plays a major role in reducing approximately 20% of salivary nitrate to nitrite. After the swallowing of the saliva, the nitrite is further reduced to NO in the stomach. The acidic environment in the stomach and the existence of vitamin C and polyphenols greatly enhances the reduction of nitrite to NO. Some part of nitrite also enters the systemic circulation. The peak plasma concentrations occur around 2–3 h after nitrate consumption. This nitrite is quickly circulated in blood and tissue and is reduced to NO in a reaction catalyzed by deoxymyoglobin, deoxyhemoglobin, xanthine oxidase, cytochrome P-450, aldehyde oxidase, the mitochondrial electron transfer complexes, and NO synthase (Benjamin et al. 1994; Duncan et al. 1995; Govoni et al. 2008; Lundberg and Weitzberg 2009; Qin et al. 2012; Wagner et al. 1983; Weitzberg and Lundberg 1998). Hypoxia and acidosis conditions have been shown that considerably enhance the reduction of nitrite to NO indicating a backup system which guarantees continuous NO production even though oxygen dependent NO synthase pathway is not functioning properly. Since the conditions of hypoxia and acidosis are likely to occur during excessive contraction of skeletal muscle, NO production via NO_3^-–NO_2^-–NO pathway may have a specific significance during exercising (Castello et al. 2006; Jones et al. 2018; Richardson et al. 1995).

Dietary nitrate has been reported to decrease blood pressure. When reduction of salivary nitrate to nitrite is interrupted, the decrease in blood pressure could be blocked which is a confirmation of the regulatory function of nitrite converted from consumed nitrate (Aboud et al. 2008; Larsen et al. 2006). Furthermore, sodium nitrate supplementation has been shown that increases the plasma nitrite and reduced oxygen consumption during a submaximal cycling exercise. Similarly consumption of beetroot juice which is a rich source of nitrate for 3 days doubled the plasma nitrite and reduced the oxygen requirements approximately 5% during exercise. Thus, it was indicated that nitrate ingestion with diet allows performing more muscle work per unite time using the same amount of energy with an increased skeletal muscle contraction efficiency. Similar results were also reported for acute nitrate treatment after 60 min of nitrate consumption and 2.5 h of beetroot juice consumption and 15 days of continued nitrate supplementation maintained the increased efficiency during exercise similar to that of acute beetroot juice consumption (Bailey et al. 2009; Jones 2014; Jones et al. 2018; Larsen et al. 2007; Vanhatalo et al. 2010). Although supplementation of nitrate in the diets of people who are moderately active have benefit to improve exercise efficiency, ingestion of nitrate have been reported to not improve exercise efficiency significantly in trained people. Since trained people could have better aerobic fitness and higher resting plasma concentrations of nitrate and nitrite than nonathletic people, improved muscular efficiency seems to be reduced in trained subjects due to already developed oxidative metabolic system and higher bioavailability of NO (Christensen et al. 2013; Jungersten et al. 1997; Longo et al. 2014; Porcelli et al. 2015; Schena et al. 2002).

Conclusion

Curing is defined as an application of nitrite, salt, seasoning and other ingredients to meat products and is an important technological procedure to give unique color, flavor and texture to meat products and for preventing microbial growth. Nitrite addition is the most effective way of preventing the growth of the *Cl. botulinum* in cured meat products. Nitrate is originally used for meat curing however microbial activity is required to convert nitrate to nitrite during processing. Since nitrite can react with secondary amines and N-alkylamides to produces N-Nitroso compounds which have been shown to be carcinogenic to animals in laboratory there has been a continuing debate regarding human health concerns due to the consumption of nitrite and nitrate in the diet for more than 40 years. N-nitrosamines can also be found in tobacco smoke and some foods including smoked fish, cheeses and beers in addition to certain processed meats. During the 1970s and 1980s manufacturers developed new processing and ingredient changes to eliminate or minimize the formation of N-nitrosamines in processed foods and beverages. Thus use of reductants in cured and processed meats and some modification in brewing methods for alcoholic beverages were introduced for the industry. In addition, regulatory changes related to addition of nitrite and nitrate in cured meat products have been placed in many countries to balance toxicological risks and the benefits of using these compound in processed foods. Furthermore, nitric oxide which is a product of nitrite has been shown an important molecule for human health in 1980s. Benefits of nitric oxide in human body includes controlling blood pressure, wound repair, immune response, neurological functions and blood flow in cardiac muscle as well as preventing some cardiovascular disease like hypertension, atherosclerosis and stroke. In addition, nitrate ingestion with diet has been shown to improve muscle work per unit time using the same amount of energy with an increase skeletal muscle contraction efficiency indicating that nitric oxide production via NO_3^-–NO_2^-–NO pathway may have a specific significance during exercising.

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The Risk of Vancomycin Resistant Enterococci Infections from Food Industry

Mevhibe Terkuran, Emel Ünal Turhan, and Zerrin Erginkaya

Abstract Several works of literature research for the contribution of Antibiotic-Resistant Enterococci (ARE) and, especially Vancomycin Resistant Enterococci (VRE) which entered into the food chain has gained importance with the increasing significance of VREs in hospital infections. Various studies conducted in Europe, United States of America (USA) and the Middle East were evaluated in terms of prevalence, epidemiology and risk factors of foodborne enterococci in VRE infections. VRE epidemiology has shown some distinctions in Europe and USA. VRE was generally isolated from animals in Europe, which was connected to the extensive/massive use of "avoparcin" as a growth promoter in animal feed in the agriculture sector. Animals fed with this feed act as reservoirs of transferable vanA type resistance. On the other hand, since "avoparcin" was not used in the USA, VRE could not be isolated in animals and healthy humans. However, hospital-acquired VRE infections are more showed in the USA than in European countries. According to numerous studies, since enterococci are used as starter culture and probiotic culture, they have no relationship genetically with the strains which include vancomycin/resistant to antibiotic/or having resistance and virülence genes. In this chapter, important features of enterococci, the role of food chain for ARE especially, VRE infections in community including strategies for future solutions about the problem are summarised.

Keywords VRE · Food chain · Enterococci · Food industry

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Introduction

Antibiotic resistance of the bacteria has become a major problem all around the world. It has been debated both food and clinical microbiology in terms of antibiotic using in food animal and agricultural process related to gaining antibiotic resistance of bacteria especially *Enterococcus* spp. Genus. They are catalase negative, Gram positive and facultative anaerobe and coc-shaped bacteria found in food natures and used as starter/co-culture microorganisms in dairy and meat products as well as inhabit human and warm-blooded animals; generally, in proximal of intestine flora of gastrointestinal tract and female genital system as a permanent flora component. Therefore, Enterococci are used in drinking water as the indicator bacteria of hygiene and fecal contamination (Gelsomino et al. 2001; Samakupa 2003). Although enterococci are not recognized Generally Recognized as Safe (GRAS) microorganism in food microbiology, they have several beneficial effects in food industry such as proteolytic, esterolytic and aroma producing components in dairy and meat production industry as well as probiotics in humans and food animals (Franz et al. 2011).

VREs have been detected and isolated in many parts of the world (Wegener et al. 1999; Hershberger et al. 2005). The extensive/massive use of antimicrobials in food animal may have undesirable consequences for human health, so it could result in a possible selection of a reservoir of opportunistic human pathogens such as Enterococci may carry antibiotic resistance determinants and including pathogenic virulence genes. Thus, in recent years the increase in VRE acquired nosocomial infections leads the possible relationship between community/food industry-acquired VRE or Glycopeptide Resistant Enterococci (GRE) strains to colonize the intestine through food and VRE/GRE strains isolated from cross infections and colonization to be detected. In medical settings; Some Chemotherapeutic agents and/or endoscopes used for diagnosis and/or treatments and VREs involved in the general circulation through the translocation from the endogenous flora as a result of the tissue damage in gastric mucosa may cause bacteremia and endocarditis which are the difficult-treated disease. Moreover, the strains being disseminated around by colonized patients persist in being in the hospital environments for a long time which results in severe nosocomial infection especially in immunocompromised and intensive care units' patients. In spite of the starter microorganisms' contribution to the food industry, because of the fact that they cause the rapid spoilage of food products stored in unsuitable conditions and, more importantly, the bacteria may gain easily resistance to antimicrobials and can easily spread lots of virulence factors, mobile genetic elements and antibiotic resistance genes to other microorganisms.

Development of new strategies/solutions for prevention of ARE especially VRE, as well as Multidrug-Resistant(MDR) Bacterial infections in the community, depend on appropriate plans and applications in clinical settings, veterinary medicine, food industry, medical-veterinary drug production sectors and international/national legislation. Those preventive strategies may be used such as alternative

antimicrobials producing instead of conventional antimicrobial agents, international standard procedures-applications of vaccination and growth promoting agents for food animals, effective disinfection programmes for hospital settings and hand washing programmes for health workers as well as regular monitoring and analyzing of all data should be considered by international/national authorities in order to prepare preventive/corrective actions. In addition, suitable and safety starter culture selection and preparing standardized regulations about this subject can be beneficial effect in both food industry and public health.

Important Features of Enterococci in Food Industry

In general, *E. faecium* and *E. faecalis* species from enterococcal group which can keep their vitality in in-vitro conditions for a long time as a result of their resistance to harsh physical conditions and their ability to spread around nature with organic elicitation, are important industrial microorganisms in food microbiology, due to both showing activity lipolytic and esterolytic in food and also utilizing citrate and creating volatile aromatic compounds. In addition, they can improve organoleptic properties such as taste, color, and the smell of some foods. Therefore, with lactic acid bacteria, they are used in fermented meat and dairy products as starter cultures (Maschieto et al. 2004).

Enterococci as Starter Microorganism

Enterococci's permitting high temperature can easily enable these bacteria to be an indicator of quality sanitation in food products. In some studies, *E. faecium* could maintain their liveness in spite of a 30-min heat treatment at 68 °C, and reported that the presence of these bacteria is inevitable in many processed and stored foods like dairy products and fermented sausages as well as food consumed raw like fresh vegetables and vegetable materials (Giraffa 2003).

Probiotic Features of Enterococci

Various studies show that enterococcus strains that will be used as probiotics can be economic, or they can be occupied from different sources through conventional methods and/or fermentation. Another issue pointed out in the studies is whether the strains got in this way have antibiotic resistance and virulence genes. *E. faecium* strains are used as probiotics in the growth of food animals, production of animal feed and the prevention of diseases in them (Çakır et al. 2002; Franz et al. 2011). These bacteria contribute to metabolism by preventing the colonization of ordinary

bacteria in intestinal flora and help the maturation and proliferation of enterocytes and muscular mucosa (Yaman and Esendal 2004). Therefore, *E. faecium* is suggested to be used in animal nutrition as an alternative to antibiotics, and it is debated that it can be used particularly in the treatment of mastitis (Marekova et al. 2003). A study reported that 15 *Enterococcus* strains gathered from different foods were identified and analyzed for virulence factors and antibiotic resistance. In another study, three *Enterococcus durans* strains were chosen to study PBMC and Caco2 cells for their immunomodulatory properties. It is reported that the strains modulated gut microbiota increasing *Faecalibacterium prausnitzii*, a functionally essential bacterium. Thus, *E. durans* EP1 not only increased *F. prausnitzii* in some cases of dysbiosis but could also be effective in gut inflammatory disorders therapy (Carasi et al. 2017).

Some studies claimed that these bacteria can be used as probiotics in gastroenteritis in humans (Hugas et al. 2003). For example; it is reported that *E. faecium* 68 (SF68) strain can be used for such purposes as strengthening the immune system, preventing diarrhea, and preventing cancer development by contributing to the consumption of carcinogen in bowels (Rinkinen et al. 2003; Linaje et al. 2004). Diarrhea duration of the patients who had diarrhea reduced when *E. faecium* was used as a probiotic; the best electrophoresis combination for producing probiotic yogurt claimed to be S. *thermophilus* and *E. faecium* species (Crittenden et al. 2003).

A similar study conducted in India and examined antibiotic resistance, biogenic amine production and the presence of *agg*, *esp*, *efa*, *afm*, *gelE*, *cylA*, *cylB*, *clyM*, *cpd*, *ccf*, *ace*, and *hyl* in *E. faecium* KH 24 strain isolated before. It was found that this strain had no antibiotic resistance; virulence genes were not detected except in *efaAfm*, and it did not produce biogenic amine. Besides, it was found that KH 24 strain had a good in vitro tolerance to biological barriers and could easily live in mice bowels. Mice groups fed with this strain had better weight control and the number of *Salmonella enteritidis* in their bowels was reduced by approximately 1 log cfu/g in comparison to the control group. A significant decrease in the coliform count (pb 0.05), lactobacilli count (pb 0.05) was observed in the test group. *E. faecium* KH 24 strain was therefore reported to be a reliable strain and it could be used as a protective culture or as a probiotic in food (Strompfova et al. 2004; Bhardwaj et al. 2010).

Another study conducted in Tunisia and reported that *Enterococcus faecium* strains were isolated from an original biotope, and analyzed for assurance and capacity as probiotics on dried Tunisian meat "Dried Ossban". It is reported that *E. faecium* strains gathered from "Dried Ossban," did not have a health risk on humans, and might be considered as candidates for future use as probiotics and bioprotective cultures for use in the food and/or feed industries (Zommiti et al. 2018).

Enterococci in Dairy Products

In the cheese maturation process, enterococci dependable affect the formation of taste, color, and aroma, in addition, to be a good starter microorganism in cheese production due to good salt tolerance, fast acid production and resistance to high temperatures (Giraffa 2003; Abeıjón et al. 2006). The strains of *E. faecium* K77D, used as a starter culture in fermented dairy products was advised by Advisory Committee on Novel Foods and Processes (ACNFP) in England (El-Din et al. 2002).

According to some studies, in Mediterranean Countries, *E. faecium*, sincerely found in milk during the production of traditional cheese like Misha and Domiatti, provides the desired aroma or contributes to the development of the sensory properties by using citrate metabolism and producing typical flavor compounds such as acetaldehyde, acetone, diacetyl and, this cheese contains nitrogen being able to dissolve in more water and improving organoleptic properties and total free amino acids (De Vuyst et al. 2003; Giraffa 2003). It is observed that *E. faecium*, providing degradation of casein in cheese production by producing proteolytic enzymes, hydrolyzes milk fat with esterases during the production of cheese (El-Din et al. 2002).

In another study, Gelsomino and his colleagues (2001) implied that cheese used in the production of cheddar cheese and *E. casseliflavus* and *E. faecalis* in the tank of milk storage were dominant species and these species described above were dominant in human feces as well.

Enterococci in Meat Products

According to some studies of biochemical activities of sausages, it was shown that the presence of enterococci in fermented meat products, particularly *E. faecium*, contributed glycolytic, proteolytic, and lipolytic activities to the sausage flavor, took on a role in "methmyoglobin reduction reactions" providing formation of red color in fresh meat and, in case of the use of *E. faecium* CTC492 as a starter culture in the production of sausages, this strain maintained its effectiveness through the end of the process (Hugas et al. 2003). On the other hand, it was pointed out that *E. faecium*, producing bacteriocins, has more advantageous than traditional chemical preservatives, impeded the growth of pathogens found in the meat products, besides its contribution to the formation of flavor, *E. faecium* strain has significant as being protective against bacterial contamination (Giraffa 2003). Thus, it was implied that these bacteria contributing to the process of food maturation as a starter retarded bacterial decrease in reproduction and putrefaction in fermented products by showing anti-*Listerial* and anti-*Clostridial* effects with the help of bacteriocins they produced (Sarantinopoulos et al. 2002; Hugas et al. 2003; Ribeiro et al. 2011). It was demonstrated that use of enterococci in industrial applications as starters, they could

colonize in products due to the bad hygiene in slaughterhouses and could become dominant by reproducing/proliferating during being hold with the design of fermentation (Giraffa 2002; Papamanoli et al. 2003). In a study of chicken meat produced with modified atmosphere, it was found that dominant microflora was formed by *E. faecalis* with *Lactobacilli* and corinabacter species during the storage of products at 3.5 °C up to 7 days (Barakat et al. 2000). In another study with German and Italian type of fermented sausages, it was reported that at the end of the maturation process, the number of enterococci in all sausages, having or not having enterococci as starter cultures, was between 10^3 to 10^5 cfu/g and enterococci were main contaminants in addition to their industrial usage (Hugas et al. 2003).

Enterococci in Food Preservation

The use of microbial metabolites such as bacteriocins use for food security instead of chemicals called as "green technology" may be considered a new bioprotective method (Ross et al. 2002). It was suggested that *E. faecium* and *E. faecalis* strains might be bacteriocins harboring technological potential in food industry because of enterocins they produced had better physicochemical characteristics and biological actions than the other bacteriocins did (Linaje et al. 2004; Franz et al. 2011). On these subjects, Bacteriocin-Like Inhibitory Substrates (BLIS), having anti-*Listerial* effect and BLIS were identified in *E. faecium* strains isolated from fermented sausages in Mexico (Alvarez et al. 2010). In another study, it has been reported that *E. faecalis* UGRA1 isolated from Spanish sheep cheese produces a 70 kb enterocin called AS-48, and the strain which can create biofilm has the competence to stick to "Caco2" and "HeLa 229" cells and *L. monocytogenes*. Advanced analyses of the strain producing a broad spectrum of bacteriocin have yielded positive results in terms of food security and it has been implied that it has a biotechnological potential like a probiotic as a protective agent in food storage (Cebrıán et al. 2012).

In a similar study conducted in Iran, it was detected that Bacteriocin *Enterococcus faecium* strain C2 (isolated from local cheese) had the most antibacterial activity. The most antibacterial effect was observed against *Listeria monocytogenes* and *Pseudomonas aeruginosa.* Considering that produced bacteriocin has a wide inhibitory range against gram-positive and gram-negative bacteria, especially pathogenic bacteria, it is recommended to use it as a substitute for chemical antibiotics (Khodaee and Nejad 2017).

Enterococci produce considerable antimicrobial compounds such as lactic acid, hydrogen peroxide and bacteriocins. Therefore, they are suggested to be used as bioprotective in the food industry. The lactic acid produced through the metabolization of carbohydrates acidifies peripheral pH and deters bacteria such as coliforms reproducing in neutral pH (Giraffa 2002; Franz et al. 2007). Despite using enterococci in food production processes, generating biogenic amines caused rotting cheese and refined meat when not stored in appropriate conditions and has provoked a dispute. Furthermore, enterococcal strains have been debated as unwanted

organisms in the food industry in terms of human health as they may develop resistance to many antibiotics used in medical treatments. So, *Enterococcus* spp. is not Generally Recognized as Safe (GRAS) (Giraffa 2002; Franz et al. 2011). Thus, the abundance of enterococci present in food is considered as an indicator for the insufficiency of sanitation conditions. According to a study; post-process contamination by *Pseudomonas* spp. and survival of enterococci supports their recommendation as additional indicator bacteria for plant hygiene, product quality, spoilage and possibly safety of High-moisture raw milk mozzarella cheese (RMMC). Thus, *Enterococcus* spp. might be used as the indicator of rot in soft cheese (Meier et al. 2018).

Importance of Enterococci in Terms of Public Health

The use of enterococci in food production sector shows that these bacteria are the part of human nutrition i.e., the body and brain health. However, enterococci have been the focus of debates in medical area lately, due to the hospital infections they have caused. These microorganisms have developed high resistance against reserve medications including vancomycin and teicoplanin that are used increasingly in clinical applications and against all other beta-lactam group antibiotics and they have been started to be isolated in hospital infections where the treatment failure was observed among patients whose immune system has been suppressed (De Fátima Silva Lopes et al. 2005; Franz et al. 2011). Another example of mechanism two may be VRE; The Enterococci, which normally colonize in the gut, might have acquired resistance to multiple antibiotics over time, making the glycopeptide vancomycin which is one of the last therapeutic alternatives.

The epidemiology of VRE shows some differences essentially between the USA and Europe. In Europe, *Enterococcus faecium* carrying the vanA resistance element (which was transferable to other bacteria) and vancomycin resistance was commonly found in the intestinal flora of farm animals as well as healthy people, but carriage of VRE in farm animals and healthy people were absent in the USA (Bonten et al. 2001). This difference has been proposed to be due to the wide spread agricultural use of avoparcin; a glycopeptide used in Europe since the 1970s, but was never approved for use in the USA. Avoparcin, which confers cross-resistance to vancomycin, has been shown to select for VRE in animals (Aarestrup et al. 1996). A large reservoir of VRE in animals presents conveniences for human infection, and the potential for resistant bacteria to colonize the human digestive tract. In addition, molecular epidemiologic studies have found that the VRE strains isolated from animals and humans are difficult to tell apart, as are the resistance elements (Woodford 1998; Jensen et al. 1998); hence at least the potential for transmission exists.

Enterococci *in Hospital Infections*

In many cases, isolation of enterococci from hospital infections in recent years has brought them into focus invariably medical area. The *Enterococcus* spp. strains causing hospital infections are isolated from hands of healthcare personnel and nursing homes and from the surfaces around the hospital. Enterococci species transported from one patient to another or even from one department in the same hospital to the other either, due to contaminated hands of health care workers or materials such as the shelves, sheets, and bedsteads may cause infections which are so difficult to treat. Particularly in patients being monitored in intensive care units, enterococci may usually cause endogenous or exogenous origin bacteriemia and endocarditis, urinary system infections, intra-abdominal infections, surgical wound infections, perinatal infections and albeit seen less meningitis and pneumonia (Chenoweth and Schaberg 1990; Morrison et al. 1997; Franz et al. 1999; Tenover et al. 2004). According to some studies, 60% of enterococcal infections are hospital originated and half of them are adhered to intensive care units. Enterococci strains were isolated in about 30% of patients in intensive care units in the USA. Commonly, *E. fecalis* is responsible for 80–90% of *Enterococcus* spp. infections, while *E. faecium* is responsible for 5–10% of them. However, the ability of *E. faecium* acquiring more easily the resistance to a broad range of antimicrobials, largely glycopeptide group antibiotics, changes the balance between hospital infections and the bacteria in favor of *E. faecium* species (Murrey 2000; Freitas et al. 2009).

Vancomycin or glycopeptide group antibiotics are preferred in treatment for difficult-to-treat and resistant Gram-positive bacteria infections, particularly in methicillin-resistant *Staphylococcus aureus* infections. Additionally, arising resistance of staphylococci and enterococci which may cause heavy infections to some antibiotics in this group was noticed in the 1980s. Similarly, there has been an increase in the incidence of VRE infections in big hospitals where patients under risk are accepted. This has aroused a series of such problems as the treatment of *Enterococcus* spp. infections and the enterococci's transmitting horizontally these resistance determinants to other vancomycin sensitive species (Pearson 2002; Hasman et al. 2005; Upadhyaya et al. 2009). Some studies in the USA have shown that the horizontal spread of *vanA* gene from VRE in patients in the hospital into Meticillin-Resistant *Staphylococcus aureus* (MRSA) has resulted in high vancomycin resistance among MRSA strains (Chang et al. 2003; Weigel et al. 2003).

The increase in the asymptomatic colonization rate of VRE/GRE strains in the community, both the exogenous and endogenous origin infections raise the importance of these bacteria in terms of hospital settings. Using of invasive instruments for treatment or diagnostics, use of antibiotics, the duration of staying in hospitals, previous stays in risky units, such as the intensive care units are vital in the increase in the prevalence of hospital origin VRE/GRE colonization. However, the importance of the strains acquired in community origin resistant *Enterococcus* spp. colonization and infections through food must not be ignored (Maschieto et al. 2004; De Fátima Silva Lopes et al. 2005; Valenzuela et al. 2009).

Antibiotic Resistance Mechanisms of Enterococci

Antibiotic resistance of *E. faecium* strains is crucial factor of their pathogenicity (Franz et al. 2001). In *E. faecium* species, that most of the virulence factors and resistance genes are encoded on a plasmid that is clear explanation of this relationship (Lukasova and Sustackova 2003). In resistant strains, the colonization of intestine is facilitated and tissue invasion is carried with the help of factors such as cytotoxin and gelatinase encoded in resistance plasmid (Aktaş and Derbentli 2009). Besides, their virulence increases because of their resistance mechanisms.

There are two types of antibiotic resistance can be improved in these bacteria; natural and acquired types (Giraffa 2002; Upadhyaya et al. 2009).

The mechanism of resistance to antibiotics in enterococcal bacteria can be analyzed in two main groups:

1. Natural (intrinsic-chromosomal) Resistance,
2. Acquired (extrinsic) Resistance.

Natural Resistance

Natural resistance of enterococci is based on species-specific. It refers to the chromosomal resistance observed in all the enterococcal species such as they are inherently resistant to penicillins, cephalosporins, lincosamides, trimethoprim-sulfamethoxazole, and aminoglycosides, including low-level resistance to quinupristin/dalfopristin. *E. gallinarum* and *E. casseliflavus* strains naturally have low-level resistance to vancomycin (Klare et al. 2003).

Acquired Resistance

The mechanism of acquired resistance, like DNA mutations or transposon and plasmid or pathogenicity islands, adapts as a result of the transfer of genome of a new DNA segment. It is the most frequent mechanism of conjugation (Murray 1998).

Despite the fact that acquired resistance in enterococci is usually found in *E. faecium* and *E. faecalis strains*, *E. avium*, *E. durans*, and other enterococcus species can also be observed having this trait. In enterococci, this resistance is encoded with *vanA*, *vanB*, *vanC*, *vanD*, *vane* and *vanG* genes and is identified with the name of the related gene. It is reported that *vanA* and *vanB* type resistance genes were firstly identified in *E. faecium* and *E. faecalis*strains (Murray 1998; Çetinkaya et al. 2000; Aktaş and Derbentli 2009).

Vancomycin/Glycopeptide Resistance

Glycopeptide antibiotics are generally prefered against nosocomial Gram-positive aerobic and anaerobic pathogens as a "last resort antibiotics" in clinical area (Morrison et al. 1997).In the mechanism of glycopeptides briefly: These antibiotics binding to "D-alany-D-alanine" dipeptide locating at the end of the pentapeptide chain linking to muramic acid in N-acetyl glucosamine-N acetyl muramic acid disaccharide forming the backbone of peptidoglycan synthesis impede the process of the glucose transglucolisation and transpeptidation required for peptidoglycan synthesis. Thus, bacterial cell wall undergoes lysis because of not being synthesized. The binding ability of glycopeptides is decreased by placing 'D-Ala-D-Lactate' or 'D-Ala-D-Serine'and ligase enzyme in peptidoglycan side chain bacteria, instead of putting 'D-Ala-D-Ala' (Çetinkaya et al. 2000; Aktaş and Derbentli 2009).

Phenotypes of Vancomycin Resistance

Inducible high-level vancomycin (MIC ≥ 64 μg/mL) and teicoplanin resistance (MIC ≥ 16 μg/mL) are defined in VanA type resistance (Murray 1998) *VanA* gene is substantially detected in *E. faecium* species. However, it is especially found in *E. faecalis*, and then *in E. durans*, *E. avium*, *E. mundtii*, *E. gallinarum*, *E. casseliflavus*, *E. raffinosus* and some species not belonging to enterococci. VanA type resistance, providing vancomycin and teicoplanin resistance among food-originated VRE, is the most common in the world wide (Linden 2007).

A moderate level inducible resistance is VanB type resistance [Vancomycin MIC ≥ 32–64 μg/mL (4–1000 μg/mL)] and it does not lead to any resistance to teicoplanin (Murray 1998; Çetinkaya et al. 2000).

VanC type resistance which is identified in both *E. casseliflavus* and *E. gallinarum* and intrinsic low-level resistance to vancomycin (MIC ≥ 4–32 μg/mL) and teicoplanin susceptibility are defined (Murray 1998; Çetinkaya et al. 2000)

VanD type resistance is recently devoloped resistance which is defined in *E. faecium* strains. *VanD* gene has shown a chromosomal localization and is known that it is not transferred due to conjugation. The strains harboring VanD type strains are resistant to both vancomycin (MIC = 64–256 μg/mL) and teicoplanin (MIC = 4–32 μg/mL).

VanE and VanG phenotypes show similar to VanC phenotypes and are only reported in *E. faecalis* strains (Aktaş and Derbentli 2009).

Pathogenicity and Virulence Factors of Enterococci

Virulence genes in enterococci are encoded in Pathogenicity Island (PAI) occupying genome and/or in plasmids. A pathogenicity island of enterococci for the first time was described in MDR *E. faecalis* in 1980 which causes hospital infection. The magnitude of this PAI whose G + C ratio is 32.2%; is about 150 kb and it encodes 129 Open Reading Frame (ORF) (Tendolkar et al. 2003; Shankar et al. 2002; Upadhyaya et al. 2009).

Alike other pathogenic bacteria, enterococci having a similar number of virulence genes and they easily develop resistance against antimicrobial agents and inhabit endogenous flora, which endorses them to become a considerable opportunistic pathogen. Enterococci carry virulence factors, such as adhesin-like antigen A and enterococcal surface protein (esp) in aggregation agents (agg), gelatinase (Gel), hemolysins, collagen adhesin (as), *E. faecalis* and *E. faecium* strains which both facilitate and also damage the colonization in the host tissue (Eaton and Gasson 2001; Franz et al. 2001; Mannu et al. 2003). *Enterococcus* spp. included the variations in the distribution of virulence genes which was reported in the molecular-based studies. In a study, it was found that spread and adhesion of *E. faecalis* and virulence genes such as cytolysinand pheromone production mechanisms were detected in higher rates than *in E. faecium* strains. It was also shown that these genes were encoded at a higher rate in clinical *E. faecalis* species than in food-originated species (Eaton and Gasson 2001). In a study, virulence factors, antibiotic resistance, bacteriocin production and properties of bile hydrolysis in enterococci isolated from raw and pasteurized milk, meat products, cheese and vegetable in Brazil being analyzed, it was found that 67.7% of the isolates hydrolyzed bile salt, 15.2% of them produced bacteriocins, 12.0% were β-hemolytic and 18.2% of them produced gelatinase, but it was failed to show antibiotic resistance (Gomes et al. 2008). However, it was claimed that virulence genes, encoded in plasmids like hemolysin-cytolysin production, adhesion ability and resistance to antibiotics, were transferred by conjugation pathway with resistance genes in enterococci, so, it was claimed that virulence genes were associated with antibiotic resistance in intestinal flora, environment, food and between strains and species (Eaton and Gasson 2001; Tansuphasiri et al. 2006).

The Prominent Virulence Factors of Enterococci

- Enterococcal Surface Protein (Esp),
- Aggregation agent,
- Microbial surface protein component which describes adhesive matrix molecules of enterococci induced collagen (MSCRAMM Ace).
- Capsules, cell-wall polysaccharides
- Lipoteichoic acid
- Superoxide

- Gelatinase,
- Seven to eight amino acids in length, small hydrophobic peptides identified as sex pheromone and encoded in the chromosome
- Hyaluronidase
- Haemolysin or cytolysin,
- *Enterococcus faecalis* antigens A (Efa)
- AS-48 (Devriese et al. 2006; Upadhyaya et al. 2009; Franz et al. 2011).

Antibiotic Resistance of Foodborne Enterococci

Numerous studies conducted in Europe, it is implied that VRE colonization frequently appears in society, and causes community-acquired asymptomatic VRE colonization due to colonizing in various animals, raw or cooked food and environmental samples and infecting people through contact. Namely, VRE in the food chain is the most critical reason for human gastric colonization (Giraffa 2002; Valenzuela et al. 2009). This view is supported by isolating increasing level *E. faecium* from clinical specimens and presenting sign for food chain whose importance is increasing in incidence, and by associating this species with the ability to live at high temperatures in beef, poultry, pork and other meat products (Wegener et al. 1997; Son et al. 1999; Gambrotto et al. 2001). In a study conducted with meat and chicken products in Spain, enterococcal strains were found in 73% of specimens and it was detected that isolates were resistant to one antibiotic at least or more than one antibiotic such as tetracycline, erythromycin, and vancomycin. Similar results were reported for 90% of pork in Sweden, 55% of chicken and 14% of pork in Denmark (Teuber et al. 1996; Guerrero-Ramos et al. 2016).

AR *E. faecium* strains are shown in dairy products, peculiarly in industrial cheese (Teuber et al. 1999; Giraffa 2002). In some studies, with cheese produced in Europe, *E. faecalis* and *E. faecium* species, resistant to penicillin, tetracycline, chloramphenicol, erythromycin, gentamicin, lincomycin, rifampin, fusidic and vancomycin, in different amounts were isolated (Teuber et al. 1996). Multidrug-resistant enterococcal species were found in both pasteurized cheese and raw milk. ARE's being found in these last products is considered as a serious risk in terms of insertion of antibiotic resistance into the food chain. In some studies, enterococcal species, high level resistant to kanamycin and gentamicin were isolated from French cheese produced from raw milk and patients in hospitals (Bertrand et al. 2000). A similar study conducted in Turkey reported that antibiotic resistance phenotype of 213 *Enterococcus* spp. isolated from traditional Turkish cheese was investigated and kanamycin and gentamicin resistance was found 98.6%, 4.2% respectively (Sanlibaba and Senturk 2018).

VRE Infections and Food Chain

Several works of literature implied that Antibiotic Resistant Enterococci (AREs) and especially VREs which entered into the food chain has gained importance with the increasing significance of VREs in hospital infections. A study conducted in Portugal reported high amounts of AREs in commercial chicken samples (Novais et al. 2005). A similar study conducted in Iran reported that thirty samples of meat, chicken, and cheese were examined for VRE during 2010. Traditional and molecular identification tests showed that all the isolates were *E. faecium* carrying vanA. None of the isolates harbored vanB. The results showed that enterococci are common contaminants in food. Indeed, this study indicates a high prevalence of multidrug-resistant enterococci in the food of animal origin in Iran (Talebi et al. 2015). Another study reported that a total of 160 samples of poultry (80), pork (40), and beef (40) products were evaluated in northwestern Spain in order to find out the prevalence of vancomycin-resistant enterococci (VRE). VRE was detected in 38 (23.8%) samples 37.5% of poultry, 15.0% of pork, and 5.0% of beef samples. It was reported that resistance or intermediate susceptibility to three or more antimicrobials of clinical significance, in addition to vancomycin. Besides, they reported that *vanA*, *vanB*, *vanC*-1, and *vanC*-2/3 genes were identified in various isolates. As a result, they reported that meat products might play a role in the spread through the food chain of VRE including several resistance and virulence genes (Guerrero-Ramos et al. 2016).

The relationship of foodborne enterococci with clinical infections has not been clearly elucidated yet. But, it is argued that foodborne enterococci, with horizontal gene transfer, may play a role in the expansion of virulence genes. Molecular-based studies show that clinical based *E. faecalis* strains had more virulence factors than food originated strains. Moreover, similar studies identified that virulence genes could be transmitted to starter cultures due to clinical type conjugation (Giraffa 2002; Valenzuela et al. 2009). A study conducted in Italy and investigated *Enterococcus* strains isolated from pork meat and stool samples and found, using PCR-RFLP and series analysis methods, that tetracycline resistance gene *tet*(M) and the presence of Tn916 transposon were correlated in all strains; it is emphasized that *Enterococcus* in the food chain can be important in transmitting antibiotic resistance. In a study conducted in Switzerland, Rizzotti et al. (2009) compared food and clinical enterococci with Pulsed Field Gel Electrophoresis (PFGE) method, found a genetically very strong relationship between the isolates, and emphasized that these isolates could spread around through food chain (Giraffa 2002; Leavis et al. 2006; Templer et al. 2008).

Various studies in Europe and the USA have studied the prevalence and epidemiology of VRE, particularly the place of VREs in infection epidemiology, causes, and identification of the risk factors. However, in the Middle East and Asia have less information about this subject (Askarian et al. 2008; Salem-Bekhit et al. 2012).

European countries and the USA show some differences in terms of VREs epidemiology. VRE was usually isolated from food animals in Europe, which was

related to the massive use of "avoparcin" as a growth promoter factor in animal feed in the agriculture sector. If food animals fed with this feed could be play as reservoir role of transferable vanA type resistance. So, VRE was found in farm animal droppings, in raw chicken meat, in fertilizer samples of pigs and poultry, and in wastewater reservoirs in the feeding area. Therefore, using of "avoparcin" was banned in the agriculture sector in 1997 (Bonten et al. 2001). On the other hand, since "avoparcin" was not used in the USA, VRE could not be isolated in animals and healthy humans. However, hospital-acquired VRE infections are shown more common in the USA than Europe (Vehreschild et al. 2018).

Identification of antibiotic resistance and pathogenicity of foodborne enterococci has become much important in the food industry. On this subject, studies conducted in recent years have dealt with these bacteria which can be found in food for a variety of reasons and/or used as starter culture; particularly their antibiotic or glycopeptide/vancomycin resistance, virulence factors and genetic similarities have been subject to much important. Numerious studies reported that some regional differences in the antibiotic resistance and virulence factors of foodborne enterococci. While some studies reported few or no vancomycin and antibiotics resistance, some other studies reported highly resistant strains. For example; a study pointed out that *E. faecium* and *E. faecalis* strains isolated from cheese did not have resistance to vancomycin (Giraffa and Sısto 1997). On the other hand, some studies investigated the presence of VRE in 101 chickens, porks, and turkey meat obtained from 18 different supermarkets and chicken droppings obtained from 50 slaughterhouses. Results implied that VRE was detected in 27.2% of the chicken samples and 16% of the chicken droppings. 11 of the VRE were identified as *E. durans*, 10 of them as *E. faecalis*, and 10 of them as *E. faecium.* Another study which searched a total of 148 *Enterococcus* species resistant to vancomycin in pasteurized meat products and fermented sausages and found that 143 *E. faecium* strains persisted in all meat products, *Enterococcus* species isolated from sausage and raw milk were resistant to tetracycline, chloramphenicol, gentamycin, and erythromycin. Another study reported that *E. faecium* strains isolated from "Dominati" cheese produced in Egypt were found to be resistant to oxacillin, cephazolin and sensitive to ampicillin, amoxicillin, and cefaclor. A similar study examined the presence of hemolysin production and resistance to vancomycin of *Enterococcus* spp. which isolated from 20 sausages and 30 cheese samples and found that there was hemolysin production in *E. durans* strains obtained from cheese and sausage samples but hemolysin production was not found in other strains. Various studies indicate that food of animal origin *Enterococcus* spp. and environment and water originated *Enterococcus* spp. were found in high quantities and they had Multiple Drug Resistance (MDR). It is emphasized that this case can play a potential role in the entrance of antibiotic-resistant bacteria and resistance genes into the food chain as well as the environment. It can also emerge as a potential public-health problem in the future. For instance; a study conducted in Hungary, it was reported that there was VRE in farms even eight years after avoparcin was banned. This finding indicates that VRE can play a reservoir role in its spread, and the technology used in farms in Hungary should be changed so that high rates of VRE can be prevented (Eaton and Gasson

2001; Giraffa 2002; Lukasova and Sustackova 2003; Hauben 2003; Çıtak et al. 2004; Karakaş 2005; Tansuphasiri et al. 2006; Hummel et al. 2007; Ghidan et al. 2008).

A similar study conducted in Turkey that Gram-positive cocciwere isolated from 50 chicken meats and their antibiotic resistance was analysed, it was showed that 50% of the enterococci strains were resistant to vancomycin. Another study which evaluated antibiotic resistance of *Enterococcus* strains isolated from meat and dairy products produced in Çukurova region of Turkey showed that all *Enterococcus* strains were found resistant to vancomycin (Yurdakul et al. 2009; Erginkaya et al. 2010).

The main question of whether foodborne enterococci are reservoirs in the antibiotic resistance and the transmission of virulence genes has made it compulsory the examination of virulence genes and antibiotics resistance of both foodborne and clinical enterococci. Various studies reported the virulence factors of *Enterococcus* spp. strains isolated from food and clinical specimens; it was found that all of *E. faecalis* strains carried virulence factors, but clinical isolates had more virulence factors when compared to the isolates that are used as a starter in foods; no virulence factors were detected in *E. faecium* strains. Another study conducted in Italy shows that the types have the ability to change genetic features through conjugation and *Enterococcus* species isolated from meat products such as salami and raw meat transfer antibiotic resistance determinants to *E. faecalis* JH2–2 species (Cocconcelli et al. 2003; Hugas et al. 2003). Another study conducted in Italy examined bacteriocin, cytolysin, hemolysin and gelatinase production of food, animal and clinic-related VRE. Although no prevalence was given, the necessity of testing antibiotic resistance and virulence features of *Enterococcus* spp. species that will be used as a starter and probiotics in the food industry has been concentrated on (Hummel et al. 2007; Sabia et al. 2008).

Preventive Strategies for VRE/ARB/MDR Bacteria Dissemination

The strategies of prevention/reduction of VRE/ARB/MDR bacteria are based on different sectors. These are clinical/medical settings and all partners of antimicrobial agent production industry, veterinary medicine, food industry as well as international and/national authorities/legislation. Some of these proactive measures are summarized below:

Implementation of Antimicrobial Stewardship Programs (ASPs) in Clinical Settings

Multidisciplinary antimicrobial utilization teams have adequate experience in their fields, including physicians, pharmacists, microbiologists, epidemiologists as well as infectious disease specialists in order to optimize antimicrobial therapy used ASPs. These programs are generally based on education, coupled with the "front-end" treatment (e.g., limiting the availability of chosen antimicrobials) or the "back-end" treatment (e.g., the criticizing of broad-spectrum empirical therapy and then predisposing or stopping therapy based on antimicrobial susceptibility testing (AST) results and clinical reply) (Moehring and Anderson 2012; Paterson 2006). ASPs have also shown a link between antimicrobial usage and the emergence of resistance such as vancomycin using and vancomycin-resistant enterococci (Harbarth et al. 2002). In 2007, the guidelines for ASPs had been suggested by The Infection Diseases Society of America (IDSA) and the Society of Healthcare Epidemiology of America (SHEA) (Dellit et al. 2007). According to various studies, ASPs have the potential to limit the emergence and spread of antibiotic resistance (Drew 2009). ASPs are included such as Antimicrobial Susceptibility Testing (AST), fast and accurate microorganism-identification, development of microbiota, education of all parts of ASPs members related to their routine treatment decisions, appropriate hospital disinfection and personal hygiene of healthcare workers (Lee et al. 2013).

The Development of Novel Antibiotics

In order to discover new classes of antibiotics, novel strategies for rational design and screening-based approaches are necessary. New strategies should be presented for the treatment of microbial diseases, such as host defense peptides, bacteriophages, vaccines, immunoglobulins, and probiotics instead of conventional antimicrobial agents (Lloyd 2012).

Veterinary Medicine

If the problem of antibiotic resistance in human medicine is wanted to solve, the first step will be a reduction of antibiotic usage in veterinary medicine, agriculture, and aquaculture. According to various studies, antibiotic-resistant bacteria/genes may be generally transferred to humans by food animals/meat products. So, to prevent the emergence and transfer of antibiotic resistance in food animals, new methods to manage infectious diseases in animal husbandry are extremely essential such as suitable use of existing vaccines, using enzymes, probiotics, prebiotics, and some

organic acids to improve their health (Castanon 2007; Potter et al. 2008; Callaway et al. 2008). In addition, developing hygiene practices in the production steps of food animals, and making use of bacteriocins, antimicrobial peptides, and bacteriophages can be used instead of antibiotics to support growth in food animals. Besides, infectious diseases may also be decreased in them (Joerger 2003; Boklund et al. 2004; Atterbury 2009). Additionally, internationally acceptable standard protocols should be prepared for the use of antibiotics in animal husbandry and about surveillance programs in order to monitor the global emergence of VRE/ARB/MDR bacteria.

Food Industry

Appropriate starter/co-culture selection is very important in the food industry. For example; the usage of enterococci needs to be safe in meat and dairy products, fermented vegetable products as well as probiotic products. Considering the safety, and according to the Qualified Presumption of Safety (QPS) list from the European Food Safety Authority (EFSA), *Enterococcus* spp. is not recommended for the QPS list (EFSA Panel on Biological Hazards et al. 2017). Moreover, these bacteria don't have GRAS status (Ogier and Serror 2008). Lately, some global organizations such as the EFSA, the Advisory Committee on Novel Foods and Processes, (ACNFP), and the Food Standards Agency permitted the use of certain strains of enterococci as food additives and supplements based on a careful case-by-case assessment. Thus, the individual strain must be considered and health risks must be removed for this certain strain (ACNFP 1996; Franz et al. 2011; EFSA 2012). In order to distinguish between safe and potentially harmful strains of *E. faecium* in animal nutrition, a system of methods is also be provided by EFSA guidance (EFSA 2012). It is intended for use as feed additive producers submitting applications to EFSA for safety evaluation.

Conclusion and Recommendation

Antibiotic resistance in bacteria has become one of the most important problems in worldwide. On the other hand, there is a very complex relationship between antimicrobial usage and antimicrobial resistance for many types of pathogen microorganisms and needs more multidisciplinary studies in order to solve this problem.

The use of antimicrobials on farms is linked to (ARG) Antibiotic Resistant Gene emergence but whether food animals are the sources of ARG transfer to human pathogens are the main question and debated human and veterinary medicine researchers as well as public health authorities. Because, ARG exposure is both food safety and public health problem and environmental exposure through air, soil, and water. Furthermore, it is important to establish a diagnostic standard such as

whole genome sequencing for ARG detection such as PFGE which is considered "Gold Standards" for assessing isolate interrelationships and Multilocus Sequence Typing (MLST) methods.

In general, some methods may be used to control antimicrobial resistance in human medicine, veterinary/agricultural area and food industry:

1. Antimicrobial usage should be reduced in both human and veterinary medicine and the use of broad-spectrum antimicrobials which are clinically important should be limited. Alternatives to AGPs, such as good farm practices and use of probiotics, prebiotics, and natural antimicrobial agents must be encouraged in the veterinary sector.
2. Forbidding the dumping of antimicrobial agents to waste into the environment and removing antimicrobial residues that exceed the standard limit in food and food products and water should happen by proper legislation.
3. To limit inappropriate administration of antibiotics, regular consultation with veterinarians can take place. Thus, the awareness/education of food/animal producers is so important.
4. The awareness of farmers regarding the implications of the unnecessary use of antibiotics in food animals has on human health and the environment is also very important, Therefore, orderly education programmes are needed for them. Foodborne enterococci (starter/co-cultures) standards with the view of food security about these subject; food-based enterococci do not have to be genetically related to strains making infection, does not have to include virulence genes which are related to antimicrobial resistant genes. Thus, proper starter culture selection in the food industry and implication of standards about this subject related to international legislation is so important.

The last but not least, in order to solve this problem is needed the cooperation from all sectors that use antimicrobials to control antimicrobial use effectively and to limit the dissemination of antimicrobial-resistant bacteria in the environment (nationally and internationally area).

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New Concept in Packaging: Milk Protein Edible Films

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Abstract Ready to eat and perishable foods need specific precautions for packaging. The main mission of food packaging is widely used for preservation of food quality, to expand the shelf-life by inhibiting oxygen, light, moisture, and ensure microbial safety for consumers. The packaging industries are studying on renewable, environmentally-friendly and biodegradable alternatives to take the place of petroleum-based packaging materials. The packaging plays an essential function in communication system of content, product differentiation and branding. Appropriate and efficient packaging such as edible films and coatings, forestall of contamination facilitates storage and transportation by averting moisture loss, solute transport, aromas loss, water absorption in the food. There is a rising interest in recent years, in a form of active packaging by proteins, lipids, polysaccharides or combinations of these. Edible films serve as a functional barrier between food and its environment, guaranteeing the safety of foodstuffs. While the oxygen barrier of casein-based films is high, whey protein films have good water vapor and oxygen permeability. Addition of plasticizers to protein-based film formulations are required to reduce film fragility and moisture barrier ability, to give specific plastic properties, to enhance processability, protraction and water vapor permeability of protein films. In this chapter, the principal functions and properties of milk protein edible films in packaging industries are summarized.

Keywords Packaging · Whey protein · Casein · Lactoferrin · Edible films

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Introduction

Food packaging is a process which is necessary to apply after processing the food. Food packaging is combination of three disciplines like food science, food processing and food preservation. Purpose of packaging technology is to facilitate foodstuffs to travel safely over long distances, to preserve from exterior harm and contamination, to ensure still be nutritious and healthy at the time of consumption and information about contents to consumers (Marsh and Bugusu 2007; Trinetta 2016). The increase in the demand of consumers to conserve food in a natural way has led the food industry to investigate alternative protection methods. The alternative methods include the use of edible biopolymers; these biopolymers could be obtained from renewable sources or industrial by-products (Wihodo and Moraru 2013; Hassan et al. 2018). Because of their advantage over unnatural films and encouraging results in food preservation, edible films and coatings have attracted great attention (Galus and Kadzinska 2015).

Besides increased consumer demand for stable, healthy and safe foods arise from raised cognizance of the detrimental environmental effects of non-biodegradable wastes from packaging; there is an increasing interest in edible packages (Debeaufort et al. 1998; De Azeredo 2012). Edible coatings are also used to diminish the usage of plastic packaging, as they are biodegradable and environmentally friendly (Park 1999; Perez-Gago et al. 2005). They are described as a thin layer of primary packaging of foods. As the name implies these films made up of edible components which is ready to eat; (i) first is the coating material may be a whole meal, (ii) synthesized as a continuous coating between food additives (Hassan et al. 2018). The other reason for using them is due to their ability to enhance the shelf life of food products. Even the prolongation of shelf life for several days constitutes a significant economic benefit for food operations (Debeaufort et al. 1998; De Azeredo 2012). Edible coatings can extend the shelf life with reduction of respiration (Baldwin et al. 1995). They present a permeable hurdle towards moisture, oxygen, carbon dioxide and solute motion, as edible films decrease water loss, respiration rate and oxidation reaction ratio of foods. Meanwhile, they do not modify the original ingredients of the food (Debeaufort et al. 1998; Park 1999; Krochta 2002; Perez-Gago et al. 2005; Hassan et al. 2018).

Even the minimum process of food causes major damage related to microbial infection, water loss, surface browning, softening, ethylene production and extended respiration (Rolle and Chism 1987). Preservatives which include anti-microbial and anti-browning agents such as ascorbic acid and citric acid used as carriers in coatings. To inhibit enzymatic activity which causes browning, some amino acids especially included sulfur compound can also be used (Dudley and Hotchkiss 1989; Hassan et al. 2018). Besides their effect as discriminating barriers to solute migration, moisture and gas edible coatings can successfully be decreased microbiological growth in all form of foodstuffs. The antimicrobial agents on this coating material are made by reducing the diffusion coefficient of food products (Aloui and Khwaldia 2016). Process of whey films (Patel 2015) is presented in Fig. 1.

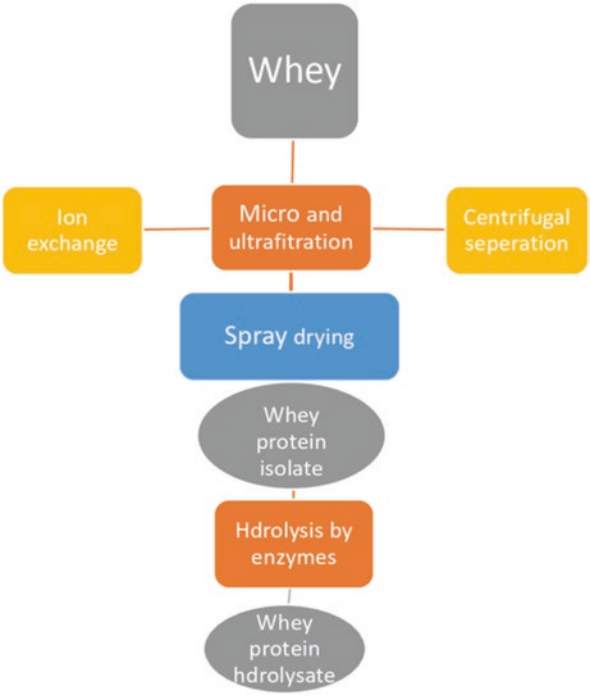

Fig. 1 Process of whey films (Patel 2015)

Feeding with the packaged products is one of the main objectives of creating edible films rather than traditional packaging, for this purpose they are generated entirely from safely to eat and renewable ingredients (Kuorwel et al. 2015). Edible coatings are firstly carried out in liquid shape but after implemented to food they are acquired as strong sheets (Falguera et al. 2011). They are applied with different methods to foods i.e. spraying and dipping. Alcohol, water or a mixture of the two can be used as solvent in the film or coating materials production (Bourtoom 2008). Another alternative advanced method is electro-spraying; which produces uniform and thin coating (Khan et al. 2012a, b, 2017). The components of proteins, polysaccharides and lipids are commonly used in edible films and coatings production (Donhowe and Fennema 1993; Cutter 2006).

Suitable mechanical, gas barrier properties, and thermal stability have led to the widespread use of petrochemical based plastics as food packaging. Although a wide variety of excellent oxygen barriers are advantageous in the presence of synthetic materials; these multilayered structures are disadvantageous due to the high technical effort (e.g. plasma or vacuum) the expensive materials used and their recycling (Selke et al. 2004). These new biodegradable polymers may be enough for food packaging applications but have some disadvantages compared to plastics for example their poor mechanical or gas barrier properties. There is a new approach to

address this problem which is biolayering technology. Biolayering technology is an innovative approach to achieving developed barrier properties of biodegradable polymers. The technology is based on microlayers made of biopolymers. Biopolymer derived from different natural sources, whey protein and casein seem very promising. The evaluation of these substances (whey proteins) considered as waste has also different importance (Regalado et al. 2006).

Protein-based films have advantages and disadvantages. These films provide barrier for gasesbut do not resist waterdiffusion. In order to develop film characteristics and their application some substances may be added. Plasticizers, such as polyethylene glycol or glycerol are added to improve flexibility of the protein network. Hydrophobic materials like beeswax or oils like oleic, are added to improve water permeability. These can affect films properties like hydrophobicity, crystallinity, surface charge, and molecular size (Oussalah et al. 2004; Hong et al. 2009). Despite their advantages, protein films can have disadvantage like susceptible to proteolytic enzymes present in meat products or allergenic protein fractions may cause adverse reactions to sensitive people (Gennadios et al. 1997).

It has been seen that milk improves the properties of edible films. Triglycerides shows low polarity; milk protein network considerably improves water vapor barrier properties. Triglycerides also lead to more opaque and relatively inflexible films and coatings (Guilbert et al. 1996). Many fats and oils were used to increase the blocking and water vapor barrier properties of edible films. Especially to modify the whey protein film structure researchers have used waxes, plant oils, acetylated monoglycerides and fatty acids (Perez-Gago and Krochta 2001; Anker et al. 2002; Shaw et al. 2002; Talens and Krochta 2005; Fernandez et al. 2007; Javanmard and Golestan 2008; Kokoszka et al. 2010; Soazo et al. 2013; Janjarasskul et al. 2014). Two other types of oil which added edible films and coatings were almond and walnut oils. Almond oil is enriched in monounsaturated fatty acids like linoleic and oleic acids. The amount of saturated fatty acids like palmitic, stearic and palmitoleic acids is very low. High oleic acid content is also very important in nutrition (Kodad et al. 2014). Walnut oil contains triacylglycerols, where in monounsaturated and polyunsaturated fatty acids are found in very high amounts. In addition to the use of these oils in the diet may have a dietary effect on human health (Martínez et al. 2010).

The milk contains a protein system composed of caseins and whey proteins are potential derivative of biopolymers. Caseins are insoluble, whereas whey proteins are soluble. Casein forms 80% of milk protein. It can be recovered in three different ways. Firstly, from skim milk via isoelectric precipitation, secondly through addition of acid, thirdly *in situ* production of acid and lastly via rennet-driven coagulation, in this process whey releases as a by-product. There are several different ways to recover whey proteins. First one is recovering via ultrafiltration, second one by centrifugation, third by thermal precipitation and after that regular filtration (Ramos et al. 2012). There are many areas where whey proteins are used. The most important ones are toddler formulae and food products which can be consumed after sport. Because of high film-forming ability of whey protein based edible substances is one of the most popular research topics in recent years. Whey protein films are characterized by three important properties: providing accurate mechanical hin-

drance, showing great gas barrier properties even at low relative humidity and exhibiting great barrier properties to aroma compounds and oils (Miller et al. 1998; Kurek et al. 2014; Hassan et al. 2018). Despite these positive properties, there are some disadvantages that limit the use of whey proteins, it is hydrophilic, and have some limitations to moisture. This chapter describes the usage of milk proteins in food packaging industries, especially focussing on packages, emulsions and edible films.

Milk Proteins

Due to the presence of several amino acid residues, which is mostly cysteine, the milk protein-based coatings provide extra advantages. They can constrain enzymatic browning of fresh-cut products; amino acid residue in the coating inhibits polyphenol oxidase activities in the food. Films from milk proteins are flexible, flavorless, transparent and provide excellent nutritional value. These features of milk protein films have gained importance (Tien et al. 2001). Due to crystallization of lactose during drying, nonhomogeneous film that adhere to emitting surfaces are formed from milk protein. For this reason, milk protein films are lead up by casein or whey protein separately (Fox and Brodkorb 2008). In the first day after the film prepared, it was observed that the properties of the protein films changed, which is observed in polymer films coated with whey protein isolate based formulations in particular. A reduction in the thermo formability of the films, over storage time, a reduction in oxygen permeability of films was determined (Schmid et al. 2012). Materials used to obtain antimicrobial edible films and coatings are shown in Table 1.

Casein

Casein is a predominant milk protein. Casein micelles form colloids composed of αS_1-, αS_2-, β-, and κ-caseins and colloidal calcium phosphate (Fox and Brodkorb 2008) with sizes commonly distributed between 50 and 400 nm (Murrieta-Martínez et al. 2018). Casein is quite a lot researched for nutritional, biological and chemical applications. Owing to casein properties like readily available, highly stable, inexpensive and non-toxic, it is utilized enormously in the food industry as a emulsifier and nutritional additive (Diak et al. 2007; Murrieta-Martínez et al. 2018).

Functional soluble caseinates can be obtained from acid caseinate (Perez-Gago and Krochta 2002). There are different ways for this purpose, firstly neutralization through the addition of alkali. Doing this is necessary for obtain edible films. Edible caseinate films can be obtained by solubilization in water followed by casting and drying. When used in casein coating materials, was shown to retard firmness loss throughout the storage of kinnows under environmental conditions with a minimum

Table 1 Materials used to obtain antimicrobial edible films and coatings

Films	Active Components	Food	Target Microorganisms	References
Milk Protein Films	Oregano, essential oil	Beef Muscle Slices	*Escherichia (E) coli* O157:H7, *Pseudomonas* spp. (10^3 CFU/cm^2)	Oussalah et al. (2004)
Sodium caseinate	Chamomile, oil	Culturemedia	*Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus salivarius*	Aliheidari et al. (2013)
Sodium caseinate	Nisin	Cheese	*Listeria (L). monocytogenes*	Ca Hoang et al. (2010)
Sodium caseinate	Chitosan	Salami	Mesophilic, Psychrottophic, Aerobic Bacteria, yeast, mold	Moreira et al. (2011)
Whey protein	Nisin		*L. monocytogenes*	Ko et al. (2001)
Whey protein	Lysozyme		*L. monocytogenes*	Min et al. (2008)
Whey protein	Lactoperoxidase		*L. monocytogenes*, *E. coli*, *S. enterica*, *P. commune*	Min and Krochta (2005); Min et al. (2005)
Whey Protein	Oregano, oils		*E. coli*, *S. aureus*, *S. enteritidis*	Seydim and Sarikus (2006)
Whey Protein	Sorbic acid		*L. monocytogenes*, *E. coli*, *S. typhimurium*	Cagri et al. (2001, 2002)
Whey Protein	Cinnamon, Cumin, Thyme	Fresh beef	Total bacteria count	Badr et al. (2014)
Whey Protein	Malic acid, Nisin, Natamycin.	Cheese surface	*L. monocytogenes*, *Ps. aeruginosa*, *Y. lipolytica*, *P. commune*, *P. chrysogenum*	Pintado et al. (2010)
WPI	0.5–1.0% *p*-aminobenzoic acid (PABA) and/or sorbic acid (SA)	Sliced Bologna, summer sausage	*L. monocytogenes*, *E. coli* O157:H7, *S. enterica typhimurium*	Cagri et al. (2002)
WPI films (casings)	*p*-aminobenzoic acid (PABA)	Hot dogs (beef 60%, pork 40%)	*L. monocytogenes*	Cagri et al. (2003)

(continued)

Table 1 (continued)

Films	Active Components	Food	Target Microorganisms	References
WPI coatings	Grape seed extract (GSE, 1.0–3.0% w/v), nisin (N, 6–18 kIU/g), malic acid (MA 1.0–3.0%; w/v) EDTA (1.6 mg/mL), andtheir combinations	Turkey, frankfurter	*L. monocytogenes*, *E. coli* O157:H7 and *Salmonella typhimurium* (10^6 CFU/g)	Gadang et al. (2008)
WPI coatings	Lactoperoxidase system (0–0.5%, w/v)	Smoked salmon	*Listeria monocytogenes* (10^2–10^4 CFU/g)	Min et al. (2005)

WPI whey protein isolate

loss of juice level and weight (Alam and Paul 2001). Casein films demonstrated high tensile strength and provide sustained release properties so it was appropriate for tablets (Alam and Paul 2001; Wagh et al. 2014). It has been reported that minimum 75% sorbitol and 20% glycerol (w/w of biopolymer) are required to generate transparent, pliable and brittle-free casein films. When casein and whey protein films compared elastic modulus and tensile strain of casein and whey protein concentrate films, values ranged from 0.9 MPa to 2.1 MPa and 19.2% to 66.6% respectively. The plasticizer concentration had more influence than the biopolymer type on the tensile properties. Package oxygen permeability was almost 856 cm^3/m^2 per day and the value was half of whey protein concentrate (WPC) films value. Casein films had better prevention of oxidation in cheddar cheese samples in storage. Thin casein films for food packaging applications possess low oxygen permeability and good strength but high sensitivity to moisture and low elasticity. Laminated casein films can be used for protection of dried fruit and vegetables from oxidation and moisture absorption (Embuscado and Huber 2009; Murrieta-Martínez et al. 2018).

Films produced using casein shows poor water vapor barrier properties and need to combine with lipids so form composite films. In such composite films, casein is supporting matrix bound and lipid provides resistance to moisture. When caseinate-lipid emulsion coatings were used to protect zucchini, and skinned carrots from moisture loss.

Besides to improve water permeability and mechanical strength covalent bonds between protein chains can be cross-linked with caseinate films and form a water-soluble three-dimensional network. There are different techniques for cross-linking. Chemical cross-linking is the most commonly used. This technique applying with reactants like glutaraldehyde or formaldehyde (Hernandez-Munoz et al. 2005; Soliman et al. 2007; Audic and Chaufer 2012). Another technique is enzymatic cross-linking with transglutaminases or peroxidases (Su et al. 2007; Han et al. 2009); and the last one is physical treatments by Irradiation. Radiation of proteins like irradiation and electron ray is a way to induce their cross-linking and to develop their efficiency such as mechanical strength (Sabato et al. 2007). Free radicals are produced during irradiation which encourage binding between adjoining molecules forming a three-dimensional network. Compared to these enzymatic cross-linking

is less used because peroxidases and transglutaminases have limited ability, efficiency and high costs (Audic and Chaufer 2012; Shit and Shah 2014).

Sodium caseinate is a milk protein consisting of a mixture of casein monomers and small aggregates. Small aggregates obtain after removing the colloidal calcium phosphate from casein micelles (Pankaj et al. 2014). Because of its capability to form feeble intermolecular interactions and its random coil structure it can compose films from aqueous solutions (Mendes de Souza et al. 2010). Sodium caseinate demonstrated great thermal stability as well as water-holding capacity. Forming ability of sodium caseinates film is based on the unsystematic bobbin structure which can form extensive intermolecular electrostatic, hydrogen and hydrophobic bonds which disperses rapidly in aqueous mixtures (Rezvania et al. 2013). Compared with sodium caseinate and calcium caseinate films, calcium caseinate edible films (CCEF) have higher thermal stability than sodium caseinate films. Because divalent calcium cations promote cross-linking with protein chains to give a tough structure to CCEF, which is also demonstrated in tensile testing. Adding carvacrol for sodium caseinate edible films decreased the thermal stability (Arrieta et al. 2012).

Casein films possess good qualities because of the tendency to provide added value to by-products of whey fraction proteins (Murrieta-Martínez et al. 2018). Rezvania et al. (2013) made edible film using sodium caseinate and stearic acid, quantities were as follows: 6 g 100 g^{-1} and 2 g 100 g^{-1}. This edible film showed low water vapor permeability. Besides sodium caseinate stearic acid was very important in controlling some properties of films like physical and mechanical properties. With increasing stearic acid ratio of drying, the consistency coefficient, elasticity, flexibility and shear thinning behavior of edible film could also increase.

Ca Hoang et al. (2010) demonstrated the effectiveness of nisin-coated sodium caseinate films against *Listeria innocua* in cheese during storage at refrigerated temperatures and found that the cells did not grow at more than 1 mm depth, mostly in surface and nisins effect was sufficient, and used for surface contaminated products.

Fabra et al. (2011) obtained sodium caseinate edible films containing 20, 40 and 60 mg/g of ferulic acid or α-tocopherol. Effects of film on oxygen barrier properties was investigated, which were determinative in the oxidation protection of lipid containing foods, while ferulic acid raised the film's rigidity even at low concentration, decreased mechanical resistance, water vapor, n-hexanal and oxygen permeability, transparency and gloss of the films. Moreover, α-tocopherol reduced water vapor permeability, mechanical resistance, transparency and gloss of the films. Kristo et al. (2008) used sodium caseinate films with preservatives (sodium lactate, nisin and potassium sorbate). They showed that especially potassium sorbate may change the water vapor and thermomechanical barrier properties of the films. Mendes de Souza et al. (2010) examined properties of lysozyme incorporated in a replaced sodium caseinate film using biochemical or chemical cross-linkers. Results indicated that with glyoxal slowly released lysozyme and reached a modulation in the antimicrobial activity against *Micrococcus lysodeikticus* and *Staphylococcus aureus* during food storage and enhanced food safety. Another work has been done with sodium caseinate-starch films. Films were containing nanoliposomes as carriers of

antimicrobial compounds, i.e. orange essential oil and D-limonene, proved that antimicrobials decreased the extensibility and mechanical resistance of the films (Jiménez Marco et al. 2014).

Monedero et al. (2010) investigated the effect of caseinates to soy protein isolated based films containing lipids which is 33% of oleic acid or 85:15 oleic acids–beeswax blend. Caseinate addition caused an increase of flexible, transparency, tensile capacity, colour softening and water vapour barrier properties of soy protein-based films.

Matsakidoua et al. (2013) prepared maize germ added composite sodium caseinate films. They obtained composite films as expected differed from the control films in some properties like surface characteristics, physicochemical and tensile properties. It was shown that the oil-incorporating films were less transparent and hydrophilic, as these properties make them more resistant to vapor permeation and water sorption than the oil-free caseinate films.

Wagh et al. (2014) studied edible films produced with casein and whey proteins, for their application and consideration of these films for packaging of Cheddar cheese. Tensile, thickness and barrier properties of the casein and whey protein concentrate films were highly influenced by the type of biopolymer, type and concentration of plasticizer used in film preparation. When compare the films, casein films showed superior barrier and tensile properties than whey protein concentrate films separately of the plasticizer. Edible film packaging did not affect the sensory qualities of Cheddar cheese.

Matricaria recutita flowers contains phenolic compounds like apigenin, luteolin, patuletin, quercetin and glucosides. Main components of flower oils are the terpenoids and chamomile oil at a concentration of 25 mg/mL; exhibited antibacterial activity towards gram-positive bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus salivarius* and *Streptococcus mutans* and also some fungicidal activity towards *Candida albicans* (Sabzevari et al. 2006; Popodopoulou et al. 2007). Aliheidari et al. (2013) prepared sodium caseinate composite films containing lipids *Matricaria recutita* essential oil, which reduced the most water vapor permeability. The presence of fatty acids and *Matricaria recutita* essential oil reduced tensile strength while increased elongation at break values.

Whey Fraction

Whey is a by-product of cheese manufacturing and evaluating this by-product is considerably important. Whey contains approximately 7% of dry matter, which approximately includes 4.5–5.0% lactose, 0.6–1.0% soluble proteins, 6–10% minerals, organic acids, and 0.4–0.5% lipids (Zhou et al. 2019). It is useful in terms of nutrients. It can be transformed and treated into different food and feed products such as bioethanol, enzymes, animal feed, biological gums, single cell protein, organic acids, exopolysaccharides and bioplastics. However, only 50% of the whey produced is used, the remaining 50% is discarded (Murrieta-Martínez et al. 2018;

Zhou et al. 2019). Evaluating cheese waste in edible packaging reduce environmental problems (Janjarasskul and Krochta 2010) and provide contribution of economic development to cheese industry and countries (Nicolás et al. 2019). One half of this amount is utilized in liquid form, 30% as powdered cheese whey, 15% as lactose and its by-products, and the remaining amount as whey protein concentrates or isolates (Spalatelu 2012). It has some distinctive characteristics i.e. amphiphilic nature, conformational denaturation and electrostatic charges. Charge density and hydrophilic–hydrophobic balance affect the conformation of whey proteins. This factors can affect the physical and mechanical properties of films and coatings (Ramos et al. 2012). Whey protein isolate (WPI) (>90%) and whey protein concentrate (WPC) (30–85%) are two important forms of whey protein and have difference in protein concentration (Jiang et al. 2018). Whey protein isolates and whey protein concentrates contain β-lactoglobulin, α-lactalbumin, bovine serum albumin, immunoglobulin G and lactoferrin (Mishra et al. 2019). Both are rich in sulphur-containing amino acids like methionine and cysteine (Jauregi and Welderufael 2010). Basic whey proteins are globular proteins, contain hydrophobic groups and embedded free thiol. Heat denaturation causes protein unfolding and expose of internal functional groups (De Wit 2009). Films have been obtained from both WPC and WPI (Fig. 1).

Heat treatment of protein solutions, that can be used for film casting, if thermal energy load is sufficient; result in protein denaturation (Schmid et al. 2014). Denaturation of the protein is begun by heat, solvent, or change in pH, form by association of peptide chains through new intermolecular interactions (Dehghani et al. 2018). WPI can self-assemble into semi flexible amyloid-like protein nanofibrils when heated at low ionic strength and low pH (Feng et al. 2018). At higher temperatures the disulphide bonds in protein are cleared resulting in a complete, irreversible protein unfolding and thus denaturation. The temperature needed for irreversible denaturation of β-Lactoglobulin and α-lactalbumin are 69 °C and 80 °C respectively. The protein aggregation by intermolecular cross-linking results in the creation of a three-dimensional network and leads to an increased structural cohesion. Moreover, denatured proteins films are more extendible and stronger but less permeable (Schmid et al. 2015). Edible films prepared from WPI displayed good aroma and oxygen barriers but poor mechanical properties (Silva et al. 2018; Jianga et al. 2019). However, films obtained from denatured protein show a high brittleness and this property influenced by the WPI molecular weight distribution. To reduce friability and extensibility of the film structure, protein based films generally require a hydrophilic plasticizing agent like glycerol (Schmid et al. 2013; Tonyali et al. 2018).

The source of milk like bovine, ovine or caprine, the type of feedstock whey (acid or sweet), the type of feed, the stage of lactation, the positioning within the season affects the profiles of whey protein products (Ramos et al. 2012). Albumin, carrageenan, casein, gelatin, cellulose, collagen, corn zein, mung bean protein, peanut protein, whey proteins, soy protein, pectin, starch and wheat gluten can be used to produce edible films but the films that producing whey protein have demonstrated mechanical and barrier properties better than competitive films produced using carrageenan, cellulose, corn zein, pectin, starch, wheat gluten and soy protein isolate.

It has more excellent oxygen permeability than the other based films such as collagen, soy protein, and wheat gluten. Whey protein films (WVP) have high water vapor permeability and low tensile strength (McHugh and Krochta 1994).

An edible whey film is a dried, greatly interacting polymer network and possesses a three-dimensional gel-type structure. WVP and coatings are flexible, colorless, odorless and transparent (Jooyandeh 2011; Ramos et al. 2012; Tsai and Weng 2019). Because of these properties, especially after a denaturation process their application in pack aging has resulted as quite encouraging (Schmid et al. 2014). Whey protein edible films obtained from non-denaturated proteins exhibited higher permeability to gases like water vapor and oxygen. Films obtained from denaturated proteins showed high ability to form intense and strong films, as this discrepancy originates from differences in structure (Schmid et al. 2013). Schmid (2013) demonstrated that enhance the hydrolyzed (h)-WPI content in WPI based films at fixed glycerol rate considerably raised film flexibility and maintained the barrier properties. Whey protein isolate based films produced with glycerol instead of h-WPI. The water vapor transmission rate and the oxygen permeability were reduced by replacing glycerol with h-WPI in films but the mechanical properties were not changed considerably.

There are some factors affecting the physical properties of composite films. The nature of the raw materials is the first, while the pH, mixing ratio, temperature, concentration and ionic strength etc. are the other process factors. Heat treatment is an important factor in film formation. Heating changes the three dimensional structure of proteins exposing internal-SH and hydrophobic groups. If the films produced in the absence of thermal processing, films would readily crack into small pieces upon drying, owing to food intermolecular interactions (Perez-Gago and Krochta 2002; Janjarasskul and Krochta 2010). Oxygen permeability values are influenced by the use of plasticizer type, glycerin or sorbitol, ratio of WPI and plasticizer, and relative humidity (Ramos et al. 2012). The ingredients in the structure of whey protein based films are as follows: glycerol used as plasticizer and the ratio 3% w/w, acetic, formic, fumaric acids 1.5% or 3%, citric acid 3%. When 1.5% acetic acid was used it was observed that whey proteins precipitate because of isoelectric pH of proteins; as a result, thick gels composed and films was not formed (Pintado et al. 2009). On other hand in the form of natamycin (0.002 g/ml), nisin (50 IU/ml), whey protein isolate (7.0%), sorbitol (1.5%), malic acid (3.0%) had best results for wrap cheese in test of mechanical and water vapour permeability and antimicrobial assay (Pintado et al. 2010).

Plasticizers are known to reduce cohesion of film-forming polymers (Guilbert et al. 1996). The mechanical strength of WPI films decreases with increasing the ratio of plasticizer to WPI; concomitantly, the water sorption increases, so the tensile strength decreases and the elongation increases (Mathews and Dufresne 2002; Piccirilli et al. 2019). For example, lecithin addition to whey protein dispersions increased the strength of heat-induced gels (Dickinson and Yamamoto 1996) and their heat-stability (Dickinson and Yamamoto 1996; Jiménez-Flores et al. 2005). The surface adhesion of whey-protein edible coatings can be improved via addition

of surfactants, for example, tween or lecithin, which decrease surface tension and improve wettability (Lin and Krochta 2005).

One of the common technique is by adding an inorganic material into the bio-based polymer matrix to make a hybrid polymer matrix. In this way the bio-based films' mechanical and barrier properties are improved. One of most preferred inorganic material is montmorillonite, if it can completely disperse it will develop various characteristics of bio-based films (Luecha et al. 2010; Wakai and Almenar 2015). Sodium montmorillonite is natural polymer nanocomposites and has chemically stable, non-toxic, thermally stable, natural and reinforcing properties (Wakai and Almenar 2015). Its high ratio and lamellar structure the crookedness of the molecule path in diffusion can be rised by nanoclays. In other words, it is sufficient to add comparatively small amounts of montmorillonite clays to achieve a significant improvement in barrier properties. The sodium montmorillonite can form stable dispersion in WPI (Azevedo et al. 2015a, b).

Whey protein isolate based layers produced by lacquering process showed great oxygen permeability with\2 cm^3 (STP) m^{-2} day^{-1} bar^{-1}, which this value can comparable with synthetic polymers, like ethylene vinyl alcohol. Also WPI films showed good mechanical and adhesion properties (Schmid et al. 2012, 2015). WPI films containing 25% glycerol were comparable again to ethylene-vinyl alcohol copolymer films in terms of barrier to δ-limonene transport but this comparison could be done under the same conditions like similar temperature and relative humidity (Perez-Gago and Krochta 2002). The whey protein isolate emulsion films manufactured with candelilla wax had no distinctive milk odor, but films were slightly sweet and adhesive. WPI films which produced with candelilla wax were opaque, without candelilla wax films looked glass clear and transparent (Kim and Ustunol 2001). Basiak et al. (2015) prepared composite films casting WPI and wheat starch. The highest values of elongation and thickness at break were observed for films obtained by incorporation of whey protein and wheat starch. It is proved that with the increasing content of WPI, tensile strength and swelling index values increased.

Edible composite films are generally formed of WPI and lipids. These films have been enhanced for some reasons. Firstly, benefit from the effective oxygen barrier of the whey protein isolate WPI matrix. The other reason is overcome the water sensitivity of the whey protein isolate WPI film by addition of hydrophobic materials (Janjarasskul et al. 2014). Because of the bilayer structure has a disposition to delaminate and crack, there is some limitations about incorporation of hydrophobic materials. These materials are added to improve moisture barrier. Moreover, the coating technique needs two different castings and handling of molten lipid at high temperature or the use of lipid solvent. Stable emulsion-based film can be hard to do because of gelation, cross-linking, solvent volatilization and lipid melting temperature steps of the structural network (Janjarasskul and Krochta 2010).

Zinoviadou et al. (2009, 2010) investigated the efficiency of antimicrobial agents which contain sodium lactate and 3-polylysine into sorbitol-plasticized whey protein isolate films. The films of beef's stored at 5 °C were prepared with sodium lactate at the rate of 1.0–1.5% (w/w) to increase water vapor permeability, water uptake of the films and maximum tensile strength. Hasanzati Rostami et al. (2010)

coated with 13% whey protein films in Kilka and indicated that whey protein can be used as edible coating for reducing the lipid oxidation during freezing storage. Wang et al. (2010) produced films using whey protein isolate and sericin and investigated that how sericin protein positively affects physical properties by incorporation into film production. It was determined that the water vapor permeability of the film reduced while increasing sericin content; other physical properties of the film such as the solubility, transparency, moisture content, light transmission and swelling were not significantly affected under the present empirical conditions.

Rodriguez-Turienzo et al. (2011) studied diversified whey protein concentrate coating formulations for frozen Atlantic salmon. They used glycerol and sorbitol. When coating applied after freezing it raised the thaw yield and reduced the drip loss. According to the study the most excellent coating for frozen Atlantic salmon preservation was whey protein and glycerol. The ratio of 1:1 whey protein based coating + glycerol.

Di Pierro et al. (2011) used an edible film composed of chitosan and whey protein as a coating material in Ricotta cheese. After coating they stored the cheese under modified atmosphere at 4 °C. It was found that the coating material obtained from the chitosan and whey protein film used in the packaging of cheese had low oxygen and carbon dioxide permeability and high water vapor permeability. Duan et al. (2011) obtained composite films from chitosan and whey protein and investigated their usefulness for encapsulation of fish oil and oil stability in films. The increment in fish oil concentration from 1.5% to 2% in the film did not change prolongation of the films but reduced the tensile strength.

Mucilage of *Salvia hispanica* is a natural gum mainly composed of glucose xylose and glucuronic acid from chia seeds. It forms a ramified polysaccharide (Muñoz et al. 2012a). Muñoz et al. (2012b) made thin films from whey protein concentrate and mucilage of *Salvia hispanica*. Whey protein concentrate and mucilage ratio of 1:3 films was better with high resistance and flexibility properties than the other films. Besides, films produced at pH 10 showed higher water vapour barrier than films produced at pH 7. Water vapour value was 0.620 ± 0.08 g mm/kPa h m^2.

Gerez et al. (2012) studied the efficiency of an uncommon microencapsulation method using whey protein and pectin to improve the survival rate of *Lactobacillus rhamnosus* CRL 1505 at low pH and bile. Beads with a pectin core and a whey protein overcoating were more stable than beads with the pectin–whey core with a WP overcoating in simulated gastric conditions.

Vitamin D3 value in fortification Cheddar cheeses reduced during ripening period. It was reported that approximately 74–78% vitamins were retained as a use of emulsifiers made from whey protein, calcium caseinate and sodium caseinate result of the (Tippetts et al. 2012). Protein based films can be applied with nisin. Whey protein films had the bactericidal properties and demonstrated high activity against *L. monocytogenes* bacteria in different studies (Ko et al. 2001; Kraśniewska and Gniewosz 2012). Hassani et al. (2012) coated kiwi fruit with whey protein and rice bran oil during 28 days of storage. This film slows down the weight loss and increases acidity in coated kiwi fruits, preserved their total soluble solid materials, and maintain their firmness, color, and their sensory attributes.

Fernandez-Pan et al. (2012) developed different antimicrobial edible films based WPI isolate and essential oils. Oils used to produce antimicrobial edible film include tea tree, oregano, mastic thyme, clove, laurel, coriander, sage and rosemary. These are effective against four potential spoilage or pathogenic bacteria of interest in food processing: *Staphylococcus aureus*, *Listeria innocua*, *Pseudomonas fragi* and *Salmonella enteritidis*. When whey protein isolate edible films incorporated oregano or clove oils the films can have the most intense inhibitory effect on the microbial growth. The films thus obtained were used to coat the fresh skinless chicken breast. The highest inhibition against bacteria obtained by films which produced oregano essential oil. Marquez et al. (2012) prepared whey protein/pectin edible films with transglutaminase for doughnuts and french fries. Their results demonstrated that these edible films reduced water vapor permeability and moisture loss. These applications were valid to both food product and applied before food frying. Bugnicourt et al. (2013) concluded that the whey coating accomplished better barrier properties than other coating materials, even closed those of synthetic barrier layers. Reproduced laminates were shown to be suitable packaging films for sensitive food products like butter cheese because of no negative sensory effects caused by packaging.

In a study, 40%, 50%, and 60% plasticizer ratios of glycerol in WPI and concentrate edible films were evaluated. Whey protein concentrate films displayed little yellow color and had higher moisture content, water activity and water vapor permeability values than whey protein isolate films. It was determined that a high lactose content was in WPC. WPI films demonstrated higher tensile strength, density and elongation values in comparison with WPC. This data apply to the same glycerol level, so whey protein isolate films were more flexible and stronger. When the content of glycerol increased, tensile strength decreased and films became weaker (Ramos et al. 2013; Murrieta-Martínez et al. 2018).

Rodriguez-Turienzo et al. (2013) prepared usage of transglutaminase in heated or ultrasound-treated whey protein coatings of frozen Atlantic salmon (*Salmo salar*). It was observed that using of transglutaminase in heated and ultrasound-treated whey protein coatings equally delayed lipid oxidation after frozen storage. Addition of enzyme did not affect this situation.

Antimicrobial packaging is a kind of active packaging; the usage of edible films to let antimicrobial constituents. For this purpose, antimicrobial characteristics of WPI films including spice like garlic, rosemary and oregano and essential oils are attractive. It was investigated to support oxygen barrier of commercial plastic film. The resulting coating were both boosted the barricade and provided rapid biodegradability. The material based on denatured whey protein and plasticizer presented fast biodegradability even after application on the commercial film. Because biodegradable packaging has a weak barrier property like gas and water vapour. Biodegradability of the whey protein films is significant since natural polymers may not degradable if cross-linked or blended with not degradable additives (Cinelli et al. 2014).

Leuangsukrerk et al. (2014) searched the effects of combination of WPI on the characteristics of konjac glucomannan-based films. The transparency of the konjac

glucomannan WPI mixtured films was raised by increasing WPI concentration. Water insolubility of the films were enhanced by WPI molecules. Blend films with the maximum concentration of WPI (0.4 g KGM: 3.8 g WPI/100 g solution) could be heat covered at 175 °C. Janjarasskul et al. (2014) produced different whey protein isolate:glycerol:candelilla wax films. Addition of the candelilla wax had a little impact on tensile properties even less than this effect on barrier properties.

Vonasek et al. (2014) encapsulated T4 bacteriophage with WPI based edible protein films. WPI films could fix phages at both ambience (22 °C and light) and refrigerated conditions (4 °C and dark). Meantime there was no major decrement in phage infectivity over a period of 1 month. The phage encapsulating with WPI film was able to effectually prevent the microbial growth.

Essential oils are found in abundance in phenolic acids and flavonoids and have many benefits, but are vulnerable to instability. Encapsulation is considered to be a solution to this in order to eliminate instability. How microencapsulation process affects the feasibility of cardamom essential oil (CEO) in WPI combined with guar gum (GG) and carrageen (CG) was investigated. The best formulations were the 30% WPI and 30% WPI + GG retaining the main components of CEO during 16 weeks. WPI microcapsules (30% and 15%) without CG or GG were the most effective in entrapping CEO and had the highest microencapsulation efficiency. These findings were related to the good emulsification properties of WPI and its ability to produce thicker and less porous microcapsules. The addition of GG and CG to WPI significantly reduced homogeneity and integrity, increased visual surface porosity of the microcapsules (Mehyar et al. 2014).

Stable nanoemulsions are used in food and pharmaceutical industries. Whey protein stabilized nanoemulsions showed good stability at high ionic strengths or at high temperatures. The result showed that the use of a comparatively high protein concentration as an emulsifier would be sufficient. Nanoemulsions may not require the use of a polysaccharide like alginate, gum arabic, carrageenan, xanthan, or chitosan as a second layer. The rate of used i-carrageenan was lower than protein concentration that there were not enough i-carrageenan molecules to cover the surfaces of the oil droplets. An explanation for this may be that, the relatively high protein concentration condition, there were enough protein molecules to adsorb the surfaces of the oil droplets to provide a high charge density on the surfaces of the droplets in the nanoemulsions (Li et al. 2014). Badr et al. (2014) investigated the activity of antibacterial whey protein edible films compared with films at different concentrations of cumin, cinnamon, thyme essential oils on meat sample during the 12 days at 4 °C. Use of whey protein edible films added 2.5% w/w cinnamon, cumin, thyme essential oil, fresh beef under refrigerated conditions had doubled shelf life.

In a study to increase the stability of folic acid, the encapsulation method applied and as encapsulation material whey protein concentrate was used. Encapsulation continued until positive interactions between protein and folic acid. The results showed that whey protein concentrate could be used as encapsulation material for the stability of folic acid and even better than commercially available resistant starch (Pérez-Masiá et al. 2015).

Nuts are healthy snacks but their shelf life is short because of bitterness. The use of edible whey protein isolate in combination with pea starch and carnauba wax was investigated on oxidation and acidity values of walnut and pine nut during storage. It forms a non-homogeneous film, as a result nuts covered with this film had good organoleptic properties at 25 °C for 12 days (Patel 2015). Soazo et al. (2015) investigated whether whey protein based edible coating have any effect at the prefreezing application. Main purpose was continuing qualitycharacteristics of strawberries. It was found that successful application in preventing weight loss was whey protein with 20% beeswax after thawing process.

Rubilar et al. (2015) prepared hydroxypropyl methyl cellulose/WPI based films. When increasing the amount of oil, moisture content was slightly reduced. It was found that a direct relationship between the increase in the amount of hydroxypropyl methyl cellulose and an exponential increase in elongation and tensile strength at break.

Trehalose is a disaccharide widely available in nature, especially in honey, bakery products and mushrooms. It has mild sweetness, moderate glycaemic index, low hygroscopicity and cariogenicity. Trehalose can protect proteins from denaturation or inactivation and damage caused by harmful chemical reactions or dehydration during frozen storage (O'Donnell and Kearsley 2012). Perez et al. (2016) produced plasticizing edible films from WPC with glycerol and/or trehalose used. Results showed that the films with trehalose were more insoluble in water than with glycerol. The films in which trehalose used being more favorable for food applications. Trehalose was also included into WPC-glycerol film formulations and so film opacity increased.

Silva et al. (2016) described functional properties of locust bean gum synergistic interactions with whey proteins. The content and production of films were as follows: 5% WPI + 0.1% LBG + 2% glycerol, heated at 75 °C for 10 min. Preserving foods from oxygen during storage or decreasing oxidative browning in processed foods can be performed by locust bean gum added WPI films. The films which including locust bean gum presented the lower oxygen permeability compared with the film which gam free films.

Galus and Kadzinska (2016a) produced rapeseed oil and glycerol added whey protein emulsion films. The use of rapeseed oil raised the hydrophobic feature of the films, reduced film solubility in water and moisture content. Galus and Kadzinska (2016b) also prepared WPI films with almond and walnut oils and their concentrations. Emulsified films demonstrated a more hydrophobic nature and this property confirmed by increasing in contact angle values and decreasing in water vapour permeability. Film characteristics were influenced by decreasing of lipid droplet size and distribution. It was determined that almond oil had a higher effectiveness and plasticizing effect in modifying whey protein films than walnut oil.

Soukoulis et al. (2017) showed that the addition of WPI increased *L. rhamnosus* GG stability. The highest cell counts were found after drying in pectin + WPC films. During storage a film offered for the greatest stability which this film includes composite whey protein, carrageenan and locust bean gum.

Catarino et al. (2017) coated two traditional Portuguese sausages which names are *paínhos* and *alheiras*. They did coating during industrial production process and used edible films which containing WPC and *Origanum virens* essential oil. After coating higher acidity and protection against color fading was observed particularly in *paínhos*, significant reduction of the lipid peroxidation was observed in *alheiras*. With this coating operation the shelf life of sausages was extended approximately 15 days. Jianga et al. (2019) indicated that transglutaminase (TGase) and nanocrystalline cellulose (NCC) had a combined effect on WPC-based films and its tensile strength and elongation value was raised. TGase promoted the enhancement in mechanical properties of WPC film strengthened with nanocrystalline cellulose (NCC).

Lactoferrin

The food industry has advantage with some techniques to prevent bacterial and fungi growth and spoilage (Davidson and Taylor 2007). The most effective results were obtained by the addition of chemicals but because of negative consumer perception against chemical additives, food industry start to research new alternatives to protect food products by replacing synthetic agents with natural compounds like edible films and active packaging (Parafati et al. 2015; Wisniewski et al. 2016; Calvo et al. 2017; Russo et al. 2017). To extend the shelf life of food and maintain product properties like quality, safety, and freshness, it is very important to select right materials and packaging technologies (Kechichian et al. 2010). In order to increase the efficiency of food preservation, the addition of active compounds to food packaging is very important (Moreno et al. 2014). The use of natural antimicrobials due to more consumer awareness is increasing compared to the use of some synthetic antimicrobials (Moreira et al. 2005; Gyawali and Ibrahim 2014). Natural antibacterials and antifungals can be obtained from different sources, for example, animals, plants and microorganisms. Among naturally occurring agents, lactoferrin is reported to possess important activity against a wide range of bacteria and fungi in a wide variety of foods (Perdones et al. 2012; Wang et al. 2013a).

Lactoferrin is an iron-binding glycoprotein which belongs to the family of transferrin proteins. It is present in bovine milk and can bind two iron atoms per molecule (Reiter and Oram 1986). The active region of lactoferrin is Lactoferricin B. It is formed by acid-pepsin hydrolysis from the N-terminal region of the molecule and contains 25 amino acid residues (Bellamy et al. 1992).

Lactoferrin firstly employed as an antimicrobial agent against a wide range of both Gram-positive and Gram-negative bacteria. Lactoferrin also has antioxidant, anti-inflammatory, immune-modulating, antiviral, anticancer and antifungal activities. So it is understood that lactoferrin could be used for the gain of active properties to biodegradable films (Naidu 2000; Aguila and Brock 2001; Wei et al. 2008; González-Chávez et al. 2009; Jenssen and Hancock 2009).

Lactoferrin effectively inhibits the growth of some bacteria, but still some other bacteria may be lactoferrin resistant because of the presence of siderophores that aid in adaptation to low-iron environments (Cagri et al. 2004). Bacteria with low iron requirements, like lactic acid bacteria, would not be adversely impacted by lactoferrin. Thus, lactic acid bacteria are used as starter culture products do not cause negativity (Reiter and Oram 1986). Both bacteriostatic and bactericidal effects of lactoferrin give this protein antibacterial properties. The bacteriostatic effect occurs as sequestrate iron making this nutrient unavailable for bacteria (Arnold and Cole 1977; Reyes et al. 2005). The bactericidal effect occurs by direct interaction with the bacterial membranes. Lactoferrin damages the outer membrane of gram-negative bacteria, so causes the release of lipopolysaccharides, which sensitize the cell to the antibiotic effect (Garcia-Montoya et al. 2012). Lactoferrin has an antimicrobial activity due to its ability to bind with iron, which results in loss of nutrients against iron in pathogens (Finkelstein et al. 1983). There are many reports about antimicrobial activity of lactoferricin against emerging pathogens like *Escherichia (E) coli*, *Salmonella* serovar Montevideo, *Salmonella typhimurium*, *Salmonella enteritidis*, *Campylobacter jejuni*, *Clostridium perfringens*, *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Payne et al. 1990; Branen and Davidson 2000; Elagamy 2000; Naidu 2000; Liceaga-Gesualdo et al. 2001).

Bellamy et al. (1992) and Jones et al. (1994) determined that the range of lactoferricin inhibitory for bacteria was from 0.3 to 150 mg/mL. It is found that *Bifidobacterium bifidum*, *Enterococcus faecalis*, *Pseudomonas fluorescens* and strains of this bacteria were highly resistant to this peptide (Tomita et al. 1991).

Lactoferricin changes membrane permeability. It is presumably due to its nature (Jones et al. 1994). Until now studies were conducted to confirm the validity of using lactoperoxidase and lactoferrin in a film. They indicated that lactoperoxidase added film demonstrated the activity against *Salmonella enterica*, *E. coli*, *Listeria monocytogenes* and *Penicillium commune* (Kraśniewska and Gniewosz 2012). Also, some essential oils obtained from garlic and oregano were introduced to whey protein-based films, which validated achieving films active against *Salmonella enteritidis*, *Staphylococcus aureus*, *E. coli*, *Lactobacillus plantarum* and *Listeria monocytogenes* bacteria. These films are used to coat the surface of dried meats and cheeses (Seydim and Sarikus 2006).

Min et al. (2005) investigated the combination of lactoferrin and edible WPI films incorporation for the inhibition of *E. coli* O157:H7 and *S. enterica*. Antimicrobial effects of lactoferrin (5–40 mg/mL) were investigated by measuring the turbidity of antimicrobial-containing media after inoculation. It was seen that the growth of *E. coli* O157:H7 and *Salmonella enterica* (4 log colony-forming units/mL) in tryptic soy broth was not prevented by lactoferrin at 20 mg/mL and 40 mg/mL respectively. Min and Krochta (2005) studied the effects of lactoferrin on the inhibition of *Penicillium commune*. Lactoferrin used both directly and incorporated into edible WPI films. Lactoferrin antimicrobial effects were examined by disc diameter, surface spreading, turbidity, and film surface inoculation tests. It was found that lactoferrin at 10 mg/mL or higher inhibited *Penicillium commune* in 1% peptone water.

Al-Nabushi et al. (2006) protect lactoferrin from contact with agents like divalent cations, encapsulated it in two types of emulsion which interfere with its antimicrobial activity. Paste-like microcapsules were incorporated in edible whey protein isolate packaging film to test the antimicrobial activity of lactoferrin against a meat spoilage organism *Carnobacterium viridans*. The film was applied to the surface of bologna after its inoculation with the organism and stored under vacuum at 4 °C or 10 °C for 28 days. The growth of *Carnobacterium viridans* was delayed at both temperatures and microencapsulated lactoferrin had greater antimicrobial activity than when unencapsulated. The results obtained in this study indicated that microencapsulation can increase the antimicrobial activity of lactoferrin in cured meat.

Brown et al. (2008) researched how to develop and characterize edible chitosan film which this film containing lactoferrin as a natural antimicrobial agent. Lactoferrin concentrations were 0.5, 1, and 2 mg in a circular disc of chitosan film. Also, two other combinations were investigated. First one was lactoferrin and lysozyme combination. This combination how would affect the growth of Listeria monocytogenes and *E. coli* O157:H7 investigated. The second one is, three concentrations, 0.28, 0.56, or 1.12 mg per disc, of lactoferrin or EDTA incorporation into the chitosan film containing lysozyme. The water barrier properties of the chitosan films containing lactoferrin were personified. It was found that if the chitosan films containing lactoferrin less than 1 mg per disc, did not alter the water vapor permeability of the chitosan film. The films antimicrobial activities against *E. coli* O157:H7 and Listeria monocytogenes were determined using the agar diffusion assay and cell count assay. It is determined that the incorporation of lactoferrin alone into chitosan film did not exhibit significant antimicrobial activity against both *E. coli* O157:H7 and Listeria monocytogenes. However, the combination of lactoferrin with lysozyme-containing chitosan film significantly decreased the growth of *E. coli* O157:H7. Additionally, the combination of lactoferrin with lysozyme in chitosan film exhibited a greater reduction in the growth of Listeria monocytogenes than did the combination EDTA with lysozyme, resulting in an approximate 3-log reduction. In conclusion, the results in this study suggest that lactoferrin alone in chitosan film is not effective against pathogenic bacteria; however, when lactoferrin is combined with other antimicrobial agents like lysozyme, the inhibitory effects of lactoferrin is comparable to EDTA and even better at higher concentrations. The combined effect of lactoferrin is not limited to Gram-negative bacteria. Therefore, as a natural chelating agent, lactoferrin potentially may be applied in the food industry to replace synthetic EDTA for more natural food products.

Elias et al. (2008) showed that different nourishing proteins can interfere with radical reactions, acting as primary or secondary antioxidants and one of these proteins is lactoferrin. Due to its chelation capability of transition metals like iron and copper, lactoferrin could act as a preventive or secondary antioxidant, which could retard the oxidation process.

Chitosan packaging films which containing different bioactive compounds were produced by Borubon et al. (2011). As bioactive compound lactoferrin, glycomac-

ropeptide and a peptide fraction from whey protein concentrate hydrolysate were used. Films barrier and mechanical properties were evaluated. SDS-PAGE was used to determine the molecular weight of protein-based compounds. The addition of lactoferrin to chitosan film resulted in an increase in elongation at break and a significant reduction in tensile strength, carbon dioxide permeability, and water vapor.

Wang et al. (2013a, b) prepared solutions with using different lactoferrin concentrations and Tween 80 and sprayed these solutions on tomato plants. As the dose of lactoferrin increased, the index of samples showing visual mold growth decreased. Consequently, it is found that when 100 mg/L lactoferrin solution was used, the lactoferrin solution can protect more than 50% of the samples.

Moreno et al. (2015) obtained glycerol plasticized potato starch films containing bioactive proteins like lactoferrin and/or lysozyme. Lactoferrin and lysozyme ratio was 0.1 and 0.2 with respect to starch. The films were characterized for some properties like physical (mechanical, optical, water content, oxygen, and water barrier), thermal, microstructural, antimicrobial and anti-oxidant. The physical and structural properties of potato starch films affected by the incorporation of proteins. Proteins modified films thermal behavior and increased the glass transition temperature. Part of the proteins are separated and transported to the surface of the film and proteins showed a certain degree of compatibility with starch chains through the bond formations. Lactoferrin incorporation extremely increased the film's brittleness, and not affected by the water content of the film. Lactoferrin enhanced water vapor and oxygen barrier properties. Lactoferrin reduced the film's gloss and transparency. The thermal degradation of blend films and isolated proteins occurred at temperatures of over 250 °C, so it can be understood that blend starch films can be thermoprocessed, according to their thermoplastic properties and following the usual practices of the plastics industries. It is observed that if lactoferrrin simultaneously applied, a synergistic antimicrobial action against *Escherichia coli* and coliforms was occurred. The films containing a blend of lactoferrin and lysozyme reduced the total coliform counts in minced pork meat but did not show significant antimicrobial activity against *Listeria innocua* and *E. coli*. Anyhow, all the films were effective at reducing lard oxidation after long storage times.

Padrão et al. (2016) developed bio-based edible antimicrobial BC films using two different sources and used lactoferrin as an active ingredient. Laboratory produced and adsorbed with lactoferrin BC films named BC1-LF and commercially acquired and adsorbed with lactoferrin BC films named BC2-LF. Physicochemical differences were observed between these different films before and after activation with the lactoferrin. The adsorption of lactoferrin caused a shift in the surface properties of the BC films towards their interaction with water, this new feature shows the impact of the protein in the BC films surface properties. Besides the positive properties of lactoferrin has gained, a negative feature is mechanical properties of the BC films were slightly impaired after lactoferrin absorption, but still, the tensile strength remained much higher than that of the pig's small intestine casing. It is observed that both films were able to reduce *in vitro* the colony forming unit viability of both *E. coli* and *Staphylococcus aureus*, reaching 1-log reduction in the *Staphylococcus aureus*. In contrast with, the fresh sausage, the bactericidal effi-

ciency was higher for *Escherichia coli* than for *Staphylococcus aureus*. It is found that the developed films were bactericidal and non-toxic. The films have the appropriate technological characteristics if want to use as a bio-based meat product casing.

Conclusion

Reusability and recyclability are new packaging approaches of consumers. The taste evaluation and storage characteristics are the main determinants of the new packaging design. As a first step, it is advised that milk protein edible films and coatings suppliers should get "no-objection" notification from the established authorities, regarding use of them as food ingredients, and also evaluate nutritional information, possible allergenicity and proper labeling. In case of nutraceutical applications, other pending regulations are to be considered, especially health concerns claim and novel foods. On the other hand, consumer acceptance that includes safety assurance, organoleptic properties, cultural background and marketing approach is very important. Marketing factors include the price of the final product, as well as annoying instructions and requirements to handle, open, and dispose of the package. Finally, cultural background encompasses reluctance to use novel materials, and prejudiced concern about the safety of their contact with items that will eventually be ingested. This issue, which has many aspects to be developed, will continue to be the subject of many researches in the future.

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Food Nanotechnology: An Emerging Technology in Food Processing and Preservation

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Abstract The advent and rise of nanoscience has led to significant development in modifying various food properties. Food nanotechnologies involve the size manipulation of the particles of a food matrix. The novel physicochemical properties at nanoscale matter enhance textural characteristics, colour, physicochemical stability, sensory attributes and involve controlled release of active agents, thus enhancing the quality and shelf life of the food product. In an advanced form, the technology is related to nanoencapsulated food additives. In addition, the application of nanocomposites promises an expansion for the use of edible and biodegradable films in active packaging to preserve fresh foods and to extend their shelf life. Also, the use of nanosensors to detect microorganisms and contaminants is particularly significant application of food nanotechnology. Apart from the benefits, the implications of nanotechnologies indicate an immediate need for regulation of nanomaterials before their incorporation into food is critical for ensuring acceptance of the technology by the consumers.

Keywords Food nanotechnology · Food processing · Food preservation · Nanosensors

Introduction

Nanotechnology is an emerging field that has led to various developments in food processing and preservation. It has various applications in food preservation, food processing, and food packaging. Reports suggest that nanotechnology has the potential to enhance the safety and characteristics of food. The technology uses nanostructured particles and their incorporation for better stability and

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functionality (Mohezar and Nor 2014). According to the European Commission, "a natural, incidental or manufactured materials containing particles, in an unbound state or as an agglomerate or as an aggregate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1-100 nm" (European Commission 2004). The size of the nanoparticles is considered to impart novel properties to the nanostructured food. Nanotechnology is a promising tool for the development of nanostructured particles, nanoencapsulation (immobilization of bioactives), nanoemulsions, nanocomposites and nanosensors. In addition, the technology of nanostructured particles is employed to improve the texture, flavor, color, and organoleptic properties of food. Nanostructures involve by top down approach or bottom up approach. Top down approach involves breaking up of larger particles into smaller nanoparticles. The examples of top down approach are milling, homogenization and size reduction. The technology of breaking up larger particles into nanoparticles increases water binding capacity, surface area and aids in better digestion and absorption. However, bottom up approach involves assembling of individual atoms to create nano-sized particles. Examples include development of nanostructured particles (nanotubes) to encapsulate bioactives (Ravichandran 2010).

The nanomaterials may be nanoparticles or linear assemblies. Linear assemblies include nanotubes, nanofibres, fullerenes, nanofibres, nanowhiskers, and nanosheets. Food matrix is processed to modify the structural properties of its particles. The modification of particles at nanoscale leads to better preservation and processing of food. Applications of nanotechnology in processing and preservation include nanoencapsulation, nanoemulsions, nanoformulations and noncomposites. These creative and innovative applications of nanotechnology have a potential to address many challenges faced by food technologists. Food nanoparticles possess active properties that can alleviate various food supply issues. Due to the associated benefits, nanotechnological applications in food have exponentially increased (Donsi et al. 2011).

Nanotechnological applications focus on food safety and innovations that are beneficial to the food industry. Some of the developed applications involve encapsulated bioactives, improved supplements, nanosensors for food packaging and novel biocomposites as packaging materials. The reviewed applications show that nanotechnology has positive impact on food safety and nutrition.

Nanoencapsulation

Nanoencapsulation involves entrapment of bioactive compounds for delivery to various sites within the body. Small size of bio actives within the capsules ensures their target site-specific delivery. Nanocapsules have better stability, solubility and encapsulation efficiency than microcapsules. Nanocapsules up to 1000 nm have the ability of precision targeting of bioactive compounds largely than microcapsules.

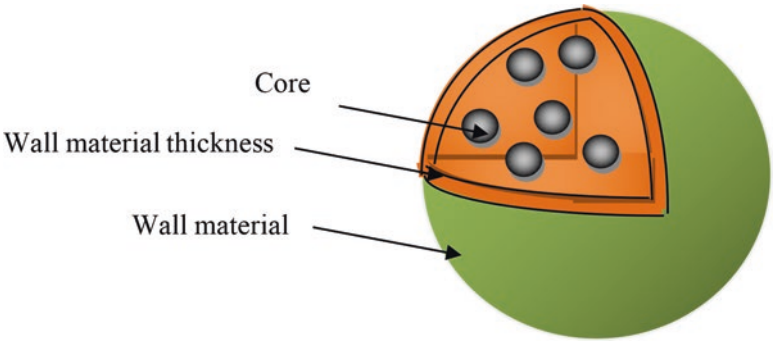

Fig. 1 Structure of a Nano capsule

The nanoscopic size provides a larger surface area and better sites of absorption and desorption (Meena et al. 2011) (Fig. 1).

The structure of Nanocapsule is categorized into two categories. They may contain active ingredient as the core material inside the secondary material, which is a shell or matrix or wall material. Alternatively, the core is entrapped within a network of matrix. The core material is released upon a trigger or by diffusion. Wall materials or encapsulating matrices are based on their thickness which is critical for their intended application. Matrices are usually biopolymers (proteins, polysaccharides, fats, liposomes) or co-polymers (e.g., poly lactic-co-glycolic acid) or metal-organic particles, or polymeric nanoparticles. Some recent matrices used in Nanoencapsulation are lecithin, gelatin, albumin, dextrin, starch, plant gums, alginates, chitosan etc. (Mozafari et al. 2006). Recently Gonzalez-Reza et al. (2015) developed nanocapsules of beta-carotene. Beta-carotenes are used to improve the color of various foods, besides it acts as an active antioxidant, protects against cancer, cardiovascular diseases and adenomas of colo-rectal (Ramoneda et al. 2011; Yuan et al. 2008). A new version of nanocapsules known as lipid core nanocapsules were formulated by dispersion of sorbitan monostreate and triacylglycerol in the core enveloped by poly (caprolactone) as polymeric wall (Venturini et al. 2011). Imran et al. (2015) developed liposomal nanodelivery systems using soy and marine lecithin for encapsulation of food preservative nisin. Nanoencapsulation of a nutritional supplement coenzyme Q_{10}, has been successfully carried out by using octenyl succinic anhydride and modified starch in rice bran oil (Cheuk et al. 2015). Campardelli et al. (2013) developed bactericidal nanocomposite by encapsulating the nanoparticles of titanium-dioxide in microspheres using super critical emulsion extraction technique. Similar studies on encapsulation of oregano essential oil using chitosan nanoparticles have been carried out. It uses a two-step method i.e. emulsion of oil in water and ionic gelation of chitosan with sodium tripolyphosphate (Hosseini et al. 2013).

Reports suggest that the encapsulation in nanoemulsion based delivery system of two antimicrobial compounds namely a terpene extract from *Melaleuca alterifolia*

and d-limonene, significantly increase the antimicrobial activity (Donsi et al. 2011). Nanoparticles added in different food types improve their flow properties, stability and color during their processing and also to enhance their shelf life.

Nanoencapsulation of live probiotic microbes for improving the gastrointestinal health is of popular interest (Alfadul and Elneshwy 2010). These encapsulated probiotics can be incorporated into various food and drink products such as fermented milk, cheese, fruit-based drink and yoghurt. Encapsulation has also led to the development of slimming products based on cocoa nanoclusters. Such were coated on the surface of nanomaterials to improve chocolate flavor through increase in surface area that target the taste buds (Handford et al. 2014). This has been considered as an effective solution for consumers in weight loss (Ranjan et al. 2014).

In recent years, nanotechnology has evolved to improve the functionality of edible films for food applications. Zambrano-Zaragoza et al. (2013) developed solid lipid nanoparticle-xanthan gum as a high-stability film-forming coating. The method of preparation was based on the hot lipid dispersion with an easy application of these edible coatings to other fruits and vegetables. This led to an increase in the shelf life of guava (*Psidium guajava L.*) (Table 1).

Nano capsules are prepared by chemical processes or physicomechanical processes, which involve building up techniques through bottom up approach that involves elementary building blocks. Chemical methods include emulsions, sol-gels, polymerizations or suspensions. Chemical methods are advantageous in providing uniformity, purity, small sized particles and more reactivity. Alternatively, physicomechanical methodologies are: (a) spray drying, (b) solvent evaporation/solvent extraction, and (c) electroencapsulation based techniques (Hughes 2005) (Table 2).

The release of bio actives from nanocapsules occurs by dissolution or diffusion through the wall or mechanical rupture of the capsule wall. Depending on the application, the nanoencapsulated product may be dry, free flowing powder, slurry or in the form of a wet filter cake (Imran et al. 2015).

Table 1 Core and shell materials more commonly used in different nanoencapsulation applications (Cano-Sarabia and Maspoch 2015)

Application	Core material	Wall material
Food	Acidulants (acetic, sorbic, lactic), flavoring agents (citrus/mint oils, oleoresin, oregan, menthol), sweeteners (sugar, aspartame), colorants (β-carotene), lipids (fish oil, linoleic oil, palmitic acid), vitamins and minerals (vitamin A, D, E, K and folic acid), salts, preservatives (gallic acid), antimicrobials and antioxidants (terpene, limonene, black pepper, catechins), probiotics (*Lactobacillus*, bifidobacteria) enzymes and microorganisms (lipase, invertase, *Penicillium roqueforti*)	Carbohydrates (sucrose, malto- and cyclo-dextrins, chitosan), gums (agar, arabic, gum acacia, sodium alginate, lipids (oils, paraffin, stearic acid, fats, beeswax, phospholipids, Stealth liposomes), celluloses, proteins (albumin, casein, gelatin, gluten, peptides, zein), synthetic elastomers (polyacrylamide, polyacrylate, polyethylene, polyvinyl alcohol, polyvinyl acetate), synthetic polymers (acrylonitrile, polybutadiene, poly(lactide-co-glycolide), silicon dioxide

Table 2 Several nanoencapsulation techniques and the steps involved in each process

Technique	Process
Sol-gel	(a) Solution of core and polymer (b) formation of sol phase (c) gelation (d) solidification
In situ polymerization	(a) Preparation of core solution (b) addition of droplets of monomer
Coacervation	(a) Formation of a three-immiscible chemical phases, (b) deposition of the coating, (c) solidification of the coating
Rapid expansion of supercritical solution	(a) Preparation of solution of core and shell materials in CO_2 (b) depressurization through a nozzle
Liposome entrapment	(a) Micro fluidization, (b) ultrasonication, (c) reverse-phase evaporation
Inclusion complexes	Preparation of complexes by mixing or grinding
Spray-drying	(a) Preparation of a dispersion, (b) Homogenization of the dispersion, (c) atomization of the dispersion
Solvent evaporation	(a) Preparation of solution of polymer and core, (b) solvent evaporation by heating
Electrocoextrusion	(a) Preparation of core solution and wall solution (b) simultaneous spraying of two solutions from two coaxial capillaries

Nowadays, Lipids, Beta-carotene, nisin, Co-enzyme Q_{10}, oregano essential oil and certain probiotics are nanoencapsulated. Chitosan, octenyl succinic anhydride, modified starch, poly caprolactone are used as shells or wall materials for nanoencapsulation. The technology of nanoencapsulation of probiotics is beneficial and of popular interest for the gastrointestinal health. In addition, applications include target crop pesticides (agriculture), nutraceuticals (omega-3 fatty acids, vitamin D, DHA etc.), encapsulated probiotics, essential oils, flavors, oils and aromas.

Nanostructured Additives

Nanoformulations include nanostructured food ingredients and additives. The structure involves formation of a 3D crystalline structure that is of nanoscale size. Nano structured food ingredients enhance solubility, facilitate controlled release, improve bioavailability, and protect micronutrients. The most important nanoformulations include nanoemulsions, micelles, and liposomes (Table 3). Applications involve nanotextured products like spreads, ice creams, yoghurts and mayonnaise (Weiss et al. 2006).

Micelles are spherical structures that form spontaneously when a surfactant is dissolved in water. The application of micelles is in encapsulation of antimicrobials, lipids, vitamins, antioxidants and flavouring agents. Water insoluble ingredients are solubilized using micelles. Micelles containing solubilized materials are microemulsions (Saiz-Abajo et al. 2013).

Table 3 Nanostructured micelles, liposomes, and additives

	Nanoparticles, nanostructured materials	Examples of applications
Food manufacturing	Nanotubes, Nano-capsules (micelles, liposomes), Nano emulsions and Nano spheres from milk protein	Encapsulation, improved solubility, protection and controlled delivery of ingredients; application for example in "nutraceuticals" and/or "functional food"
	Nano-lycopene	Antioxidants for food supplements and food
	Nano-β-carotene	Coloring agent for beverages
	Nano-silicon dioxide	Food additive
	TiO_2, SiO, CaO, ZnO, MnO	Confectionery coatings

Liposomes are lipid vesicles that are also spherical and form aggregates. They can be as small as 20 nm. They are formed by polar lipids (such as phospholipids), which are used to encapsulate proteins. Both fat-soluble and water-soluble ingredients are encapsulated in liposomes. They are highly stable and retain their functional properties (Imran et al. 2015).

Nanoemulsions are 50–200 in size and are oil in water emulsions. The small size of droplet gives nanoemulsions its transparency. They are quite stable for longer periods and demonstrate rheological and textural attributes when added to food (Yuan et al. 2008).

Nano additives include vitamins, fatty acids and various biopolymers which are nanostructured ingredients and serve as a vehicle for carrying the functional ingredient. In addition, many additives are used to fasten the production process, stabilize color, and produce an improvement in taste (Alfadul and Elneshwy 2010).

Nanocomposites

Nanocomposites are a multiphase system in which the continuous phase comprises of polymer with discontinuous nanodimensional phase or nanofiller. It is a form of active packaging used to extend the shelf life of food. Numerous biopolymers find use to develop bionanocomposites. Majorly, starch and polylactic acid derivatives, polybutylene succinate (PBS), zein, gluten, gelatin and polyhydroxy butyrate are biopolymers for nanocomposites. Biopolymers form the continuous phase of nanocomposites and nanofillers form the discontinuous phase. Bionanocomposites are biodegradable and have better oxygen and water vapor barrier properties.

The disadvantage of using biopolymers is poor mechanical and barrier properties. Moreover, nanofillers such as metal nanoparticles of SnO_2, ZnO, TiO_2, MgO_2 etc. enhance the barrier and antimicrobial properties of bionanocomposites. Ethyl vinyl alcohol (EVOH) enhances the oxygen barrier properties (Sorrentino et al.

2007). Sometimes antimicrobials incorporated in bionanocomposites improve the antibacterial properties. Encapsulated antimicrobials in bionanocomposites improve the stability and extend the shelf life of food. De Moura et al. (2012) developed cellulose based bactericidal nanocomposite containing nanoparticle of silver. El-Wakil et al. (2015) developed wheat gluten based nanocomposite by evaporating wheat gluten, titanium-dioxide, and cellulose nanocrystals nanoparticles. Guar-gum-based nanocomposite films have also been developed which exhibit an improved mechanical and barrier properties (Saurabh et al. 2015). Rouhi et al. (2013) studied the physical properties of bio nanocomposite films obtained from fish gelatin incorporated with zinc oxide nanorods. Imran et al. (2012) successfully encapsulated an antimicrobial peptide nisin using soy lecithin nanoliposomes, and incorporated into HPMC (Hydroxy Propyl Methyl Cellulose) to form antimicrobial films. Investigations report, the antimicrobial films effectively inhibited the growth of pathogenic *L. monocytogenes.* There has also been a comparative study between nano and micro solubilisates. It was shown that nanosized solubilisates have a significantly higher antimicrobial property.

Nanosensors

Nanosensors are essential tools used in smart packaging system to detect the presence of microbial contaminants and adulterants. These sensors respond to various changes of contamination, spoilage and degradation. In addition, nanosensors also detect the presence of pathogens, chemical contaminants and freshness of food. Various time-temperature indicators and gas detectors are used as sensors in food packaging. Time-temperature indicators indicate the temperature of food during its journey. Gold nanoparticles are widely used as sensors for time temperature indication. Gas detectors are also used to detect the presence of gases produced by action of microbe. Sensors involve the use of SnO_2 nanoparticles, ZnO-TiO_2 nanocomposites (Chowdhary et al. 2008).

Nanosensors are under research and development stage. For example Opel. It produces opal film by incorporating 50 nm carbon black nanoparticles. Later, opal films can be used as biosensors that display color change in response to food spoilage (Dhineshkumar et al. 2015). The nanotechnological company, pSiNutria developed a nano-based tracking technology, including an edible BioSilicon, which can be placed in foods for monitoring purposes and pathogen detection (Momin et al. 2013). Sometimes the sealing defects in the food packaging remain undetected and the sensor plays a crucial role by indicating any undesirable change that might have occurred during its journey and thus makes consumers alert towards the spoilage of food. Oxygen-sensing links derived from nanoparticles of titanium-dioxide were used as tamper-proofing device (Alfadul and Elneshwy 2010) (Table 4).

Table 4 Nanoparticles in the food industry and applications

	Nanoparticles, nanostructured materials	Examples of applications
Packaging	Nanocomposites, nanoparticles (silver, titanium dioxide, nanoclay silicon dioxide)	Foils, packaging containers, PET bottles
Safety and sensor technology	Nano-silver	Sensors to detect pathogens, chemicals, poisons
		Antibacterial coating in domestic appliances

Nanotechnology and Food Safety

Food safety implies that all the food products should be protected during processing, handling and distribution from any chemical, biological, physical and radiation contamination. So far, the present work has focused on the nanotechnological applications concerning food processing and packaging. It is well known that nano technology has greatly revolutionized the non-food sectors. However, despite their potential benefits, the future of food nanotechnology is uncertain. The consumer's attitude is particularly sensitive when it comes to the food and beverages they consume. Numerous data gaps need to be filled so that product safety can be well demonstrated to the public. These data gaps basically include lack of information regarding: the interaction of bio molecules nanomaterial and cellular components, the interrelationships in between nano particle characteristics, migration of nano material through polymer films and pharmacokinetic properties, consistent and appropriate methods to identify, quantify and characterize nano materials in complex food matrices and biodegradability of nano materials or toxicity to ecologically important organisms (Duncan 2011).

According to European Union regulations for food and its packaging., for introduction of new nano technology, specific safety standards are required. In the United States, the United States Food and Drug Administration (US FDA) regulate most of the food packaging and nanofood, whereas the Environmental Protection Agency (EPA) regulates organic chemicals. However, EPA nor FDA has recognized nanomaterials as new chemicals (Badgley et al. 2007).

Conclusion

Nano technology has numerous applications in food and offers tremendous opportunities for innovations in food industry. The applications show considerable advantages in improvement of encapsulation techniques, nanosensors and packaging materials. However, a broad research needs to be carried about the properties, environmental fate and life cycle of nanoparticles to determine how long they survive, distribute, and degrade. The lack of synergistic relation between nanotechnology and food safety could hamper the progress of the nanotechnology. In addition, an

accurate and quantitative method for the measurement of particles in complex systems is yet to be established. The significant benefits have stumbled upon the risk factors associated with nanoparticles. In addition, the implications of nanotechnologies indicate an immediate need for regulation of nanomaterials before their incorporation into food is critical for ensuring acceptance of the technology by the consumers.

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Nanoparticles in Food Packaging: Opportunities and Challenges

Süleyman Polat, Erdal Ağçam, Burcu Dündar, and Asiye Akyildiz

Abstract Packaging is the last stage of food processing and a successful package is one that protects a product or contents from environment for a period of time with a reasonable cost. The package affects the quality of foods by controlling the degree of factors connected with processing, storage and handling that can act on components of food.

Use of nanotechnology in science brings great opportunities to many industries including food packaging industry. Recently, various engineered nanomaterials such as nanoclays and metallic nanoparticles have been introduced to food packaging as functional additives. Their positive effects on developed packaging materials have been extensively reported.

Nanoclays and metallic nanoparticles are also promising in active packaging technology, an innovative technology for food preservation based principally on mass transfer interactions between systems "food/packaging". These nanoparticles have been applied to packaging system using different ways. This chapter aims to give an overview about the use of nanoparticles for food packaging and introduce the nanoclay and metallic nanoparticle types. Recent developments on active packaging produced by the use of nanoparticles are summarized. Migration studies and their safety issues are also discussed.

Keywords Metallic nanoparticles · Nanoclay · Migration · Silver · Zinc oxide · Safety

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Introduction

Packaging is the last stage of food processing and a successful package is one that protects a product or contents from an environment for a period of time with a reasonable cost. The package controls the degree of factors relevant with processing, storage and handling which can act on components of food. Conventional food packaging has four major functions which are containment; protection/preservation; communication and utility. The term "containment" means to contain products to enable them to be moved or stored. Products should be contained for delivery from their production place to market or store. Products which are likely to be lost some existing components or contaminated by the environment can be transported with proper packaging.

Fresh or processed foods is exposed to physical damage (shock, vibration, compressive forces, etc.) and environmental damage (caused by exposure to water, gases, light, odors, microorganisms etc.) during storage and transportation. An appropriate packaging ensures protection and reduces these types of damage to the packaged foods. The protection function is crucially important for shelf stable foods in a can or pouch, in order to be stable, especially against microorganisms during the time that the package provides protection. The communication functions of food packaging mainly provided with label. According to country regulation that produced food, the label should contain some information such as the net quantity of the contents, name and address of the manufacturer, nutritional information, distributor, and notice for allergens. So the package identifies the product, also with a special design (shape, color, recognized symbols) behaves like a silent salesman.

Sometimes utility function of packaging is termed "convenience". The packaging industry should respond to consumer demands change with their life styles. The popularity of food products that offer simplification and convenience have grown in population. For example, microwaveable entrees, oven-safe meat pouches, steam-in-pouch vegetables and pump-action condiment have been introduced and the packages that provide product visibility, resealable and easy disposal have got the importance. Glass, paper, metal, paperboard and polymers (plastics) are the main materials used by food packaging industry. These materials can be used alone or in a combination (composite material or multilayer packaging material). Due to its functional properties (heat sealable, flexibility, transparency etc.), low weight and costs, plastic is the most used material in packaging of foods (Shin and Selke 2014).

As mentioned above, the main functions of the packaging are accepted as to protect the food from contamination and spoilage and enable to transport it. Therefore, the basic expectations from food packaging are that it should be inert (not to release substances into food); don't change the taste, odor, and composition of the food. While, technological developments including nanotechnology contribute to new functions to the food packaging. Active and intelligent packaging methods introduced in food industry. Active packaging method may be defined as incorporation of certain additives into package to maintain or extending product quality and shelf-life, whereas intelligent/smart packaging method is defined as

those packaging systems with the aim of monitoring the condition of packaged foods to give information about the quality of the packaged food. With the help of these new methods, the package can inform the consumer about the condition of the food and may even interact with the food by releasing or absorbing substances. So, food contact material legislation have been revised in many countries and apart from inert packaging, intelligent food contact and active food contact materials have been introduced (Prasad and Kochhar 2014).

Advancement in use of nanotechnology brings great opportunities for many industries including food packaging industry. Recently, various nanoparticles (such as nanoclay and metallic nanoparticles) have been introduced to food packaging as functional additives. Their positive effects on developed packaging materials have been extensively reported. According to the studies, these nanoparticles improved certain properties of packaging material and introduce new functionalities to active packaging method.

In particular, metallic nanoparticles such as silver, zinc oxide, silicon dioxide and titanium dioxide have been extensively studied; silver and zinc oxide with antimicrobial functions have already been commercialized in some applications (Duncan 2011; Ayhan et al. 2015). These nanoparticles are known to be efficient antimicrobial agents because of their unique properties such as high-temperature, stability and low volatility. However, there is a concern with regard to usage in food packaging materials, whether the nanoparticles transfer from the packaging and cause negative health effects. Present chapter aims to give an overview of the use of nanoparticles for polymer based food packaging, introduce the nanoclay and types of metallic nanoparticles and their application methods. Recent developments on active packaging produced by the use of inorganic nanoparticles are summarized, and migration studies on nanocomposite packaging films and their safety issues are also discussed.

Nanotechnology Usage in Food Packaging

Packaging plays a crucial role in maintaining food quality and reducing product waste. There is an increase in the amount of packaging used, but still poor packaging of food has a significant impact on food loss or wasted food amounts. The use of nanotechnology in food packaging is considered to be highly promising, because it has contributed the development of materials with new properties. The combination of nanoparticles with food packaging materials is known to improve packaging performances, such as ultraviolet, oxygen, carbon dioxide, moisture, and volatile barrier attributes and mechanical properties like tensile strength, elongation, young modulus, yield strength, thereby extending the shelf-life of foods. Even use of the nanoparticles in low fractions into traditional packaging materials can improve the initial properties of material (Cushen et al. 2014b; Ayhan et al. 2015). However, these improvements may change depending on some parameters such as the polymer type, the amount, type and properties (shape, size, production method) of

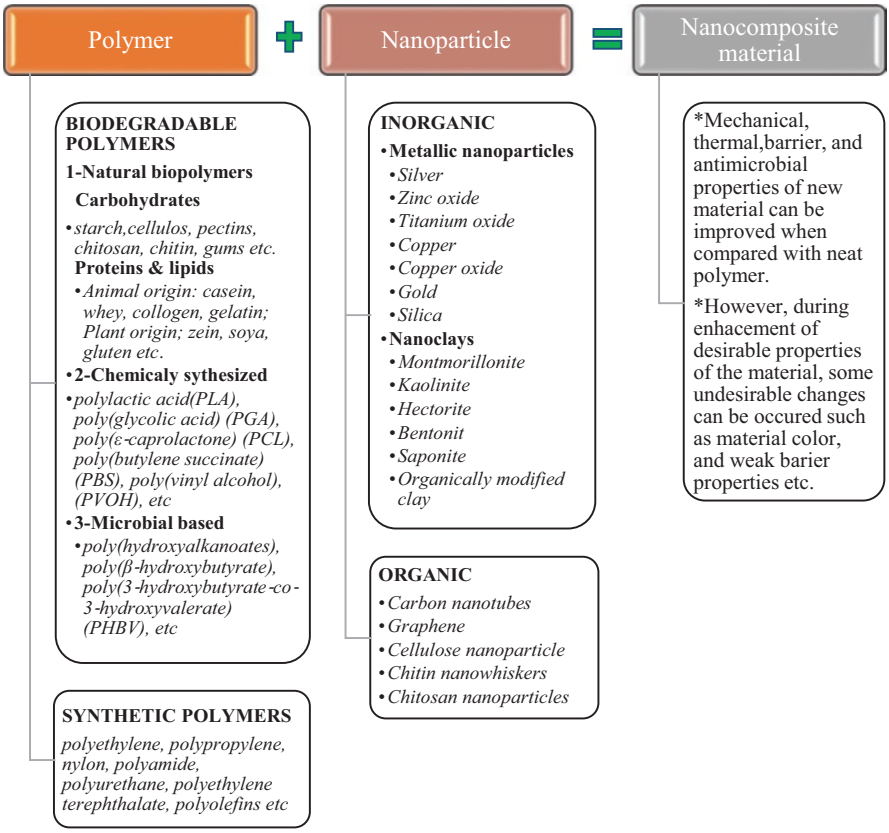

Fig. 1 Classification of polymers and nanoparticles used to produce nanocomposite material

nanoparticle, the method used for the production of the material and the process conditions. Various polymers and nanoparticles can be used for nanocomposite packaging material (Fig. 1).

Figure 1 shows the classification of polymer and nanoparticles which are used for the production of nanocomposite materials. Due to its functional properties such as light weight, cost and heat sealable, plastics (synthetic polymers) are the most used material for food packaging. But extensive usage of the plastics give rise to billion tons of plastic waste is being generated into the environment annually. Generally, decomposition of the plastic based packaging materials is not possible and recycling of them is very difficult. The environmental pollution caused by these products has led to an increasing interest in developing biodegradable packaging materials produced from renewable resources. Efforts are being made worldwide to develop renewable and biodegradable substitutes for non-biodegradable (synthetic polymer based) food packaging materials. Biodegradable polymers, such as polylactide and polycaprolactone, represent an alternative to the conventional synthetic

polymers. However, their cost and the performance of package produced from them restrict their extensive usage.

Nanotechnology is also used in the production of new food packaging materials. The European Union defines 'nanomaterials as "a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range of 1–100 nm. This definition is a reference for determining and legally clarifying whether a material should be considered as a 'nanomaterial in the European Union (Rijk and Veraart 2010; Störmer et al. 2017).

Mostly inorganic nanoparticles are used in the production of new food packaging materials in food industry. The purposes of inorganic nanoparticle addition to polymer based food package are given below:

- Improvement of barrier properties via the incorporation of nanoclays.
- Active food packaging applications to increase shelf-life of food, active substances such as metallic nanoparticles (especially nanosilver, zinc oxide and copper) are used
- Enhancement of physical characteristics to make the packaging more durable to environmental conditions (UV blockage, thermally stable material).

The positive effect of addition of nanoparticles to polymers (biodegradable or non-biodegradable) has been extensively reported. Researchers have emphasized that addition of metallic nanoparticles to polymer contribute the material' antimicrobial and UV barrier properties; and addition of nanoclays improved the material barrier properties against O_2, CO_2, and water vapor permeability. However, when the nanoparticles added to polymer, it is possible that some properties of the material may be weakened, while developing certain features of it. For instance, the addition of ZnO nanoparticles can weaken the mechanical properties of the polymer, while improving the UV barrier properties of polymer. Addition of silver nanoparticles can reduce the light permeability of the material, whereas increase the antibacterial properties of the material at the same time. As mentioned earlier, the enhancement of material properties depends on the amount, type and properties of the added nanoparticle (such as shape, size, production method, surface area) and process conditions used for production of the material.

Production of Nanoparticles

The synthesis methods of nanoparticles is very important as their properties are affected greatly with size and shape. There are two main approach to synthesize nano-size structures: top-down and bottom-up. Nanoparticles are produced with these two approaches. The first one is dividing large compounds to obtain smaller portions (top-down approach), while the other one is condensing of matter in gaseous phase/solution (bottom-up approach). In the first approach, mostly in physical

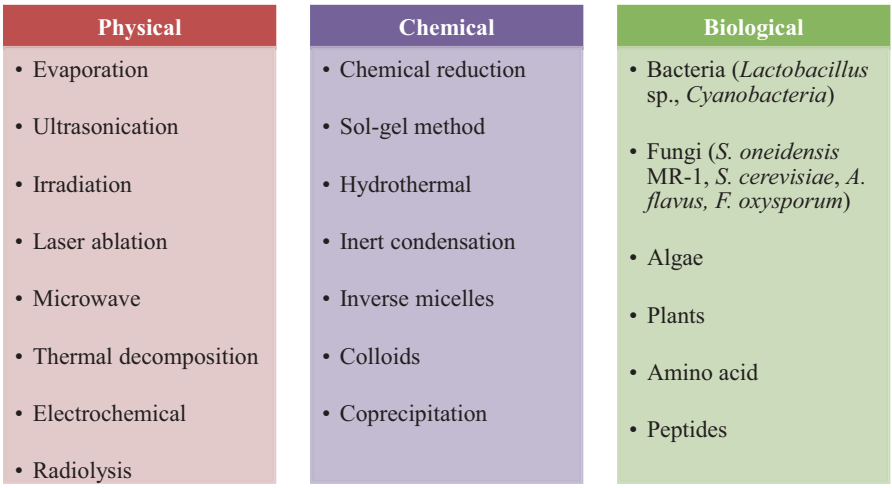

Fig. 2 Synthesis methods of nanoparticles

synthesis, materials are breakdown for obtaining matters at submicron size, while atom or molecules are combined with molecular structures in the bottom-up approach, mostly in chemical or biological synthesis. The earlier method to synthesize nanoparticles mostly applied in metallic ones, especially gold nanoparticles which are one of the most studied nanomaterials in many different fields from electronics to foods (Kalpana and Rajeswari 2017; Carrillo-Inungaray et al. 2018).

Nanoparticles can be produced with physical, chemical or biological methods which are used recently in nanotechnology (Fig. 2).

There are different kinds of chemical synthesis reported in the literature such as colloidal method, photochemical, electrochemical and radiochemical reduction, sol-gel method, solvothermal, hydrothermal and sonochemistry synthesis. In chemical methods, reduction of a dissolved metal salt is ensured with the aid of a reducing agent (sodium borohydride, sodium citrate, citric acid, ascorbic acid, carbohydrates, hydrazine, hydroxylamine compounds) in the presence of a stabilizer or capping agent.

Physical methods containing evaporation and condensation can be used for producing some metallic nanoparticles but the energy consumption is higher. Also, laser ablation of metal plates in a solution, and the laser irradiation of colloidal solution can be used to obtain contaminant-free NPs.

In the biologic methods which are more environment friendly than other methods, plant extracts are used to obtain NPs surrounded by proteins or other biomolecules. Microorganisms can be used to produce NPs in different shapes (cubic, spherical, octahedral, tetragonal, wormhole and irregular) by intracellular or extracellular routes. Additionally, microbial enzymes with antioxidant or reducing properties can be used for reduction of metal compounds.

Some molecules have also been utilized for metallic nanoparticle (Ag, Au, Pt and Cu) production with different methods like the peptide mediated reduction of silver ions, the photo reduction of DNA-metal ion complex and the simple seeded mediated growth method (Huang et al. 2007; Kalpana and Rajeswari 2017). While biologic methods are better to control the size of nanoparticles, chemical methods should be preferred for large-scale productions of nanoparticles (Kalpana and Rajeswari 2017; Tamayo et al. 2019). The size is one of the key parameter of the nanoparticles, because it has a crucial role on properties of nanocomposite material. Depending on their size, antibacterial properties, UV barrier properties of material can change.

Inorganic Nanoparticles

Nanoclays

Clay materials are in silicates group (phyllosilicates) and organized into several classes such as kaolinite, smectite, vermiculite, chlorite and micas, as it can be seen at Table 1.

Table 1 Classification of silicates (Khalid et al. 2016)

Silicates
1. Tectosilicates (zeolites, quartz, feldspars)
2. Phyllosilicates (sheet silicates)
(a) 1:1 Phyllosilicates (kaolinite, serpentine)
(b) 2:1 Phyllosilicates
• Talc-pyrophyllite,
• Smectites
– Dioctahedral smectites (montmorillonite, beidellite, nontronite)
– Trioctahedral smectites (saponite, hectorite, sauconite)
• Vermiculites
• Chlorites
• Micas
(c) 2:1 inverted ribbons (sepiolite, polygorskite)
3. Other silicates

Clays consist of silicon, aluminum or magnesium, oxygen and hydroxyl with various cations. These ions and OH groups are organized into two dimensional structures as layers. Thus clay minerals are also called layered silicates or phyllosilicates because of their structural framework. The silica and alumina layers are 1 nm thick and joined together in various proportions and stacked on top of each other in certain way with a variable interlayer distance. The clay minerals can be classified into three different types based on the condensation ratio of silica to alumina sheet as 1:1, 1:2 and 2:2 types (Ke and Stroeve 2005; Azeez et al. 2013).

Nanoclays are nanoparticles of sheet mineral silicates with layered structural units that can compose complicated clay crystallites by stacking these layers (Lee and Diwakar 2012). Montmorillonite (MMT) and halloysite (HNT) are nanoclays with platelet and tubular structure respectively. Both clays have been extensively investigated in food packaging applications as fillers and carriers of active compounds (Tornuk et al. 2018). The MMT belongs to the structural family known as the 2:1 phyllosilicates, and HNT is 1:1 phyllosilicates.

The chemical composition of MMT is approximately $Na_{1/3}(Al_{5/3}Mg_{1/3})Si_4O_{10}(OH)_2$ (Pinnavaia and Beall 2000), however it can be varied depending on geographic location and deposit strata. Montmorillonite owes special attention among the smectite group due to its ability to exhibit extensive inter layer expansion or swelling, because of its special structure as shown in Fig. 3.

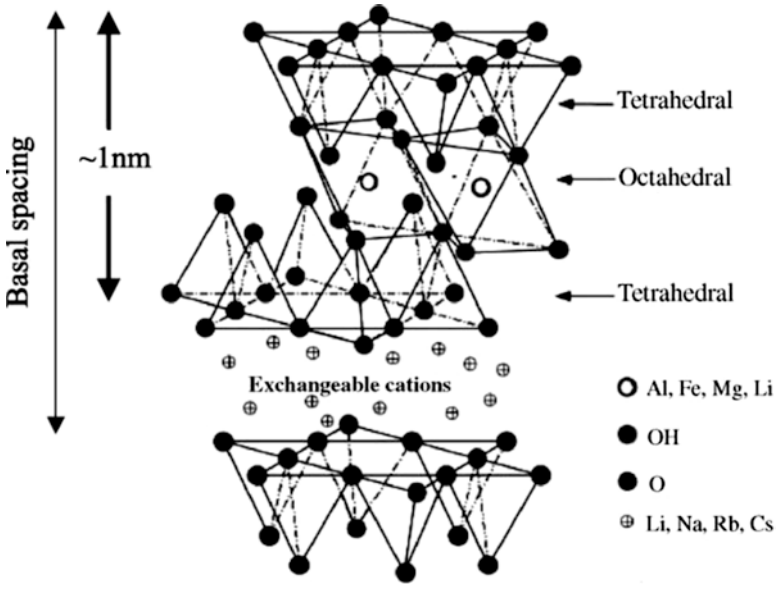

Fig. 3 Basic structure of a 2:1 phyllosilicate (adapted from Ray and Okamoto 2003)

The crystal structure of montmorillonite consists of a single aluminum hydroxide octahedral sheet is located between two layers of silicon oxide tetrahedral sheets (Yalcin and Cakmak 2004). So that the apical oxygen atoms of the tetrahedral sheets are all shared with the octahedral sheet. An overall negative charge is provided by isomorphous substitution of aluminum for silicon in the tetrahedral sheet and iron/magnesium for aluminum in the octahedral sheet. As the surface between the layers is negatively charged it attracts cations such as Ca^{2+}, Fe^{2+} and Na^{+}. They form layer positively charged between the negatively charged areas. The silicate layers of MMT are planar, solid about 1 nm in thickness with high aspect ratio and large active surface area (700–800 m^2/g) (Azeez et al. 2013). The enormous surface area available for interaction between polymer and clay allows polymer chains to transfer stress into filler particles. Additionally, high aspect ratio particles may help to recuperate the barrier properties of membranes by increasing the tortuosity of the material.

Metallic Nanoparticles

Metallic nanoparticle usage has recently gained increasing interests due to antimicrobial activities of the nanoparticles. Properties of metallic nanoparticles depend on source of metal (Ag, Zn, Cu, TiO_2), synthesis method, size (0–100 nm) and shape (spherical, rods, wires etc.). The possible antimicrobial action mechanism of the metallic nanoparticles has already been discussed (Fernández et al. 2010; Espitia et al. 2012). These are:

1. release of antimicrobial ions from nanoparticles and interaction of nanoparticles with microorganisms
2. damaging the integrity of bacterial cell
3. formation of reactive oxygen species (ROS) by the light radiation (Fernández et al. 2010; Espitia et al. 2012).

Especially for TiO_2 nanoparticles, UV activation is needed for antimicrobial effect (Zhang et al. 2014). Metallic nanoparticles have been applied different ways on food by researchers (Fig. 4).

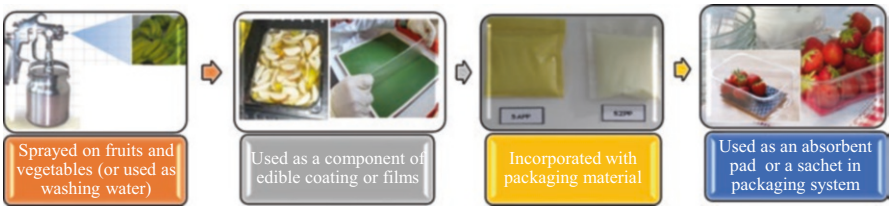

Fig. 4 Application of metallic nanoparticles

Ouzounidou and Fragiskos (2011) sprayed nano Cu on parsley leaves and reported that Cu solution increased shelf life of parsley. Hedayati and Niakousari (2015) prepared gum arabic/silver nanoparticle coating solution and applied on green bell peppers. The coating significantly hindered growth of microorganisms and physicochemical losses, and also enhanced the shelf life of the peppers. Hu et al. (2011) produced PE based nanocomposite film and packaged ethylene-treated mature kiwifruit. Fernández et al. (2010) used cellulose/nano silver absorbent pads and located in trays of fresh-cut melon and reported that absorbent pad application shows lower microbial load. Packaging application using metallic nanoparticles has a great importance in food industry, especially because of their inhibiting effect on some microorganisms. The antimicrobial mechanisms of metallic nanoparticles in dense polymers or thermoplastics used in food packaging materials mainly based on the releasing of ions.

The metallic nanoparticles are also used in intelligent/smart packaging which contains chemical or bio-sensors to be able to monitor the changes in quality or safety of foods. Leakage, level of some particular gases like carbon dioxide, pH, storage time and temperature can be determined by smart packaging systems and biosensors. Fu et al. (2008) investigated a biosensor based on an Au/silicon nanosize-rod for the detection of *Salmonella*.

Production Methods of Nanocomposite Packaging Materials

The nanocomposite food packaging materials are produced according to different methods combining inorganic particles with polymers. The efficiency of these methods is related to inorganic nanoparticle addition processes. These processes are involved in melt blending, in situ-polymerization or solution blending as techniques to incorporate the nanoparticles. According to the form and the final destination of the package, other techniques are introduced in the transformation process such as extrusion-blow molding, compression molding, injection molding or evaporation (Fig. 5).

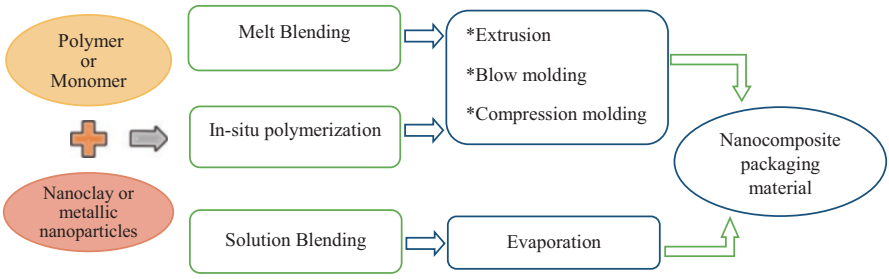

Fig. 5 Nanocomposite packaging material production with different production methods

The production method of nanocomposites has an important role in how the inorganic nanoparticles are distributed through the polymer matrix. The possible distribution of the clay nanoparticles and its possible effect on barrier properties of the resulting materials has been reported (Alexandre and Dubois 2000). An important task in the preparation of nanocomposite material is to achieve uniform dispersion of nanoparticles in the polymer matrix (Guo et al. 2018).When the higher amount of nanoparticle is used, the agglomeration of nanoparticles may be occurred. Generally, this requires chemically modifying the nanoparticle surface so that more homogeneous dispersion occur within polymer. Each of the production method has its own advantages and disadvantages.

Solution Casting

Solution casting is a solvent based process in which the polymer are soluble inside a proper solvent and then nanoparticle mixed with this solution and homogenization is applied for a time. Then solution is poured in a flat mold and solvent is evaporated. This technique is mostly favorable for lab scale production of nanocomposite films. The major advantage of this method is that it offers the possibility to synthesize intercalated nanocomposites based on polymers with low or even without polarity (Azeez et al. 2013). The drawbacks of this technique is that the intercalation process only occurs for certain polymer/nanoparticle/solvent combinations and plentiful amounts of organic solvents are typically required, which may be environmentally unfriendly and costly production (Guo et al. 2018).

Melt Blending

The melt blending technique involves the melting of polymer granules to form a viscous liquid and then the nanoparticles are dispersed into the polymer matrix by high shear rate combined with diffusion at high temperature (Chen 2011). Generally, extruders (it might be single or twin screw) are used for compounding to achieve a uniform dispersion of nanoparticles in polymer matrix. Then, produced nanocomposite granules are diluted with neat polymer to get desired nanoparticle ratio in polymer matrix, and a second process is applied (such as extrusion blow molding, injection molding or compressing molding) to create packaging material (Fig. 2). This technique is considered industrially feasible, because existing equipment which are used for producing food packaging material can be adaptable to nanocomposite material production, and compatible with current industrial processes such as extrusion and injection molding, especially for synthetic polymer based materials fabrication. When compared with solution casting method, the absence of solvents reduces the environmental concerns and provides better mixing of polymer and nanoparticle fillers. There are some important parameters for the production of

the nanocomposites with the melt blending technique. These are extruder processing conditions such as type of extruder, rate of feed, temperature profile, screw speed, die pressure, mixing time and the presence of oxidative environment, material grades and contents, as well as the chemical nature of the nanoparticles and polymers (Guo et al. 2018).

In Situ Polymerization

In situ polymerization is a widely-applied synthesis technique, which provides uniform dispersion and is easy to modify by changing the polymerization conditions. In this method, nanoclays are initially dispersed in monomer solution, then the monomer solution is subjected for subsequent polymerization for producing nanoclay/polymer composite. This polymer composite can also called as hybrid polymer materials, while for metallic nanoparticle/polymer composite production; metallic nanoparticles can be synthesized in situ by using the polymer matrix as the reaction medium. After the clay or metallic particles grafted with thin layer of polymer can be combined with neat polymer matrix to provide desired amount of nanoparticles in final material and secondary processing technique is applied to produce packaging material (Guo et al. 2018).

Tornuk et al. (2018) incorporated the nanoclay particles into LLDPE pellets to produce active nanocomposite films using a twin screw extruder and blown film unit. Huang et al. (2006) added ZnO nanopowder through microinjection molding to investigate the mechanical properties of PP. Lepot et al. (2011) added ZnO nanoparticles without compatibilizer or coupling agent in a PP matrix and investigated its effects on mechanical and barrier properties after biaxial stretching. Zapata et al. (2011) investigated polyethylene nanocomposites containing silver nanoparticles produced via in situ polymerization.

Metallic nanoparticles are also combined on polymer by surface coating method to produce antimicrobial polymer/metal composite coatings. TiO_2 nanoparticles was coated onto OPP film using a bar coater and its antibacterial properties were investigated in vitro and in vivo conditions (Chawengkijwanich and Hayata 2008). Colloidal silver particles have also been coated on paper using ultrasonic radiation, and the coated paper demonstrated antibacterial activity against *E. coli* and *S. aureus* (Gottesman et al. 2011). However, this technique has mainly been used for producing medical instruments, devices and packaging (Cometa et al. 2013; Palza 2015). The coating can be made by using electrochemical or plasma based methods (Fig. 6). Similar to this type technique is used for commercial production of ultrathin SiO_x (protective layer) coated PET bottles. This multilayer packaging material create a barrier for oxygen sensitive foods and carbon dioxide loss from carbonated beverages.

In addition to the above mentioned methods which are widely used for nanocomposite packaging material, some other methods such as electrospinning, sol–gel,

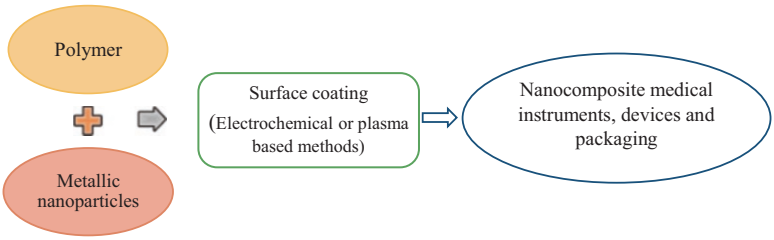

Fig. 6 Surface coating technique

solid-state intercalation have been developed to produce nanocomposites. However, the strict limitations of these methods restrict their wide applications.

Studies on Nanocomposite Food Packaging

Numerous studies and developments about nanoparticle usage in food packaging technology are gaining interest day by day. These studies pointed out that different kind of nanoparticles including metals or metal oxides (silver (Ag), gold (Au), iron (Fe), iridium (Ir), zinc oxide (ZnO), silicon dioxide (SiO_2), titanium dioxide (TiO_2), titanium nitride (TiN), alumina (Al_2O_3), iron oxide (Fe_3O_4, Fe_2O_3), copper (Cu), copper oxide (CuO) and palladium (Pd)) can modify the properties and performance of polymer which was used in packaging. However, commercially most available nanocomposites are based on Ag nanoparticle and nanoclays (Garcia et al. 2018). The results of recent studies about usage of metallic nanoparticles and nanoclays on the polymeric materials are shown in Tables 2 and 3. One of the metallic nanoparticles, titanium dioxide (TiO_2) used in the outer layer of high-density polyethylene bag to decrease the effect of light on carotenoid degradation. Color and astaxanthin degradation was delayed by TiO_2 addition to packaging film (Colín-Chávez et al. 2014). The shrimps packaged in low density polyethylene films containing nano-SiO_2 had better sensory quality and water holding capacity with delayed deterioration, less endoenzyme activity. Nano-SiO_2 using ensured antimicrobial activity and extended the shelf life (approximately 33%) (Luo et al. 2015). Nano-sized metals and their oxide forms are used in food packaging mainly on the purpose of enhancing antimicrobial activity. Inorganic nanoparticles including metallic nanoparticles and nanoclays bind to the cell wall of microorganism and damage it by generating reactive oxygen species. The reactive oxygen species may be responsible for inactivation of proteins, DNA damage and releasing of ions (Hoseinnejad et al. 2018).

Biodegradable nanocomposite films (whey protein isolate/cellulose nanofibre) containing TiO_2 (1%, w/w) reduced the bacterial growth and preserved the sensory qualities of lamb meat. Therefore, edible nanocomposite films with metal oxide nanoparticles are recommended in red meat (Alizadeh Sani et al. 2017).

Table 2 The recent researches about metallic nanoparticles usage

Size	Metal	Matrix	Results	References
Nanosize (rutile)	TiO_2	HDPE + marigold extract, blown film	Barrier properties against ultraviolet are enhanced; Doubled the shelf life of the carotenoids; Tripled the shelf-life of soybean oil with packaging;	Colín-Chávez et al. (2014)
<250 nm	SiO_2	Low density polyethylene (LDPE) (titanate crosslinking)	Gas barrier properties were improved; The shelf life of Pacific white shrimp extended	Luo et al. (2015)
Nanosize	Ag	Starch film and/or with a quaternary ammonium salt (clay)	A low concentration of Ag gave antibacterial properties and enhanced clay dispersion Ensure bacteriostatic effect Enhanced the clay dispersion in film Changed the surface polarity	Abreu et al. (2015)
50 nm	SiO_2	PP (surface treated with EVA)	Oxygen gas and water vapor permeability reduced Tensile strength significantly increased	Li et al. (2016)
50 nm	CuO (1%)	Low density polyethylene (LDPE)	Antimicrobial effect on the growth of coliform bacteria in the cheese	Beigmohammad et al. (2016)
40–60 nm	Ag + TiO_2 + SiO_2	LDPE, blown film	Rate of the respiration and ethylene scavenging were lowered; The packaging protected the nutrient content of mushrooms for 14 days	Donglu et al. (2016)
Nanosize (anatase)	TiO_2 + nanocellulose	Whey protein (added rosemary oil), cast film	Inhibition effect on bacteria Preserved the organoleptic qualities of lamb. The shelf life is extended 9 days.	Alizadeh Sani et al. (2017)

(continued)

Table 2 (continued)

Size	Metal	Matrix	Results	References
40 nm (anatase)	TiO_2	Low density polyethylene (LDPE)	Developed barrier properties, Lowered O_2 and increased CO_2 permeability; Ethylene generation is decreased.	Li et al. (2017)
10–15 nm (anatase and rutile)	TiO_2	Gelatin/agar, cast bilayer film	Inhibited the oxidation of fish oil.	Vejdan et al. (2017)
20 nm	TiO_2	PET, bottle	Decreased the permeability of water vapor; Decreased migration of additive from the packaging to a food simulant.	Farhoodi et al. (2017)
<25 nm	ZnO	Chitosan-CMC-oleic acid, cast film	Decreased water vapor permeability; Inhibited microorganisms; Increasing the shelf life of bread to 35 days.	Noshirvani et al. (2017)
60 nm	ZnO	BPAT, cast film	Enhanced mechanical and gas barrier properties; antimicrobial effects	Venkatesan and Rajeswari (2017)
7–9 nm	ZnO + Al,	Coating PLA, extruded film	Antibacterial activity against *E. coli*	Valerini et al. (2018)
<100 nm	TiO_2, TiO_2 + Ag	PLA, cast film	Antimicrobial effect; Preservation of the freshness; Extended shelf life for cheese to 25 days	Li et al. (2018)
22 nm	ZnO	Biodegradable nanocomposites of cationic starch (CS)	The water vapor permeability and the UV light transmittance are reduced; $\Delta E*$ and opacity of the nanocomposites enhanced	Vaezi et al. (2019)

Table 3 shows that montmorillonite, illite and kaolinite are the most used clays in the recent researches. The improvement in tensile strength of the nanocomposite after using a low amount of montmorillonite (MMT) may be relevant with the uniform dispersion of MMT in the matrix, while the decrease in tensile strength in high MMT levels may be caused by aggregation of clay particles (Rostamzad et al.

Table 3 The recent researches about nanoclays

Nanoclay	Matrix	Results	References
A synthetic iron containing kaolinite	HDPE film	The oxygen fighting role of clay: active performance by trapping and reacting with molecular oxygen and a passive barrier performance by imposing a tortuous diffusion path; Relatively inexpensive	Busolo and Lagaron (2012)
Halloysite nanoclay (HNC)	Polysaccharide-based bio-nanocomposite films	Decreased water vapor permeability and oxygen permeability; Increased tensile strength and glass transition temperature Heat seal strength was increased. Elongation at break was decreased. Uniform and smooth surface morphology obtained	Alipoormazandarani et al. (2015)
Montmorillonite (MMT)	Fish myofibrillar protein (FMP) film	Water gain, water vapor permeability and solubility of the film improved; Improved the tensile strength and elongation of nanocomposites.	Rostamzad et al. (2016)
İllite, scoria and hydrotalcite	Nano-biohybrids (nanoclays and antibacterial natural extract)	Nano-biohybrids have higher antibacterial efficacy than extract itself	Kim et al. (2016)
MMT (Cloisite 30B)	Hydroxypropyl methylcellulose (HPMC)-based films	Increased the water barrier; Strengthened the mechanical properties (elastic modulus and tensile strength)	Klangmuang and Sothornvit (2016)
Na^+ MMT, HNC and Nanomer®I.44P	Fenugreek seed gum (FSG)/clay nanocomposite films	Improved gas barrier and thermal properties of film; Improved tensile strength properties, Decreased elongation at break values of the film; SEM images showed especially lower levels (up to 5%) of nanoclay reinforcements provided film with smooth structure Water vapour permability of the films were not improved	Memiş et al. (2017)

(continued)

Table 3 (continued)

Nanoclay	Matrix	Results	References
Na^+ MMT	Plasticized banana flour film(PBF)	The lower hydrophilicity of nanoclay improved the water barrier properties	Orsuwan and Sothornvit (2017)
Two different MMT (Cloisite® Na^+; Cloisite® Ca^{2+})	Chitosan film	Extending the shelf life of the poultry meat; Reduced lipid oxidation by 50% Reduced microbiological contamination by 6–16%	Pires et al. (2018)
MMT nanoclay	Food-grade edible films with Salvia macrosiphon	Mechanical and thermal properties were considerably improved; The lowest water vapor permeability was obtained in the composite film, Highest elongation at break and tensile strength. Increased the hydrophobicity	Davachi and Shekarabi (2018)
MMT nanoclay	Films of nano-clay-loaded LDPE	Improved thermal and barrier properties; Increased the stability of LDPE; Accelerated the UV oxidation of LDPE	Han et al. (2018)
MMT and HNC	LLDPE based active nanocomposite films with thymol, eugenol and carvacrol	The films had strong in vitro antibacterial activity on pathogens (*S. typhimurium*, *L. monocytogenes*, *S. aureus*, *E. coli* O157:H7 and *B. cereus*)	Tornuk et al. (2018)
MMT	Biodegradable nanocomposites of cationic starch (CS)	Reduced the permeability of water vapor and the UV light transmittance	Vaezi et al. (2019)

2016). Nanoclay (modified montmorillonite) increased the water barrier, and also strengthened the mechanical properties of the hydroxypropyl methylcellulose (HPMC) film (Klangmuang and Sothornvit 2016).The thermal resistance increased with the increasing MMT content of the zein based films, while the mechanical and barrier properties changed non-linearly. The properties of the zein nanocomposite films highly affected by the preparation technique of MMT (Luecha et al. 2010).

The migration from package is dependent on the storage time and temperature as well as the morphology of nanocomposites used (Farhoodi et al. 2014). In spite of the results in the literature showed that the mechanical, thermal and barrier properties of the films generally positively affected by nanoclay or metallic particle usage in package; migration studies are considered as insufficient. The lack of studies on

humans and toxicology properties of nanomaterials is of concern about the use of nanotechnology in packaging of foods. Nano-packaging of foods with prior determined and limited health risk and quality changes may enhance the consumer acceptance, food quality and safety appreciably.

Commercial Applications of Nanocomposites

The number of nano-enabled consumer products is increasing rapidly with parallel of manufacturer number. Also, production and distribution of nanotechnology products is increasingly global with the help of shopping malls or over the internet. Although there is a concern about nanotechnology usage in food sector, it has a commercial application in food packaging area. Commercially available packaging materials that contain nanoclay or metallic nanoparticles were listed by many authors (Bumbudsanpharoke and Ko 2015; Drew and Hagen 2016), even the specifications of some products are available on the internet (http://www.nanotechproject.org/cpi/). Some examples of commercial nanocomposite packaging materials (nanoclay or metallic nanoparticle) are given below:

- Nanoclay based bottles:
  - Aegis OXCE, (Honeywell International Inc. USA)
  - Imperm Nylon, (Mitsubishi Gas Chemical Company Inc. USA)
- Nanoclay based bags:
  - Debbie Meyer BreadBags (USA)
  - Aisaika Everfresh Bag (Japan)
- Nanosilver based food containers:
  - FresherLonger™ Miracle Food Storage (USA)
  - BlueMoonGoods™ Fresh Box (USA)
  - Nano-silver Storage Box (Baoxianhe) (China)
  - A-DO Global (Korea)
  - Nano Silver NS-315 Water Bottle (A-DO Global, Korea)
  - Nano-silver Salad Bowl (Changmin Chemicals, Korea)
  - Nano Silver Baby Mug Cup (Baby Dream® Co., Ltd.)
- Nanosilver based bag
  - FresherLonger™ Plastic Storage Bags (USA) and
- Nano zinc oxide based film
  - Nano Plastic Wrap (SongSing Nano Technology Co., Ltd. Taiwan) also has been commercialized.

Search on new packaging materials such as increasing the shelf life of food products are still going on. A study on evaluation of the current state of nanocomposite materials research has shown that the USA and the Asian countries are the leaders according to published article numbers and patent applications.

Migration Studies on Nanocomposite Packaging Materials

Migration of substances from packaging materials to food is restricted with a legal regulation. Many countries have developed their own system of regulation, while some countries recognize the regulations of other countries and accept the safety of a packaging material when it complies with such recognized regulations. Responsible authorities related in consumer's health have already been taken measure for consumer protection. Overall migration limit (OML) and specific migration limit (SML) terms are defined for food contact materials including food packaging. The OML has been defined the maximum permitted amount of non-volatile substances released from a material or article into food stimulants, SML has been defined as the maximum permitted amount of a substance released from a material or article into food or food simulants in regulations. In many countries, the overall migration limit is accepted as 10 mg/dm^2 of packaging. This value is also accepted as equal 60 mg/kg of foodstuff, for a cubic package containing 1 kg of food. The SML can be determined according to the acceptable daily intake (ADI) or the tolerable daily intake (TDI) values that are confirmed by the governments or scientific committees on food. In the estimation of SML, it is accepted that a 60 kg person eats 1 kg of packed food that containing the substance in the maximum permitted quantity, every day throughout lifetime.

As mentioned earlier, nanocomposite food packaging is a new generation of packaging technology based on nanomaterials. The use of nanoparticles (metallic and clay) in food packaging materials are developing. The challenges for authorities is that the migration behavior of nanoparticles from the packaging may be different from that of traditional materials, and also migrated nanoparticles may be more reactive and exhibit a different toxicological profile (Rijk and Veraart 2010). Thus, some nanomaterials are not permitted in the EU due to limited available toxicity results (Cushen et al. 2014b).

Migration Tests

Migration tests are an important view of safety evaluation of food packages. According to regulations of many countries any material which will be in contact with food is subjected for migration tests. However, regulations of nanocomposite food packaging materials are limited.

In Europe, a new food packaging material produced from plastic and whether or not contains nanoparticles should be subjected to migration test according to Commission Regulation (EU) No 10/2011. The regulation not only contains SML, but also other restrictions specifically mentioned and it contains specifications related to the substances that possibly migrate to food. Although the best approach is to perform migration test with real food matrices, the determination of migration (especially specific migration value) is analytically difficult and time consuming with the real food (Huang et al. 2015c). To overcome this challenge, the regulations have proposed standardized test conditions (including testing time, temperature and test medium (food simulant).

Six types of food simulants are introduced and it is mentioned that for determining the appropriate food simulant, the chemical and physical properties of food should be considered.

- Food simulant A (ethanol 10% (v/v)),
- Food simulant B (acetic acid 3% (w/v)),
- Food simulant C (ethanol 20% (v/v)),
- Food simulant D_1 (ethanol 50%),
- Food simulant D_2 (vegetable oil that has a specific fatty acid composition or isooctane),
- Food simulant E (poly(2,6-diphenyl-p-phenylene oxide, particle size 60–80 mesh, pore size 200 nm), respectively.

Food simulants A, B and C represent foods that have a hydrophilic character and are able to extract hydrophilic substances. Food simulant B represents acidic foods which have a pH below 4.5. Food simulant C represents alcoholic foods with an alcohol content of up to 20%, and foods that has slight lipophilic character, while food simulant D_1 represents alcoholic foods with an alcohol content of above 20% and oil in water emulsions. Food simulant D_2 represents foods which contain free fats at the surface. Food simulant E is assigned for represent to dry foods. Also, water is considered as a food simulant represents many food products, such as bread, fresh fruits and vegetables, meat and fish etc., in some countries (Huang et al. 2015c).

In the migration tests, packaging material and food/food simulant are contacted at specific conditions (time and temperature). The contact conditions should be determined according to the intended use of the food package or represent worst foreseeable conditions of using the packaging material (Council Directive 97/48/EC; EC 2011).

Nanoclays and metallic nanoparticles has been used in various polymers (synthetic or biopolymers) and their migration tests have been carried out under different test conditions and medium (food or food simulant). Recent studies on migration of metallic nanoparticles and nanoclay in nanocomposites (NC) are summarized Tables 4 and 5.

Tables 4 and 5 showed the special analytical techniques such as Atomic absorption spectrometry (ASS), inductively coupled plasma mass (ICP-MS), atomic emission (ICP-AES), optical emission spectrometry (ICP-OES), Transmission electron

Table 4 Migration studies on metallic nanoparticles containing nanocomposite food packaging material

Migrant	Polymer type	Food/simulant	Contact conditions	Test method	Result	References
Ag	PVC	Chicken meat	5, 20 °C 1–4 days	ICP-MS	8.85 mg/kg or 0.84 mg/dm^2	Cushen et al. (2013)
Ag	• PP based commercialfood containers • LDPE based commercial bag	Water, 3% AA, 10% EtOH, olive oil	20 °C 1 h to 10 days	ICP-MS SEM, TEM-EDX	The migrated silver was detected as ionic form and nanoparticle form. Higher migration level in 3% AA was determined No migration in olive oil	von Goetz et al. (2013)
Ag	• Polyolefins based food containers • LDPE based bag	50% EtOH, 3% AA	40 °C for 10 days 70 °C for 2 h (three cycles)	ICP-MS, SEM-EDX	Migration was higher in AA. Migration values were much higher when heating in a microwave oven than in a conventional oven	Echegoyen and Nerín (2013)
Ag, Cu	PE	Chicken breasts	8.13–21.8 °C 1.1–3.1 days	ICP-MS	Effects of time and temperature on the migration were not significant	Cushen et al. (2014a)
Ag	PE	Water, 3% AA	40 °C 10 days	ICP-AES, TEM	The initial amount of Ag and simulant type were significant effect on migration rate.	Cushen et al. (2014b)
TiO_2	PE	3% AA, 50% EtOH	25, 70, 100 °C 1–8 h	ICP-MS, LPSA	The maximum migration amounts into AA was 12.1 ± 0.2 μg kg^{-1}, while into EtOH was 2.1 ± 0.1 μg kg^{-1}at 100 °C. Increasing additives in the film increased migration. The researchers noted that cut edges of the film can be contribute to migration.	Lin et al. (2014)
ZnO+ Ag	LDPE	Water	40 °C 10 days	ICP-MS	ZnO migration more than Ag migration.	Panea et al. (2014)

(continued)

Table 4 (continued)

Migrant	Polymer type	Food/simulant	Contact conditions	Test method	Result	References
Ag	Commercial PP based containers and LDPE based bags	W, 3% AA,10% EtOH, 95% EtOH	10 days at 20 °C, 10 days at 40 °C, 2 h at 70 °C	ICP-MS, AF4-ICP-MSSEM-EDX	Silver ions and nanoparticles were distinguished during migration tests. Chlorine and sulphur atoms could play a role on AgNPs transformations during migration test	Artiaga et al. (2015)
Ag	Commercial PE containers and Commercial PE cling film withcoated silver	Bread, Apple, Cheese, Milk powder; Orange juice, Carrot, Ground beef, Butter/W, 3% AA	40 °C 7–10 days	AAS, ICP-MS	Too low levels of Ag were released from containers into real food samples and food simulants. While, higher levels of migration have been detected for samples exposed to nano-silver coated films. No chemical or biochemical changes observed for the food samples.	Metak et al. (2015)
Ag	PE based commercial containers, HDPE based commercial bag PE based commercial bag	W, 3% AA,10% EtOH	40 °C 10 days	ICP-MS, sp-ICP-MS,TEM, EDS	The total content of silver in the containers varied from 13 to 42 µg/g. The highest total Ag migration was observed for AA (3.1 ng/cm^2 after 10 days) Nanoparticle release was observed for all food container brands. The released particles were detected as spherical particle.	Mackevica et al. (2016)
Ag	PP based containers, dishes, cups, cutting boards PE based bags	W, 4% AA,20% EtOH	10 days at 5 °C 10 days at 40 °C 30 min at 60 °C30 min at 95 °C	ICP-MSUltra-filtration	Migrations of Ag and Zn were highest in AA. The Ag that migrated from nanosilver products into AA was in the ionic form, and those into W and EtOH were in the nanoparticle form.	Ozaki et al. (2016)

ZnO	PE based film	3% AA, 10% EtOH, isooctane	10 days at 40 °C 2 days at 20 °C	AAS with graphite furnace	Films containing 3% or more ZnO nanoparticles were determined inappropriate for packaging of acidic foods.	Polat et al. (2018a)
ZnO	PP based film	3% AA, 10% EtOH, isooctane	10 days at 40 °C 2 days at 20 °C	AAS with graphite furnace	Migration values were changed depending on initial concentration of ZnO Max migration value was detected as 14.06 mg/kg in AA.	Polat et al. (2018b)
ZnO	PP based films	3% AA	40 days at 20 °C 25 days at 40 °C 24 days at 70 °C	ICP-AES	Three types of polypropylene nanocomposite films with or without coupling agent were tested. The coupling agent decreased migration. The higher migration rate was detected for homopolymer of polypropylene.	Chen and Hu (2018)

Table 5 Migration studies on nanoclay containing packaging materials

Migrant	Polymer type	Food/simulant	Contact conditions	Test method	Result	References
Cloisite 20A	PET bottles	3% AA	90 days at 45 °C	ICP-OES	Migration was increased with storage time and temperature. Aluminum migration was detected as 0.34 mg/kg Silicon was detected as 9.5 mg/kg	Farhoodi et al. (2014)
MMT	BOPP/ nanocomposite-adhesive/BOPP Multilayer film	W, 3% AA, 15% EtOH, olive oil, grapeseed oil and coconut oil	20, 25, 40, 70 °C 2, 6 h 1, 3, 7, 10 days	ICP-OES	The migration of Si increased with storage time and temperature Migration was higher in AA. They suggested a numerical model based on Fick's diffusion theory.	Huang et al. (2015b)
Nanoclay	Commercial LDPE based bags	10% EtOH 3% AA	10 days at 40 °C 2 h at 70 °C	ICP-MS	Aluminum migration was observed for both samples 51.65 $ng.cm^{-2}$ for the Aisaika brand 24.14 $ng.cm^{-2}$ for the Debbie Meyer bag	Echegoyen et al. (2016)
Cetylpyridinium bromide (CPB)-modified MMT	LDPE	10% EtOH	35 day	Electrical conductivity	The organoclay surfactant migration was detected. The migration increased with time.	Muñoz-Shugulí et al. (2019)

microscopy (TEM), Laser particle size analysis (LPSA), energy dispersive X-ray spectroscopy (EDS), Ultrafiltration can be used for the determination of nanoparticles migration. These techniques are different from common chromatographic techniques applicable to conventional polymer additives. The chromatographic techniques are inappropriate or severely limited because they cannot measure particle size, shape and aggregation of nanoclay or metallic nanoparticles. Because of its high selectivity and sensitivity, ICP-MS is more favored than ICP-OES and ICP-AES in migration studies of nanoparticles. However, basic ICP-MS does not differentiate between nanosized elemental metal and metal ions, which in case of nanosilver migration studies means dissolved silver ions and dispersed silver in its particulate form. Single particle (sp) ICP-MS is recommended as a new technique able to distinguish quantitatively between dissolved and particulate species. However, the sp-ICP-MS technique has also not respond fully to detect metal and metal ions quantitatively in some nanoparticle migration studies. Because, metal species can be changed reversibly the oxidation state from metal to metal ions by the chemical environment. Such a redox change may also be occurred when handling and concentrating migration solution for preparation to TEM measurement. So, extreme care needs to be taken when preparing the samples for the analytical techniques which are used for migration tests (Störmer et al. 2017).

Ozaki et al. (2016) investigated the migration of silver from food-contact plastics, six nanosilver-labelled products and five silver ion (Ag^+) labelled products, to various food simulants (water, 4% acetic acid, 20% ethanol). They detected highest Ag and Zn migration values in 4% acetic acid. With the increase of migration time and temperature, the release of Ag and Zn increased. Moreover, they observed that the Ag migrated from nanosilver products into the acetic acid was in the ionic form, and those into the water and 20% EtOH were in the nanoparticle form. Chen and Hu (2018) studied ZnO migration from three types of polypropylene (homopolymer (PPH), block copolymer (PPB) and random copolymer (PPR) of polypropylene) nanocomposite films with or without coupling agent to 3% acetic acid and reported addition of the coupling agent could decrease ZnO migration, because the coupling agent could improve the interface between ZnO and PP molecular chain segment. Moreover, the migration rate of ZnO changed depending on the degree of crystallinity of polypropylene and higher migration values were detected for PPH, PPB and PP, respectively.

Studies demonstrated that migration of nanoparticles from food packaging material into food or food simulants may be changed by multiple factors including temperature, time, the initial concentration of nanoparticle in polymer matrix, polymer properties, position of the nanoparticles in the packaging material, interaction between the nanoparticles and the materials, sampling type of material, contact type of material and the nature of the food/food simulants. Generally, the nanoparticles have the potential of migrating into food, especially when in contact with more acidic substances. Studies also showed that sampling method of packaging materials and contact type has an effect on migration values, because sampling of the material (cut edges) may create new surface areas for migration.

According to migration studies, it can be concluded that there is no standard techniques for the detection and characterization of nanoparticles migrated from polymer matrices to foods. Thus, the different techniques should be used with a proper sampling method that allows sensitive chemical detection and quantification as well as accurate physical determination of particle sizes, and also distinguish between dissolved and particulate nanomaterials even in complex matrices.

As a summary the challenges in migration studies are;

- There are no standard test conditions for nanocomposite materials
- Authorizations of a material in its bulk form do not imply authorization of the nanoform (especially for metallic nanoparticles)
- Current migration limits do not apply to nanoparticles
- There are difficulties for detection of metallic nanoparticles migration with the current analytical methods

Health Aspects of Inorganic Nanoparticles

Consumer safety has become a serious concern, because of the increasing interest in the use of nanoparticle based products. Nanoparticles may have differential toxicity, because they have different physicochemical properties, such as chemical, optical, magnetic, and structural. Even in cases where nanoparticles do not show any acute toxicity, questions of long-term effects remain unanswered on bioaccumulation and in food chains (Tiede et al. 2008). Therefore, the generalization of potential toxicological effects of nanomaterial-based products are extremely difficult (Vega-Villa et al. 2008). The type of nanoparticles and related chemical, physical, and morphological properties affect their interaction with living cells. Moreover, they determine the route of clearance from the gastrointestinal system and possible toxic effects (Borel and Sabliov 2014). Nanoparticles bio-distribution occurs through systemic circulation to organs such as liver, kidneys, spleen, heart, lungs, and brain. The biotransformation of the nanoparticles occurs through interactions with proteins and lipids in tissues such as the liver and intestine (Vega-Villa et al. 2008). It is known that the liver, kidneys, and colon are primarily responsible for excretion of nanoparticles and their possible metabolites (Bertrand and Leroux 2012; Bouwmeester et al. 2009).

Several research groups around the world started to probe potential toxicological effect of nanoparticles on human health and biological systems. The evidences collected from the studies have shown that there are reasons to suspect that nanoparticles may have potential toxic effect on biological systems (Bouwmeester et al. 2009). Clinical and experimental studies showed that reactive oxygen species can be catalyzed by nanoparticles and thus, causing oxidative stress and subsequent inflammation by the aid of interaction with the reticulo-endothelial system (Nel et al. 2006). Lordan et al. (2011) conducted the cytotoxicity of two different nanoclays (the unmodified (cloisite-Na^+) and organically modified (cloisite-93A)) in

human hepatoma cells frequently used as in vitro alternatives to primary human hepatocytes. The results showed that cloisite-Na^+ induced intracellular reactive oxygen species (ROS) formation which coincided with increased cell membrane damage, while ROS generation did not play a role in cloisite-93A-induced cell death due to low accumulation in the cell culture. In addition, the nanoclays aggregated differently in the cell culture medium and appeared to have an effect on their mechanisms of toxicity. They concluded that the studied nanoclays are highly cytotoxic, and therefore pose a possible risk to human health. Finally, they suggested that the effect of size, shape, composition and aggregation-dependent interactions of nanoclays on biological systems with particular attention given to food packaging applications to be studied.

The effects of Nano-silver particles (Ag-NPs) on traits of productivity, oxidative stress and some of important blood parameters in broiler chicks was conducted by Ahmadi (2012). The findings indicated that no significance in growth performance of the chicks was detected, while significant change was found in the feed efficiency. The blood parameters such as ALT, AST, ALP, TP, albumin, gamma globulin, triglyceride, and cholesterol were significantly changed in the exposed broiler chicks. Another interesting result was on oxidative stress enzymes responsible from production of reactive oxygen species have been reported. These enzyme activities increased significantly in nano-silver exposed chicks when compared to untreated samples. Arora et al. (2009) used primary liver and fibroblasts cells that were suitable for in vitro toxicity studies in order to determine interactions of silver nanoparticles. They indicated that morphology of primary liver and fibroblasts cells remained unchanged up to 100 μg/mL and 25 μg/mL, respectively. The antioxidant defense mechanisms were also probed in terms of lipid peroxidation levels, concentration of glutathione (GSH) which is responsible from reducing damage effect of oxidative stress in cell and finally, superoxide dismutase activity (SOD) catalyzing superoxide radicals into molecular oxygen or hydrogen peroxide. The effect of different doses and sizes of nano-silver particles, as well as biocompatible coating on the proliferation of mouse germline cells was carried out in a research study by Braydich-Stolle et al. (2010). According to the results, reduction in proliferation of the cells treated with any type particles was not observed for exposure lower than 10 μg/mL and also these concentration levels did not appear to be stressed the cells, because there was not detection of ROS production or any signs about cell apoptosis. However, significant decrease in cell proliferation was observed for the exposure higher than 10 μg/mL after 24 h incubation. Moreover, particle size and type of coating affected significantly this mechanism; especially smaller particles caused greater decrease than larger ones. Hackenberg et al. (2011) evaluated possible toxicological effect of Ag-NPs on human cells. They exposed the human cells in solution having concentrations of 0.1, 1 and 10 μg/mL for 1, 3, and 24 h and showed that significant change was found for cell DNA treated with higher Ag-NPs and longer treatment time. Huang et al. (2015a) treated the mouse brain neural cells with 3–5 nm Ag-NPs to investigate whether these particles can pass through the cells and biochemical responses of the cells were monitored. Their results showed that 3–5 nm Ag-NPs are able to cross the cell membrane, which induced the gene expres-

sion related with inflammatory process and phagocytosis. Moreover, in Ag-NPs exposed cells, stress-responsive gene and immune reaction genes were activated. Taken into consideration of all findings obtained from the mouse brain cells exposed with Ag-NPs, the researchers concluded that the progress of neurodegenerative disorders might evolve due to induced neuroinflammatory response and amyloid (Aβ) deposition. Moreover, the researchers highlighted that it is necessary to figure out the daily usage and distribution of Ag-NPs in the environment with future studies.

In the food industry, TiO_2-NPs are commonly used due to anticaking properties or antimicrobial effects (Smolkova et al. 2015). The experimental studies generally indicated that DNA damages (as oxidation or breaks) were determined in the cells exposed with TiO_2-NPs (Magdolenova et al. 2014). The particle size of TiO_2 in agglomerates showed different toxicity in human or animal cells. The results indicated that no genotoxic effect was detected for the cells treated with TiO_2-NPs dispersed with agglomerates less than 200 nm, while larger agglomerates caused DNA damages in the exposed cells (Magdolenova et al. 2012). Kreyling et al. (2017) injected aqueous suspension of TiO_2-NPs agglomerated with size of 70 nm for conducting bio-kinetics of the nanomaterials in rats. The bio-kinetics of TiO_2-NPs were investigated in liver, spleen, kidneys, lungs, heart, brain, uterus, blood, carcass, skeleton, and soft tissue and the exposed rats were also monitored from one-hour to 4-weeks in terms of TiO_2-NPs accumulation. They reported that TiO_2-NPs were in detectable level for all studied organs. After 24 h, the highest accumulations of TiO_2-NPs were determined in liver with 95.5%, followed by spleen with 2.5%, carcass with 1%, skeleton with 0.7% and blood with 0.4%. The findings showed that the concentration of TiO_2-NPs reduced remarkably in blood after 24 h from the exposure, while the distribution showed constant tendency until 28 days in other organs and tissues. In another study, 15 post-mortem humans were surveyed to present TiO_2-NPs accumulation in livers and spleens. The results showed that the nanoparticle counts in liver and spleen ranged from 1×10^9 to 21×10^9 TiO_2-NPs/kg tissue and from 1.2×10^9 to 56×10^9 TiO_2-NPs/kg tissue, respectively. In addition, at least 24% of TiO_2-NPs detected in the organs were smaller than 100 nm (Heringa et al. 2018). Sycheva et al. (2011) studied genotoxic and cytotoxic effects of TiO_2 on in vivo mice treated orally with the size of micro- (160 nm) and nanoparticles (33 nm) in dose of 40–1000 mg/kg bw. The circulation of TiO_2 particles was also followed in the cells of six organs, namely; liver, brain, bone marrow, colon, testis and for stomach. The results showed that the micro-TiO_2 induced DNA damage and micronuclei in treated bone marrow cells, while nano-TiO_2 induced DNA damages both liver and bone marrow cells. Moreover, a significant reduction was determined in sperm count and function of mice treated with high-dose TiO_2. They explained these undesirable effects with genotoxic and cytotoxic mechanisms related with inflammation or oxidative stress caused by TiO_2 particles in the exposed cells and highlighted that over exposure of TiO_2 particles has a potential health risks and dose-dependent studies must be conducted to determine acceptable daily exposure of micro- or nano-scale of TiO_2 particles.

The toxicological effect of Zn-NPs at different concentrations (500 μg/L and 2000 μg/L) on the fish brains were studied by Saddick et al. (2017). The exposed

fish results showed that there are significant increases in the enzymes activity associated with antioxidant mechanism. Moreover, for treated fishes, 96 h lethal concentrations (LC_{50}) of Zn-NPs were calculated as between 5.5 and 5.6 mg/L. The effects of zinc-copper alloy nanoparticles (Zn/Cu-A-NPs) on human lung cells were investigated with in vitro study in terms of cyto- and genotoxicity. They reported that the exposure of Zn/Cu-A-NPs caused significant DNA damages in the lung cells and presence of Zn/Cu-A-NPs in the medium induced significant formation of intracellular reactive oxygen species. Due to potential toxic effect of Zn/Cu-A-NPs on the biological systems (Kumbıçak et al. 2014). Lanone et al. (2009) conducted a comparative study about the toxicity of 24 nanoparticles that are of equivalent spherical diameter and different elemental compositions using various cytotoxicity assays. They used different cells originated from human tissue in order to investigate toxicity of the nanoparticles. Their results demonstrated that the highest toxicity responses were from cells exposed to Cu- and Zn-based nanoparticles, while the moderate toxicity responses were obtained for cells treated with Ti-, Al-, Ce and Zr-based nanoparticles. However, no detectable toxicity was determined for tungsten carbide nanoparticles. The researchers also stated that there is no significant correlation between equivalent spherical diameter or specific surface area of nanoparticles and cytotoxicity. They concluded that choosing of cytotoxicity assays and cell types, due to different sensitivities, are very important points to be clearly put forth possible toxicity effect of nanoparticles. Wang et al. (2016) demonstrated the possible effects of diets containing ZnO-NPs between 0–5000 mg/kg on growing mice, Zn metabolism and biodistribution. They fed the mice with ZnO-NPs and change in the metabolism was monitored during 34 weeks. The biodistribution findings showed that a significant accumulation was detected in kidney, bones, liver and pancreas of mice fed with 5000 mg/kg ZnO-NPs. However, exposing ZnO-NPs between 50–500 mg/kg in the diets for long term showed minimal toxicity in the mice. As a result of long term exposure (34 weeks), the lowest body weight was determined for the mice exposed with diet of 5000 mg/kg ZnO-NPs. Moreover, in the mice treated with the same diet concentration, the relative weights of lung, brain and pancreas increased significantly. Compared to untreated mice, expression of mRNA associated with Zn metabolism induced significantly in mice receiving 5000 mg/kg ZnO-NPs.

Conclusion

In spite of the toxicological properties of inorganic nanoparticles, the research studies on nanocomposite food packaging materials and number of commercial food contact materials containing inorganic nanoparticles are in continuous extension. According to the research results, the positive effects of inorganic nanoparticles on the properties of food packaging materials are incontrovertible. However, the migration features of these nanoparticles are a matter of debate. Some of the migration studies performed on food simulants showed that the migration values of nanocom-

posite materials are lower than the detection limits of the analysis technique although an exposure risk to these nanoparticles still suspected by other studies with an ambiguity related to the toxicological form of these particles. Thus, specific migration limits of each inorganic nanoparticle should be set up in addition to the improvement of the test method sensitivity in order to overcome to these weaknesses. Finally, to deal with the expected increase of using nanocomposites in food contact material the governments and independent associations are forced to design urgently science-based regulations.

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Ultrasound: A Food Processing and Preservation Aid

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Abstract Ultrasound is a form of green technology that has diverse applications in food processing, preservation and quality control. It is an emerging technology that modifies various properties of food products. The principle phenomenon behind ultrasound is cavitation and mass transfer. High frequency ultrasound is used to monitor the composition of food whereas low frequency ultrasound induces physical and chemical/biochemical changes in food. The technology employs a non-invasive technique to estimate the composition of food. Various frequencies of ultrasound can be significantly utilized to modify physiochemical properties of food during freezing and crystallization, defrosting/thawing, drying, meat tenderization, pickling and emulsification/homogenization. The technique is also useful in extraction of bioactive components, sterilization, defoaming, depolymerization and inactivating enzymes. Ultrasound minimally affects the quality of food products and is an inexpensive technology with the only limitation to certain foods which contain small air bubbles.

Keywords Ultrasound · Preservation · Processing · Frequency · Minimal

Introduction

Ultrasound is mechanical energy that involves frequency spectrum. It is a sound wave with frequency beyond threshold of human hearing >20 kHz. The propagation of ultrasound takes place in the form of vibrations. Vibrations result in displacement and oscillation of molecules from their mean position. Such vibrations propagate along the direction of waves. Ultrasound waves undergo various compression and rarefaction cycles which leads to various physical and chemical changes in the

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medium through which it travels. Motion of waves transfers energy from one point to another without the transfer of matter. Frequency is the key component that generates changes in the medium. However, other factors such as wavelength (m), frequency (Hz), amplitude (μm), velocity (m/s) and intensity (W/cm^2) also affect the nature of the ultrasound wave. Depending on the echodensity of the material an ultrasound may be absorbed, reflected or allowed to pass through which leads to various chemical and mechanical effects in the medium through which it is passed. The chemical effect of ultrasound is radical formation and mechanical effect involves shear gradients. Such effects of ultrasound waves are appropriately used in various biomedical, biotechnological and food processing applications for distance measurements, cleaning, mixing, detection and other manufacturing operations (Delia et al. 2018).

Ultrasound has recently emerged as a novel food processing technique over the various available classical technologies. It is of considerable interest in food is for its low energy consumption and production of very less toxic compounds. The key feature of ultrasound is its non thermal nature. Nonthermal techniques specifically cause minimal degradation of food, enhance shelf life, and are hence preferred. Ultrasound can induce various favorable physiochemical properties to food. It has many applications in food such as extraction of bioactives, degassing, microbial and enzyme inactivation, crystallization, filtration, starch polymerization, fat separation, emulsification and mass transfer. The process enhances the shelf life of food with minimal nutrition loss (McClements 1995a). Till date ultrasound has been widely used in the non thermal and minimal processing of food. Ultrasound works on the principle of cavitations phenomenon which occurs due to compressions and rarefactions of ultrasonic waves. It is the formation of implosive bubbles at the microstructural level which leads to various chemical and mechanical effects in food matrix. The microstructrural effects are likely to occur at bubble interface, inside the bubble and in the bulk itself. Promising effects include alteration of functional properties in various food products, inactivation of microbes or enzymes and starch polymerization etc. Thus, the structural changes lead to physical, microbial and enzymatic stability.

Principles of Ultrasound

Ultrasound waves are sound waves generated in specific frequency ranges. They are pitched above the human hearing range. The physical parameters that characterize ultrasound are velocity, frequency and wavelength. Ultrasound is considered as a pressure wave in one dimensional propagation and can be illustrated as a sinusoidal wave with pressure amplitude along the x-axis and time or distance along the y-axis (Fig. 1). The basic properties of ultrasound are based upon wavelength, frequency and velocity.

Wavelength is the distance of wave (i.e. one complete cycle) that propagates through a medium. It depends on the frequency of oscillations and velocity of the

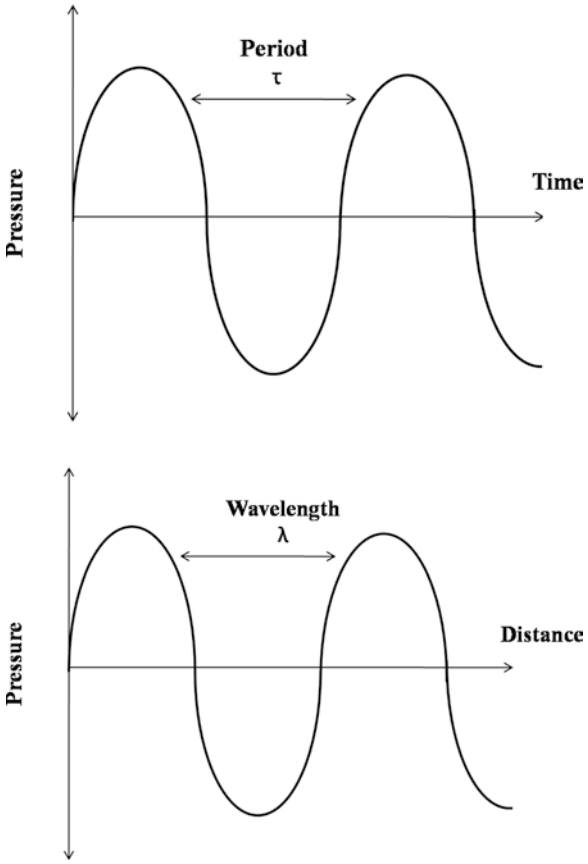

Fig. 1 Sinusoidal wave on time and distance axis. The time to complete one cycle is the period *(τ)*. The distance to complete one cycle is the wavelength *(λ)*

wave in the medium. Frequency is the number of oscillations per unit time whereas velocity depends on the physical properties of the medium such as density, compressibility and bulk modulus (Hwang, 2009).

The relationship between wavelength, frequency and velocity is given by

$$c = f\lambda$$

Also, velocity of the wave through a medium can be determined from the density (ρ), compressibility (K) and bulk modulus (β) of the medium

$$c = \frac{1}{\sqrt{K_p}} = \sqrt{\frac{\beta}{\rho}}$$

Types of Ultrasound

Ultrasound can be classified into low power and high power ultrasound on the basis of frequency (either low or high) (Hwang, 2009).

Low Power (High Frequency)

A frequency of higher than 100 kHz and intensity less than 1 W/cm^2 are employed. It is used to monitor the composition and physicochemical properties of food. LPU is based on the principle of velocity, attenuation coefficient and acoustic-impedance. It is used to disperse aggregated materials, generate emulsions and disrupt cells. Recent applications include degassing, enzyme inactivation, filtration and inducing oxidative reactions (Knorr et al. 2004). High frequencies impart physical and chemical changes due to immense pressure, shear and temperature gradient through the medium of propagation.

Principle of Low Power Ultrasound

Ultrasonic velocity (v) is determined by density (ρ) and elasticity (E) of the medium, according to the Newton–Laplace equation

$$v = \left(E / \rho\right)\%¿ \qquad where\, v = \lambda f \, or \, d / t$$

Attenuation (A) and acoustic impedance (z) are expressed as

$$A = A_o e^{-ax}$$
$$R = A_T / A_i = z_1 - z_2 / z_1 + z_2 \left(\text{where } z = \rho c\right)$$

A_o = is the initial (unattenuated) amplitude of the wave.

X = is the distance traveled

R = is the ratio of the amplitude of reflected wave to the incident wave

z_1 and z_2 = are the acoustic impedances of two materials (Fig. 2).

The relationship between velocity, attenuation and impedance with the physicochemical properties of food determines the basis for ultrasonic analysis. Such relationship is established either by plotting a calibration curve between the physiochemical property and ultrasonic property. Examples include monitoring the attenuation of ultrasound wave to determine the degree of homogenisation in milk which further estimates the degree of emulsification and creaming (McClements, 1995b). Velocity and attenuation can also determine the stability of mayonnaise, edible oils and fruit juices (Mason et al. 1996).

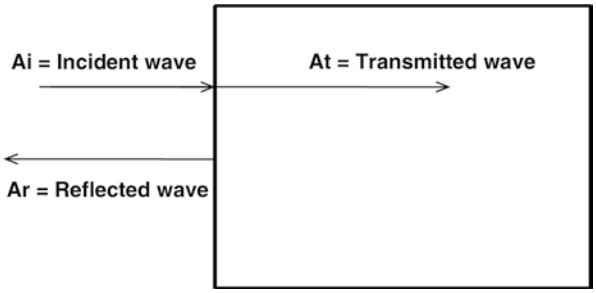

Fig. 2 Reflection and transmission of an ultrasonic wave from a boundary between two materials

High Power (Low Frequency)

A frequency of 20–100 kHz and intensity higher than 1 W/cm^2 are applied to food, so as to induce physical and chemical/biochemical changes by cavitation. It is based on the principle of energy, intensity, pressure, velocity and temperature and supports various food processing operations such as freezing, emulsification, extraction and drying.

High power ultrasound generates complex effects in propagating food material which alters the physiochemical properties of food by cavitation. One of the major applications of high power ultrasound is for cleaning purpose. However, the earliest approaches for ultrasound were for the use of disrupting biological cell walls. Studies show that use of ultrasound can reduce the production time of yogurt by 40%. Additional effects of ultrasound include the disruption of cell walls to release cell contents which leads to increase in mass transfer (Chendke and Fogler, 1975). Zayas (1986) reported an increase in the yield of enzyme rennin from calf stomachs by ultrasound. Furthermore, power ultrasound can accelerate ice nucleation process (Zheng and Sun, 2006).

Principle of High Power Ultrasound

$$P_a = P_{amax} \cdot \sin(2\pi ft)$$

P_a is the acoustic pressure (a sinusoidal wave), which is dependent on time (t), frequency (f) and the maximum pressure amplitude of the wave. Pa max is related to the power input or intensity (I) of the transducer

$$I = P_{amax} = 2\rho v$$

(*where ρ is the density of the medium and v is the sound velocity in the medium*)

Mechanism of Sonication Process: Cavitation Phenomenon

A sonic wave passes through a medium as a longitudinal wave; regions of alternating compression and expansion are created. These regions of pressure lead to occurrence of cavitation, and gas bubbles are formed inside the medium. Cavitation is affected by the viscosity of liquid, temperature and ultrasound frequencies. During the expansion cycle, the bubbles of cavitation have a large surface area to increase the process diffusion, which causes the bubble to expand. Stable cavitation arises from small bubbles and generation of bubbles creates microcurrents which causes microstreaming (Fig. 3). A point is attained where the ultrasonic energy cannot retain the vapour phase in the bubble and leads to rapid condensation. The condensed molecules collide and create shock waves. The shock waves create high temperature and pressure of 5500 °C and 50 Mpa, respectively and finally the bubbles collapse. This is known as quasiadiabatic high-energy process.

There is no interaction of ultrasonic waves directly with the food matrix and energy concentration in the form of bubbles is the fundamental reason behind it. Compression causes positive pressure which forces molecules to come close together and rarefaction exerts negative pressure which separates the molecules. There are two cavitation phenomena, stable and transient cavitation. Stable cavitation occurs at low intensities and chemical effects achieved are temporary. The bubbles oscillate for a certain number of pressure cycles around the equilibrium radius and grow slowly. In transient cavitation, bubbles grow rapidly to become unstable and implode violently during the compression phase. This type of cavitation takes place at high intensities and chemical effects occurred is permanent (Delia et al. 2018).

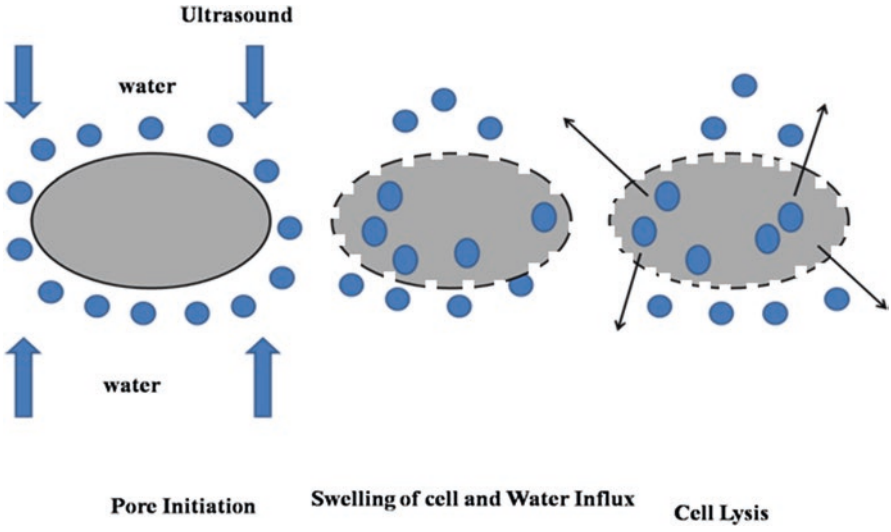

Fig. 3 Cavitation phenomenon

Variables Affecting Ultrasound

Various variables affect the ultrasonic waves and their application in food (Fernández and Muñoz, 2017)

(a) **Frequency:** The low intensity of ultrasound is produced from high frequencies and high intensity ultrasound is produced from lower frequencies.
(b) **Amplitude:** The intensity of ultrasound waves is directly proportional to the amplitude of waves. Increase in the amplitude of waves at source leads to an increase in the intensity of sonication.
(c) **Solvent:** Water is used as a solvent in ultrasound applications because of its polarity. The polarity helps in the implication of process and reduction in energy consumption. It also generates vapor pressure which increases the number of cavities generated and enhances the process of sonication. Also, water has the highest values of surface tension which leads to maximum cavitation intensity.
(d) **Temperature:** High temperatures of solvent improve diffusion of solutes from the matrix to the medium. And at low temperatures cavitation is stabilized

Ultrasound Generation

Ultrasonic wave producing system consists of a transducer and a processor. Transducer is the key component of the ultrasonication device. Transducers convert electrical energy to mechanical energy. The received mechanical signal is than converted back to electrical signal which is then digitized by a ultrasound processor. And the three main types of transducers include fluid-driven, magnetostrictive and piezoelectric transducers (Christensen, 1988).

Ultrasound Application

Ultrasound can be applied to food in three different ways

(a) **Direct application**—Applying directly to the food. This method is involved in the quality analysis of food
(b) **Coupling with the device**—Coupling the food with the device. It is used to induce changes in food.
(c) **Submergence in an ultrasonic bath**—The method involves submerging the food in the ultrasonic bath. It is used for microbial inactivation

Methods of Ultrasound

Ultrasound can be used for the preservation of food in combination with other treatments to improve the inactivation efficacy (Guerrero et al. 2017).

(a) **Ultrasonication** (US) involves application of ultrasound at low temperature. It is suitable for heat sensible products and requires long treatment time (Ajlouni et al. 2006).
(b) **Thermosonication** (TS) is ultrasound combined with heat. When used for sterilization purpose, lower temperatures and processing times are required to achieve the same lethality values as with conventional processes (Ugarte-Romero et al. 2006, 2007).
(c) **Manosonication** (MS) is combination of ultrasound and pressure. It inactivates enzymes and/or microorganisms by combining ultrasound with moderate pressures at low temperatures (Arroyo et al. 2012).
(d) **Manothermosonication** (MTS) is a combination method of heat, ultrasound and pressure. Applied temperature and pressure, bubble implosion in the media which increase the level of inactivation (Caminiti et al. 2012).

Effects of Ultrasound on Physical and Technological Properties

Ultrasound is a technological alternative applied to food processing and preservation. It has various chemical and mechanical effects on the properties of food. Ultrasound improves the qualitative characteristics of food and is also used for the non destructive analysis of food. The effects induced in food are due to cavitation. The interaction of cavitation bubbles and acoustic energy with food occurs at the microstructural level which induces various macroscopic changes in the functional and physiochemical properties of food. It involves better penetration of cavitation bubbles into the food which disrupt the cellular structure and modify the properties of food. Ultrasound is known to enhance the extraction of bioactives, freezing, dehydration, filtration etc. It is also known to facilitate defoaming and degassing, nucleation and crystallization, and stabilize emulsions and homogenization. Also, ultrasound employs its non destructive nature to evaluate the quality of food products. It is also used to enhance the quality and shelf life of food products through microbial and enzymatic inactivation (Purkait et al. 2018). Research studies show novel applications of ultrasound in oxidation of unsaturated oils, aging of alcoholic beverages, hydration of acetylene, decalcification of bone and hydrolysis of esters (Mason, 1999).

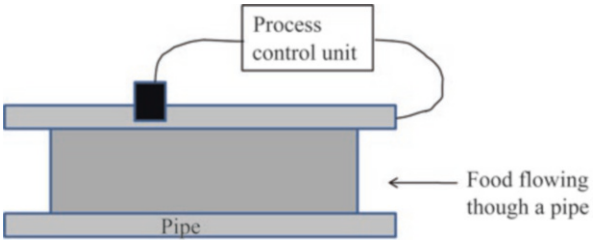

Fig. 4 Online measurement of food flowing through a pipe

Applications of Ultrasound in Food Processing

On-Line Measurements

Ultrasound measurements are rapid and reliable measurements, non-invasive and non-destructive, robust, low cost, easily automated and hygienic. Ultrasonic transducer is set into the wall of a pipe through which the sample flows (Fig. 4). The time taken for a pulse to travel across the sample (t) is measured using a digital timing device, and the ultrasonic velocity (c) is calculated from knowledge of the inside diameter of the pipe (d):

$$c = 2d / t$$

Determination of Composition and Microstructure

Application of ultrasound relies on their being a significant change in the ultrasonic properties of a material as its composition changes. The accuracy depends on how accurately the velocity can be measured. Measurements of the ultrasonic velocity and attenuation as a function of frequency are used to determine the particle size distribution in various food (Awad et al. 2012).

Foreign Body Detection

If an ultrasonic pulse is propagated into a sample it will be reflected from any boundaries it encounters, providing there is a large enough difference in acoustic impedance between the food and the foreign body. For example, the distance of the foreign body from the surface of the can is determined by measuring the time of-flight of ultrasonic pulses reflected from the foreign body and from the can wall.

$$d_2 = d_i.t_2 / t_i$$

(*where* d_2 = *final diameter,* d_i = *initial;* t_2 = *final time,* t_i = *initial time*)

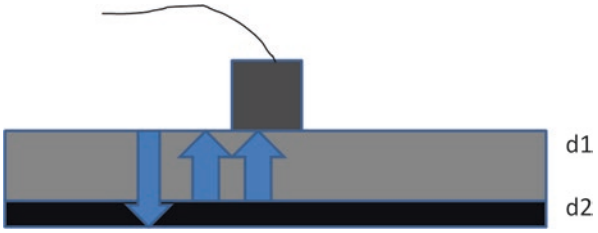

Fig. 5 Thickness measurement using ultrasound

Thickness and Level Detection

An ultrasonic transducer is pressed against the side of a material and the time taken for a pulse to travel across the material and back is measured (Fig. 5).

If the velocity of ultrasound in the material is known then the distance can be calculated:

$$2\mathrm{d} = ct$$

(*where d=distance, c=velocity, t=time*)

Defoaming/Degassing/Deaeration

Common method for degassing/defoaming involves: boiling and reducing pressure. This may however, involve thermal degradation of product. But use of ultrasound treatment involves a small temperature change. Rapid vibration of gas bubbles brings them closer by acoustic waves and bubbles so as to make them grow to a size sufficiently large to allow them to rise up through the liquid against gravity, until they reach the surface. LPU has been used for long to degas carbonated beverages before bottling. Ultrasonically assisted degassing is rapid in aqueous systems, but more difficult in very viscous liquids such as melted chocolate (Chemat et al. 2011; Ojha et al. 2018).

Depolymerization

Depolymerization means the degradation of polymers. There are two possible mechanisms:

(a) Mechanical degradation of the polymer from collapsed cavitation bubble
(b) Chemical degradation as a result of the chemical reaction between the polymer and high energy molecules such as hydroxyl radicals produced from cavitation

Table 1 Effect of ultrasonication on textural properties of fruits and vegetables

Food	Processing conditions	Effect on textural attributes
Apple slices	90 W, 3.5 min	Minor differences in texture
Organic fresh lettuce	40 kHz, 5 min	No significant change occurs in texture
Dried apple slices	24 kHz, 200 W, 50% and 100% amplitude	Ultrasound reduces the time of drying and allows elimination of more water from the apple slices
Edamame	58-W, 0.7 min, 50%, −20 °C	Water-holding power of edamame after thawing is 92%
Dried apples (Fuji)	50/60 Hz, 117 V and 185 W	Ultrasound treatment resulted in lower moisture content, higher glass transition temperature and rehydration rate, and less penetration of calcium ions in dried apples

Sonication (LPU) can lead to several improvements in various properties: increased solubility and foaming ability. However, the application of low-power ultrasound causes a temporary change. Although high powers cause depolymerization and result in a permanent change in their rheology.

Cooking

Ultrasound improves heat transfer characteristics. Table 1 shows the effect of ultrasound on the textural attributes of certain fruits and vegetables. Ultrasound cooking results in greater cooking speed, moisture retention and energy efficiency. It may improve the textural attributes of cooked meat. Muscles processed using US have two to five times less cooking losses than those cooked by boiling and convection (Charoux et al. 2017). Applications of ultrasound to induce physical and chemical changes in meat and meat products has proved as an alternative to chemical or thermal processing. Ultrasound induces membrane cell disruption which leads to meat tenderness by weakening the physical structure of muscle or indirectly, by the activation of proteolysis either by release of cathepsins from lysosomes and/or of Ca++ ions from intracellular stores so that it may activate the calpains (Jayasooriya et al. 2004).

Demoulding and Extrusion

The device for demoulding industrial food products couples with the mould and the ultrasonic source in order to enhance removal of the product by virtue of the high-frequency relative movement between the contact surfaces of the mould and of the

product contained in the latter. This technique allows surface coatings to be eliminated of any residual material in the mould to be cleaned automatically. Ease of removal makes the cleaning process easier.

Cutting

Ultrasonic cutting uses a knife-type blade attached through a shaft to an ultrasonic source. One of the widespread application of ultrasound is the cutting of fragile and heterogeneous products (such as cakes, pastry and bakery products) and fatty (cheeses) or sticky products. The use of vibration prevents the adherence of the product to blade so as to reduce the development of micro-organisms on the surface. The accuracy of the cut produces a reduction in losses relative to the cutting (due to cracks, crumbs, etc.) and a better standardization of the weight and dimensions of portions.

Freezing

Sonication enhances the nucleation rate and rate of crystal growth. It creates a large number of nucleation sites in the medium throughout the ultrasonic exposure. Cavitation bubbles act as nuclei for crystal growth and thus increases the number of nucleation sites. Under the influence of ultrasound, conventional cooling provides much more rapid and even seeding, which leads to a much shorter dwell time, number of seeds is greater, and the final size of the ice crystals gets smaller and so the cell damage is reduced. Table 2 shows the effect of freezing assisted by ultrasound on various fruits and vegetables (Xu et al. 2017).

Table 2 Effects of ultrasound on frozen food quality (Charoux et al. 2017)

Food matrix	Effect
Red radish	The samples treated by ultrasound-assisted freezing show significant reduction in drip loss and phytonutrient (anthocyanin, vitamin C, and phenolics) loss, as well as better textural preservation and higher calcium content
Broccoli	Microstructure and firmness of broccoli tissue get preserved and drip loss also gets significantly reduced by the application of ultrasound
Mushroom	Drip losses reduce by 10% using ultrasound Highest textural hardness values reach with the samples treated by ultrasound Polyphenol oxidase and peroxidase enzyme activities significantly get reduced with increase in ultrasound power
Pear (*Pyrus pyrifolia*)	Improved texture profile of ultrasound pretreated freeze-dried samples compared to control

Table 3 Effects of ultrasound on dehydrated food quality (Rodríguez et al. 2014; Szadzińska et al. 2017)

Food matrix	Main results
Apple	A significant difference between the loss of total polyphenol content, flavonoid content, and antioxidant activity of samples treated and not treated with ultrasound The use of ultrasound promotes notable changes in the microstructure (SEM observation) of apple samples
Green pepper	Total color change between the control and treated samples: (DE) ¼ 11.51 Vitamin C retention: 69% Due to long-lasting hot air drying the dried biomaterial strongly deformes and shrinks

Drying

HPU improves heat and mass transfer phenomena in drying processes. Acoustic dehydration relies on cavitation and also on the effects of compressions and expansions induced by soundwaves passing through the food medium (Table 3). There is a direct increase of the drying effect with the acoustic intensity when the other thermomechanical parameters (temperature, flow rate, suction) are kept constant

Brining, Pickling and Marinating

Most current salt-brining or pickling-fermentation processes are prone to enzymatic changes, structural damage and bloating. The effects are detrimental to preservation. Ultrasound reduces the pickling time of products especially those foods with a crunchy texture. It also allows a low level of sodium chloride compared with the pickles. And, hence there is no need to 'desalt', repack to reach the desired finished product salt level. Also, low power ultrasound is the best suited method for fermentation. Although, only a few reports for use of US in food exist.

Sterilization/Pasteurization

Ultrasound can accelerate the rate of sterilization of foods when used in combination with heat, thus lessening both the duration and intensity of thermal treatment and the resultant damage. The advantages of ultrasound over heat is the minimal flavour loss, homogeneous treatment and significant energy savings.

Emulsification/Homogenization

The process of emulsification requires an energy input by means of mechanical agitation or ultrasonication to facilitate the formation of small droplets. With ultrasonication, the collapse of cavitation releases and forms high energy microjets near interfaces, and facilitates emulsification. Compared to mechanical agitation, the use of ultrasound produced smaller and more stable droplets.

Sonocrystallization

Power ultrasound influences the crystallization of liquids and melts. This is known as sonocrystallization. Sonocrystalliszation increases the nucleation rate due to high-pressure pulses associated with collapsing cavitation bubbles and modifies their polymorphic crystallization. US modifies the microstructure, texture and melting behavior of foods such as chocolate. Also, it induces primary and secondary nucleation, which yields small crystals and increased hardness. This effect could be tuned by controlling sonication time, power, duration of the acoustic pulse and crystallization temperature (Ojha et al. 2018).

Ultrasound Assisted Extraction

A major application of HPU is for facilitating the extraction process of a variety of food components (such as herbal, oil, protein, polysaccharides) as well as bioactive ingredients (such as antioxidants) from plant and animal resources (Table 4). The action of HPU is due to acoustic cavitation, which generates high shear forces and microbubbles that enhances surface erosion, fragmentation and mass transfer resulting in high yield of extracted materials and fast rate of extraction (Lavilla and Bendicho, 2017).

Microbial Inactivation

Thermal treatment of food to deactivate microorganisms (Table 5), often leads to *unwanted flavors and loss of nutrients.* And so, ultrasound deactivation of microbial flora can be an advantage to preserve the characteristics of food (Fig. 6). The phenomenon of acoustic cavitation is involved in the inactivation of microbes. And the factors which affect the effectiveness of microbial inactivation include amplitude of ultrasound waves, exposure or contact time, volume and composition of the food processed and treatment temperature.

Table 4 Effect of ultrasonication on extraction of bioactives

Product	Treatment conditions	Bioactive compounds
Blueberry juice	13, 43, 73 J/mL, 20 kHz	Retention of anthocyanin
Strawberry juice	25 kHz, 0, 15, 30 min	Enhancement of bioactive compounds
Red grape juice	0–135 Hz, 20–40 min, 25–50 °C	Retention of total anthocyanin content
Blackberry juice	Amplitude level 40–100%, 20 kHz,10 min	94% anthocyanin retention
Carrot juice	24 kHz, 120 μm amplitude at 50 °C, 54 °C and 58 °C for 10 min, 2204.40, 2155.72, 2181.68 mW/mL	Retained carotenoids (>98%)
Grapefruit juice	30, 60 and 90 min, 28 kHz, 20 °C	Improved total antioxidant activity
Cantaloupe melon juice	376 W/cm^2 10 min	30% reduction in phenolic compounds
Red grape juice	Amplitude level 24–61 μm, 20 kHz, 0–10 10 min	Retention of anthocyanin content, cyanidin (97.5%), malvanidin (48%), delphenidin (81%)
Strawberry juice	Amplitude level 60, 90,120 μm, 20 kHz, 3, 6, 9 min, 25, 40, 55 °C	Less degradation of anthocyanins (0.7–44%)
Pineapple juice	376 W/cm^2 and 10 min	30% increase in phenolic compounds
Cranberry juice	Amplitude level 60, 90,120 μm, 20 kHz, 3, 6, 9 min, 20, 40, 60 °C	More percentage of aromatic compounds
Prebiotic cranberry juice	600 and 1200 W/L + high pressure (450 mPa, 5 min)	Increased anthocyanin content (24%)
Pear juice	750 W, 20 kHz, 10 min, 25, 45, 65 °C	Increased retention of phenolic compounds
Blueberry juice	13, 43, 73 J/mL, 20 kHz	Retention of anthocyanins

Thermosonic, manosonic and manothermosonic methods are found to be the best treatments to inactivate microbes, as they are more efficient and effective in killing microorganisms. Ultrasound minimizes the flavor loss, produces homogeneity and is energy efficient than heat pasteurization (Piyasena et al. 2003). However the bactericidal effectiveness of ultrasound depends on the type of micro organism tested, amplitude of the ultrasonic waves, exposure time, volume of food being processed, the composition of food and the treatment temperature (Davies, 1959).

Spore Inactivation

Ultrasound alone is ineffective against spores. However, combining the effect of ultrasound and pressure can result in better spore inactivation. A treatment at 500 kPa for 12 min can inactivate over 99% of the spores. Increasing the amplitude

Table 5 Effect of ultrasonication on inactivation of microorganisms (Bansal et al. 2018)

Microorganism	Medium	Treatment conditions	D value (min)/ log reduction
Escherichia coli	Orange juice, apple juice	20 kHz, 37.5 μm, 30 °C, 15 min	Total inactivation
Staphylococcus aureus	Orange juice	30 kHz, 30 min, 50 °C	5.5
Escherichia coli O157: H7	Spinach leaves	21.2 kHz, 2 min	2.2
Pichia fermentans	Tomato juice	20 kHz, 61.0 μm, 10 min, 40 °C	5
Cronobacter sakazakii	Apple juice	20 kHz, 64 °C, 117 μm, 200 kPa	2.7
Saccharomyces cerevisiae	Pipeapple, grape and cranberry juice	24 kHz, 117 mm, 6 min, 60 °C	5
Escherichia coli	Apple juice	24 kHz, 100 mm, 2.9 min, 50 °C	4.9
Saccharomyces cerevisiae	Saline solution	20 kHz, 35 °C, 124 μm, 5 min	3.1
Escherichia coli O157:H7, Salmonella enteritidis	Mango juice	25 kHz, 60 °C, 7 min	5
Enterobacter aerogenes, Bacillus subtilis, Staphylococcus epidermidis	Suspension	850 kHz, 20 °C, 20 min	4.2, 2.5, 4.4
Bacteria, yeast and mold count	Strawberry	33 kHz, 25 °C, 60 min	2, 1.22
Alicyclobacillus acidoterrestris spores, Saccharomyces cerevisia	Apple juice (commercial and natural)	20 kHz, 95.2 μm, 30 min, 44 °C	3, 6.4

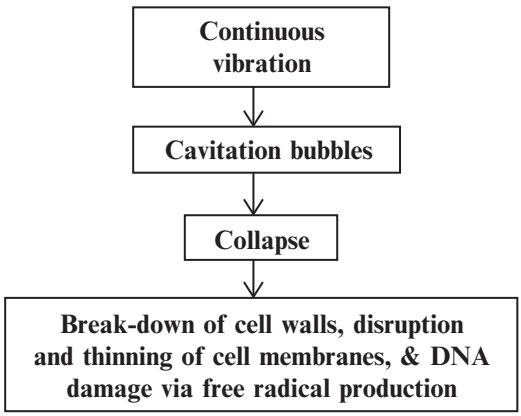

Fig. 6 Microbial inactivation using ultrasound

of ultrasonic vibration i.e., the acoustic power also increases the level of inactivation. Finally, increasing the thermal temperature of the treatment results in greater rates of inactivation compared to thermal treatment alone (Berger and Marr, 1960).

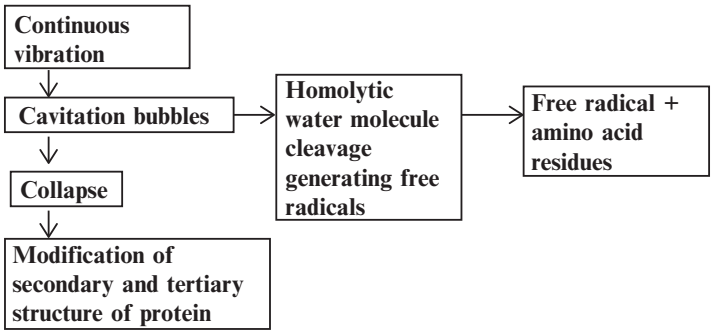

Fig. 7 Enzyme inactivation using ultrasound

Recently, a hurdle approach involving ultrasound was used to inactivate *B. cereus* in potatoes (Luo et al. 2016).

Enzyme Inactivation

Heat treatment is a commonly used method to eliminate enzymes which side by side destroys various nutrients and causes loss of food quality (Fig. 7). Recently, ultrasound emerged as a technique for effective inactivation of enzymes (glucose oxidase, peroxidase, pectin methyl esterase, protease and lipase and poly-phenoloxidase) without the application of (Table 6) temperature and pressure (Noci, 2017).

Surface Decontamination

Ultrasound is also used in the decontamination of various objects used in a food industry. Objects that can be cleaned include large crates, plastic baskets, shackles and conveyer belts. The cleaning process involves acoustic cavitation phenomenon, the formation, growth and implosive collapse of cavities (gas bubbles) in liquids that release high amounts of highly localized energy. And their implosion cleanses surfaces from contaminants.

Figures of Merit

(a) Ultrasound is inexpensive to purchase and operate.
(b) Ultrasound involves rapid and reliable measurements, in a non-destructive and noninvasive manner.
(c) Measurements can easily be automated.

Table 6 Effect of ultrasonication on enzyme inactivation (Bansal et al. 2018)

Product	Treatment conditions	Enzyme inactivation
Tomato	0.3 min at 72 °C, cavitation intensity 0.008 mg/L/min	Pectinmethyl esterase (89%)
Orange juice	1.05 W/mL, 10 min	Pectinmethyl esterase (62%)
Pear juice	750 W, 20 kHz, 10 min, 25, 45, 65 °C	Complete inactivation of peroxidase, pectinmethyl esterase, polyphenol oxidase
Lemon juice	80 °C, 220 s	Endogenous enzymes (>90%)
Pineapple juice	376 W/cm^2 and 10 min	Phenoloxidase (20%)
Citrus juice (lemon and strawberry)	Manothermosonication (70 °C, 300 kPa, 2 min)	Pectin esterase (94%)
Citrus juice (lemon and strawberry)	Manothermosonication (80 °C, 200 kPa, 5 min)	Pectin esterase (96%)
Tomato	Manothermosonication (62.5 °C, 200 kPa)	Pectic enzymes (90%)
Tomato juice	24 kHz, 60–70 °C	Pectinmethyl esterase and Polygalacturonase
Tomato juice	Manothermosonication (20 kHz, 2 kg pressure, 117 lm amplitude and 70 °C	Pectic enzymes (undetectable)
Tomato juice	Manothermosonication (20 kHz, 2 kg pressure, 117 lm amplitude and 70 °C	Polygalacturonase (62%)
Purple cactus pear juice	Amplitude level 40% and 60% for 10, 15, 25 min; 80%	–
Purple cactus pear juice	Amplitude level 80% for 3,5,8,10, 15, 25 min	–
Blackberry juice	Amplitude level 40–100%, 20 kHz,10 min	–
Orange juice	Amplitude level 40%, 70%, 100%, 20 kHz, 2,6, 10 min	–
Orange juice (calcium added)	Amplitude level 89.2 μm, 20 kHz, 2,4, 6, 8, 10 min	–
Strawberry juice	25 kHz, 0, 15, 30 min	
Red grape juice	0–135 Hz, 20–40 min, 25–50 °C	
Carrot juice	24 kHz, 120 μm amplitude at 50 °C, 54 °C, and 58 °C for 10 min, 2204.40, 2155.72, 2181.68 mW/mL	
Grapefruit juice	30, 60, and 90 min, 28 kHz, 20 °C	
Cantaloupe melon juice	376 W/cm^2 10 min	

Conclusion

Ultrasound is a form of green technology that has diversified applications in food analysis, processing, and quality control. Low power ultrasound is a non-invasive technique that estimates the composition of food whereas high power ultrasound induces mechanical, physical and chemical/ biochemical changes in food.

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A Natural Way of Food Preservation: Bacteriocins and Their Applications

Halil İbrahim Kaya, Burcu Özel, and Ömer Şimşek

Abstract Since the consumers demand foods produced without additives, new friendly preservation strategies become significant in processing of foods. Bacteriocins are ribosomally synthesized peptides produced from many bacterial strains which are approved as natural due to being degraded by digestive enzymes. In Lactic acid bacteria (LAB), many strains have been identified as bacteriocin producers. In fact, nisin was approved by Food and Drug Administration (FDA) to be used as food additive in some foods. Lacticin and pediocin producers, *Lactococcus lactis* and *Pediococcus acidilactici*, respectively, have been used as protective cultures in food system. Bacteriocins produced by some LAB have shown wide antimicrobial activity against food related pathogens species such as *Bacillus, Listeria, Staphylococcus* and *Clostridium*. However, in recent years bacteriocins having specifically narrow-spectrum antimicrobial activity have been introduced.

Bacteriocins are used either directly in food systems or by the addition of producer strains. In this way, it has been possible to prevent pathogenic microorganisms in various fermented food products. However, the effectiveness of the LAB bacteriocins may reduce due to their adsorption on to the hydrophobic surfaces and degradation with proteases. Therefore, the combinational usage of bacteriocins with other preservation methods, such as high hydrostatic pressure, pulse electrical field or essential oils, were reported successful at inhibiting pathogens including the Gram negatives.

In the first part of the chapter, the general introduction to bacteriocins and new generation bacteriocins are discussed. In the second part, the applications of bacteriocins in different food systems have been explained and the combinational usage of bacteriocins together with different preservation methods have been exemplified.

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Introduction

All living organisms produce antimicrobial proteins, many of which are referred as antimicrobial peptides due to their small molecular masses (Chikindas et al. 2018). Bacteriocins are ribosomally synthesized antimicrobial metabolites with a peptide or protein structure that are extracellularly secreted into the environment, and that usually inhibit closely related strains (Delves-Broughton et al. 1996; Zou et al. 2018). Gram-positive (Gm+) and Gram-negative (Gm−) bacteria, and even archaea, have been found to produce bacteriocins (Juturu and Wu 2018). Bacteriocins act to inhibit or stop the growth of prokaryotic microorganisms, and are also effective against pathogenic and antibiotic-resistant bacteria (Zou et al. 2018). Bacteriocins have been reported to be effective against human and animal pathogens, including methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci (VRE), and to show no toxicity (Ahmad et al. 2017; Zou et al. 2018). Bacteriocins also play a role in the formation of useful bacterial populations (Balciunas et al. 2013). The first identified bacteriocin was colicin, which is an antimicrobial protein produced by *E. coli* that was found at 1925 by Gratia (Balciunas et al. 2013; Cavera et al. 2015; Juturu and Wu 2018). The most known antimicrobial metabolites of lactic acid bacteria (LAB) are also bacteriocins. The bacteriocin produced by *Lactococcus lactis* subsp. *lactis*, identified in 1933 by Whitehead, was named nisin (Juturu and Wu 2018). The bacteriocins produced by LAB have found wide use, especially in the food industry regarding as being starter culture in fermented foods which have been generally recognized as safe (GRAS) and have a qualified presumption of safety (QPS). The second reason for their use in this area is the bactericidal effects of LAB bacteriocins against many deterioration and pathogenic bacteria in the nanomolar range. Finally, they do not affect the sensory qualities of food (Alvarez-Sieiro et al. 2016; Field et al. 2018). In 1988, nisin was approved by the U.S. Food and Drug Administration for use in cream cheese (Juturu and Wu 2018; Zou et al. 2018). Nisin—produced by *Lactococcus lactis*—is used commercially in many countries, including the United States and Canada, while micocin®—a combination of pediocin, produced by *Pediococcus acidilactici*, and three other bacteriocins (carnocyclin A, carnobacteriocin BM1 and piscicolin 126) that are produced by *Carnobacterium maltaromaticum* UAL307—is also used commercially in the United States and Canada (Zou et al. 2018). All of these bacteriocins produced by bacteria are used for food preservation purposes, either through direct use of the producing strains, or through the addition of partially purified forms (O'Shea et al. 2013). In addition, commercial preparations containing bacteriocin-producing LAB are used as biopreservative starter cultures.

The first section presents fundamental characteristics of bacteriocins and address the new-generation bacteriocins and their classification. The mode of action of bacteriocins is elucidated; their use in food systems are discussed, and examples of their use together with other preservation methods are presented.

Classification of Bacteriocins

Bacteriocins are classified based on the producing microorganism, their amino acid composition, the presence of post-translational modifications, molecular mass, heat stability, mode of action and antimicrobial spectrum (Juturu and Wu 2018; Zou et al. 2018). Bacteriocins were first classified by Klaenhammer (1993), who identified the bacteriocins produced by LAB. This classification was later modified and updated by Cotter et al. (2005) and Heng and Tagg (2006). Alvarez-Sieiro et al. (2016) produced a revised classification of bacteriocins, and most recently, Varella Coelho et al. (Varella Coelho et al. 2017) classified bacteriocins into five different groups based on their molecular structures and molecular masses (Zou et al. 2018; Vasilchenko and Valyshev 2018).

Bacteriocins of Gram (+) Bacteria

Gram-positive bacteriocins are divided into three main groups based on their biosynthesis mechanisms and biological activity (Alvarez-Sieiro et al. 2016). The first group contains ribosomally synthesized and post-translationally modified peptides (<10 kDa), the second group contains non-modified bacteriocins (<10 kDa), and the third group contains non-modified bacteriocins with molecular masses greater than 10 kDa.

Group I

The first group consists of bacteriocins that are synthesized as a result of enzymatic modifications, and contains unusual amino acids, glycosylated units and cyclical peptides (Vasilchenko and Valyshev 2018).

Group Ia or lanthipeptides contain different usual amino acids, lanthionine (Lan) and beta methyl lanthionine (MeLan) derivatives in their structures (Arnison et al. 2013). Lantipeptides are modified post-translationally, and so come in four different types. However, of these different types, only Type I and Type II are referred to as lantibiotics (Cotter et al. 2005; Wiley and van der Donk 2007; Heng et al. 2007). Type I lantibiotics have a net positive charge and a hydrophobic polypeptide structure, and display antimicrobial activity by creating pores on the membranes of target cells. Type I lantibiotics are further divided into the Ia and IIa groups. This division is based on the modification enzymes and differences in the biosynthetic pathway (Pag and Sahl 2002). Type II lantibiotics, on the other hand, are not charged or have a negative charge. They have a globular peptide structure, and display antimicrobial activity by inhibiting specific enzymes (Twomey et al. 2002). LAB produce many different types of lantibiotics. One example of a Type I lantibiotic is nisin, which has been studied extensively and is used as a preservative in many countries (Nes et al.

2007), while two nisin variants (nisin A and nisin Z) are produced by the bacterium *Lactococcus lactis*, and another variant, called nisin U, which is 78% similar to nisin A, is produced by *Streptococcus uberis* (Wescombe et al. 2006). The most extensively studied Type II lantibiotic is lacticin 3247, which is produced by *Lactococcus lactis*. Many lantibiotic mode of action target the Lipid II molecule in the cell wall. Other examples of this group are lacticin 481, mutacin A and duramycin (Oscariz and Pisabarro 2001; Siegers et al. 1996).

In the Group Ib bacteriocins, which are circular from head to tail, the N-terminal and C-terminal ends are linked with a peptide bond, giving them a circular molecular structure. All members consist of alpha helix sections. Similar to other bacteriocins, these bacteriocins are also ribosomally synthesized, and they are clearly separated from the circular antimicrobial peptides that are enzymatically synthesized, such as gramicidin S and mycosubtilin (Mogi and Kita 2009). NMR and X-ray studies have shown that carnocyclin A and enterocin AS-48—produced by *Enterococcus faecalis*—are belong to this group (Kawulka et al. 2003; Martin-Visscher et al. 2009; Jimenez et al. 2005; Van Belkum et al. 2011). These peptides are usually resistant to heat and proteolytic enzymes, and display antilisterial activity. Of the circular bacteriocins identified to date, six are produced by LAB (gassericin A, reutericin 6, enterocin AS-48, enterocin 4, carnocyclin A, lactocyclicin Q), and the other two are circularin A, produced by *Clostridium beijerinckii*, and butyrivibriocin AR10, produced by *Butryrivibrio fibrisolvens* (Cotter et al. 2005; Rea et al. 2011; Martin-Visscher et al. 2009). Despite their similar structure, circular bacteriocins have two different mode of action: carnocyclin A creates pores on bacterial membranes, depending on voltage, while enterocin AS-48 creates dimers on bacterial membranes, modifying their structure.

Group Ic, known as sactibiotics, are peptides containing alpha carbon linked sulphur. Subtilosin A and thuricin CD and H are examples of this group. Although subtilosin A is a cyclic bacteriocin with a negative charge produced by *Bacillus subtilis*, it is included in this group due to its post-translational modification (Kawulka et al. 2003, Martin-Visscher et al. 2009). Thuricin CD is produced by *Bacillus thuringiensis* 6431 with two-peptide containing alpha-carbon cross-links between cysteines, and has an antimicrobial effect against *Clostridium difficile* (Rea et al. 2011). But thuricin H is produced by *Bacillus thuringiensis* with a single-peptide having 4S-alpha carbon linkages. Subtilosin A shows inhibitory effect by targeting the cell membrane and forming temporary pores, whereas thuricin H has no effect on cell membrane permeability.

Group Id consists of linear azole-containing peptides (LAPs) including thiazole and methyloxazole heterocyclic circular structures with cysteine, serine and threonine derivatives, generated by ATP-dependent cyclodehydration and then by flavin mononucleotide-dependent dehydrogenation. Streptolysin S, produced by *Streptococcus pyogenes*, is one of the example for this group (Molloy et al. 2015).

Group Ie are known as glycocins and contain glycolized residues. Glycocin F—produced by *Lactobacillus plantarum*—is the first defined glycocin produced by LAB. Glycocin F consists of two alpha helices connected via disulfide bonds (Acedo et al. 2018; Amso et al. 2018).

Group If are lasso peptides containing negatively charged residues that surround the C-terminal linear section of the polypeptide and the amide bond in the first amino acid of their core peptide chains. Lasso peptides display antimicrobial, antiviral and anti-cancer activity. Any lasso peptide that is produced by LAB have not been reported to date (Acedo et al. 2018; Mesa-Pereira et al. 2018).

Group II

This group consists of bacteriocins without modifications and with molecular masses below 10 kDa (Alvarez-Sieiro et al. 2016). Group II bacteriocins are small, heat-resistant, ribosomally synthesized antimicrobial peptides. As they do not undergo post-translational modification and as they contain only the genes required bacteriocin, immunity and transport, group II bacteriocins have a simpler structure than lantibiotics. These bacteriocins are divided into four sub-groups. Group IIa consists of strong antilisterial bacteriocins like pediocin, Group IIb consists of two-peptide bacteriocins, Group IIc consists of bacteriocins without precursor groups, and Group IId consists of other linear bacteriocins that are dissimilar to pediocin (Rea et al. 2011; Alvarez-Sieiro et al. 2016).

Group IIa bacteriocins—the largest of the Group II sub-groups—are called pediocin-like bacteriocins which inhibit mainly *Listeria* (Ennahar et al. 1999). These are smaller than 10 kDa, and resistant to high temperatures and extreme pH values. To date almost 30 pediocin-like bacteriocins have been reported to be produced from different strains of LAB (Nissen-Meyer et al. 2009). Among the LAB strains *Pediococcus* have been found the most capable of producing pediocin-like bacteriocins. Within this genus, the species that are reported to produce pediocin mostly are *P. acidilactici*, *P. pentosaceus* and *P. damnosus.* Pediocin PA-1, enterocin A, leucocin A-UAL 187, mesentericin Y105, sakacin P and curvacin are the bacteriocins well characterized and identified (Drider et al. 2006; Hastings et al. 1991; Hechard et al. 1992; Henderson et al. 1992; Nieto-Lozano et al. 2010; Tichaczek et al. 1992; Aymerich et al. 1996). The members of this group have similar amino acid sequence homologies, and differ from other groups in that they contain an intact amino terminal sequence and a cysteine disulphide bridge at the amino terminal end. The effect mechanism of Group II bacteriocins consists of three main steps (Alvarez-Sieiro et al. 2016). Pediocin attaches to the receptors of the sugar-carrying mannose phosphotransferase system (Man-PTS), passes through the cytoplasmic membrane and finally forms the pore complex. The potential effect mechanism of pediocin-like bacteriocins, which are known for their antibacterial qualities and show bactericidal effect against Gram-positive bacteria, is via an inhibition effect that targets the cell membrane and requires a specific attachment region. The amino acid sequence YGNGV of Group IIa bacteriocins attaches to a membrane receptor that recognizes only this sequence (Papagianni 2003). The results of the mutants and analogues of Group IIa bacteriocins have shown that the entire sequence and the three-dimensional structure play a very important role for interaction. The three-dimensional structure is critical especially for bacteriocins that act by attaching to

specific regions of the cell membrane, as it enables bacteriocin molecules to pass through the wall. Once they pass through the cell wall and reach up to contact with the cytoplasmic membrane, bacteriocin molecules disrupt the function of the cytoplasmic membrane. As a result, membrane permeability changes, the membrane transport mechanism and the proton-motive force (PMF) are ceased leading to cell lysis. Consequently, energy generation and biosynthesis of such basic molecules as proteins and nucleic acids are stopped at cell level (Hill et al. 2011). Pediocins usually have a narrow antimicrobial spectrum, and are known in particular to be highly inhibitive against *Listeria*. However, these bacteriocins have been found to have antimicrobial effects of various degrees also on other Gram-positive bacteria. Spectrum studies have shown that besides *Listeria*, pediocins have moderate or low antimicrobial effects against bacteria such as *C. sporogones*, *C. thiaminolyticum*, *B. cereus*, *E. faecalis* and *S. carnosus* (Anastasiadou et al. 2008; Drider et al. 2006).

Group IIb consists of two-peptide bacteriocins, which have three basic characteristics. They have a stronger antimicrobial activity with two peptides, however have able to with single peptide (Alvarez-Sieiro et al. 2016). They contain an immunity protein. In addition, two consecutive bacteriocin structural genes are found together following the immunity gene in their genetic organisation (Nes et al. 2007). To date, 16 two-peptide non-modified bacteriocins have been identified (Nissen-Meyer et al. 2009). The three-dimension structure studies demonstrated that two-peptide bacteriocins have been found to have functions that resemble those of the single-peptide bacteriocins in Groups IIa and IId (Rea et al. 2011). Lactococcin G—produced by *Lactococcus lactis*—requires both peptides to act whereas thermophilin 13—produced by *Streptococcus thermopilus*—can act with only one peptide although its activity increases with two peptides. Lactococcin G acts by attaching to the receptors on the membranes of sensitive bacteria, with its helix-helix structure penetrating the membrane and resulting leakage (Alvarez-Sieiro et al. 2016).

Group IIc consists of bacteriocins produced without leader peptides. The N-terminal, which acts as the region of recognition in the secretion and modification of the bacteriocin and keeps it inactive within the producing cell, is synthesized without a leader peptide. Enterocin L50 is a well-studied and well-characterized two-peptide, plasmid-coded bacteriocin with leaderless peptide (Perez et al. 2018; Towle and Vederas 2017).

Group IId contains different bacteriocins non-modified, linear and dissimilar to pediocin (Cotter et al. 2005; Nissen-Meyer et al. 2009). These bacteriocins contain a large variety of antimicrobial peptides that are isolated from various sources. Lactococcin A was the first bacteriocin isolated in this group and unlike other bacteriocins, it has no homologous sequence (Nissen-Meyer et al. 2009). Bacteriocins produced by *Propionibacterium* species are also classified in this group (Heng et al. 2007). Lactococcin 972 is a heat-resistant bacteriocin with pH stability attaching to the Lipid II molecule on the cell membrane. Lactococcin A, on the other hand, acts by targeting the cell membrane and attaching to the receptors of the Man-PTS system (Martínez et al. 2000, 2008; Daba et al. 2018).

Group III

This group contains non-modified bacteriocins with molecular masses exceeding 10 kDa, and effect mechanisms that may or may not be bacteriolytic. Group III bacteriocins are antimicrobial peptides called bacteriolysins, which are not heat resistant and have large molecular masses. The bacteriocins in this group have different domains. Helveticin J—produced by *Lactobacillus helveticus*, zoocin A—produced by *Streptococcus zooepidermicus*, enterolysin A—produced by *Enterococcus faecalis*, millericin B—produced by *Streptococcus milleri*, dysgalacticin—produced by *Streptococcus dysgalactiae* subsp. *equisimilis* W2580, and linocin M18—produced by *Brevibacterium linens* are included in this group (Joerger and Klaenhammer 1986; Valdes-Stauber and Scherer 1994; Simmonds et al. 1997; Beukes et al. 2000). Among the bacteriolytic bacteriocins, enterolysin A and zoocin A contain an endopeptidase domain in their N terminals and a substrate recognition domain in their C terminals (Alvarez-Sieiro et al. 2016; Balciunas et al. 2013). They display antimicrobial activity by attaching to the peptidoglycan layer of the target cell's wall acting without causing cell lysis but show bacteriostatic effect. Dysgalacticin in this group acts by attaching to the glucose and/or Man-PTS system, preventing sugar intake and causing small molecules inside the cell to leak through the membrane (Ahmad et al. 2017; Alvarez-Sieiro et al. 2016). Caseicin, produced by *Lactobacillus casei*, is also in this group, and shows its effect via a different mechanism, by inhibiting the protein and DNA biosynthesis mechanisms (Alvarez-Sieiro et al. 2016). The protein lysostaphin, which is not produced by LAB, is also included in this group (Bastos et al. 2010; Rea et al. 2011).

In the light of this information, the main characteristics of bacteriocins synthesized by LAB can be briefly stated as follows:

1. Their inhibitory spectrum is specific to the target microorganism.
2. The inhibitory effect is related to cell-specific receptors.
3. The genetic determinant of bacteriocin production and the bacteriocin immunity of the producing cell usually originates in the plasmid DNA. It has also been reported that in some species, bacteriocin production originates in the chromosomal DNA (Barefoot and Klaenhammer 1983; Juven et al. 1991; Hastings et al. 1991).

Bacteriocins of Gram (−) Bacteria

Bacteriocins produced by Gram-negative bacteria are different to those produced by Gram-positive bacteria based on their structures and antimicrobial effect mechanisms. The first bacteriocin identified was at *Esherichia coli*, a Gram-negative bacterium. The bacteriocins of Gram-negative bacteria are divided into two main groups as microcin and colicin produced by *Escherichia coli* (Vasilchenko and Valyshev 2018). Gram-negative bacteria other than *E. coli* have also been found to

produce bacteriocins similar to microcin and colicin. Among these, pyocins and lumicins are colicin-like bacteriocins produced by *Pseudomonas* species and *Photorhabdus luminescens*. Pesticins are colicin-like bacteriocins produced by *Yersinia pestis*; klebicins are colicin-like bacteriocins produced by *Klebsiella pneumoniae*; and microcin E492 is a microcin-like bacteriocin also produced by *Klebsiella pneumoniae* (Rea et al. 2011).

Colicins

Colicins are the most extensively studied Gram-negative bacteriocins. The first bacteriocin—identified by Gratia in 1925—was colicin V produced by *Esherichia coli*. Colicins usually have large molecular masses, varying between 30 and 80 kDa. In terms of their structural and functional characteristics, colicins have three domains. The receptor attachment domain plays a role in interacting with receptors on the external membrane; the N terminal domain plays a role in interacting with carrier proteins; and the C terminal cytotoxic domain plays a role in creating the antimicrobial effect (Yang et al. 2014; Vasilchenko and Valyshev 2018; Rea et al. 2011). Colicins are usually coded by plasmids, whereas some are coded by chromosomal DNA. Toxin protein, immunity protein and lysis protein are coded by the colicin gene set (Rea et al. 2011; Yang et al. 2014). Colicins act on the target cells via three different mechanism: first forming channels on the membrane of the target cell, depending on voltage; second acting as a nuclease in the cytoplasm; or third damaging the peptidoglycan layer (Rea et al. 2011). Colicins first require the recognition to be able to inhibitory by using two different proteins called as Ton and Tol. These proteins are intact at the internal membrane and supply energy from the proton motive force to the receptors. Colicins are classified into two groups, based on the membrane systems they need as recognition regions. Group A requires Tol protein systems to penetrate the cell membrane, whereas Group B requires the Ton protein system as the recognition region to be able to act on the target cell's membrane. Colicins A, E1-E9, K, N, U and S4 are classified Group A, whereas Group B harbours colicins B, D, Ia, M, 5 and 10. Apart from these colicins, colicin-like bacteriocins are produced also by species other than *E. coli*. Cloacin DF13, klebicin, marcescin 28b and alvecin are included in Group A, whereas pesticin and pyocin S1–5 are included in Group B (Brown et al. 2012; Behrens et al. 2017).

Microcins

Microcins are bacteriocins that have smaller molecular masses than colicins. Their molecular mass can vary between 1 to 10 kDa, and they are resistant to proteases, extreme temperatures and pH values. Similar to colicins, they can be plasmid or chromosomal coded. Microcins are synthesized as precursor peptides, after which some modified post-translationally to be activated. They have many bactericidal mechanisms by creating pores or acting as a nuclease. Microcins are classified into

two groups based on their molecular masses and the presence of post-translational modifications. Group I contains peptides that have molecular masses of less than 5 kDa and modified post-translationally. These peptides are the microcins B17, C7-C51, D93 and J25. Group II are peptides with larger molecular masses compared to Group I, varying between 5–10 kDa. Group II is further divided into two sub-groups, IIa and IIb. Group IIa consists of peptides that are encoded on the plasmids, non-modified post-translationally and with disulfide bonds, whereas Group IIb consists of linear peptides with chromosomal coded, having C-terminal siderophores and modified post-translationally. Microcins L, V and N are included in Group IIa, whereas microcins E412, M and H47 are in Group IIb. Group I microcins inhibit vital bacterial enzymes. They target enzymes such as DNA gyrase, RNA polymerase and aspartyl tRNA synthase. Microcin J25, in addition to inhibiting the RNA polymerase, targets mitochondria and the respiratory system. Group II microcins target the internal membrane or membrane components. Microcin H47 targets the ATP synthase enzyme, whereas microcin E492 forms channels on the internal membrane and targets the mannose permease system (Yang et al. 2014; Vasilchenko and Valyshev 2018; Rea et al. 2011).

Mode of Action of Bacteriocins

Different bacteriocins produced by a great diversity of producers display their antimicrobial effects against Gram-positive and Gram-negative bacteria in many different mode of action. The main mode of action of bacteriocins are interruption of the transcription, translation, replication and cell wall biosynthesis which are vital for living cells (Oscariz and Pisabarro 2001). These differences in the effect mechanisms of bacteriocins result from their different chemical structures (Ahmad et al. 2017).

The Mode of Action of the Gram-Positive Bacteriocins

Bacteriocins usually inhibit closely related strains, and those that have this type of bioactivity are called to have a narrow spectrum. In recent years, many bacteriocins have been found to be active against various bacteria of multiple genera, and have been included among the wide spectrum bacteriocins. In terms of their mode of action, Gram-positive bacteriocins can be divided into three groups, as inhibitors of cell wall synthesis, destroyers of the cell membrane structure and inhibitors of septum formation (Fig. 1) (Cavera et al. 2015).

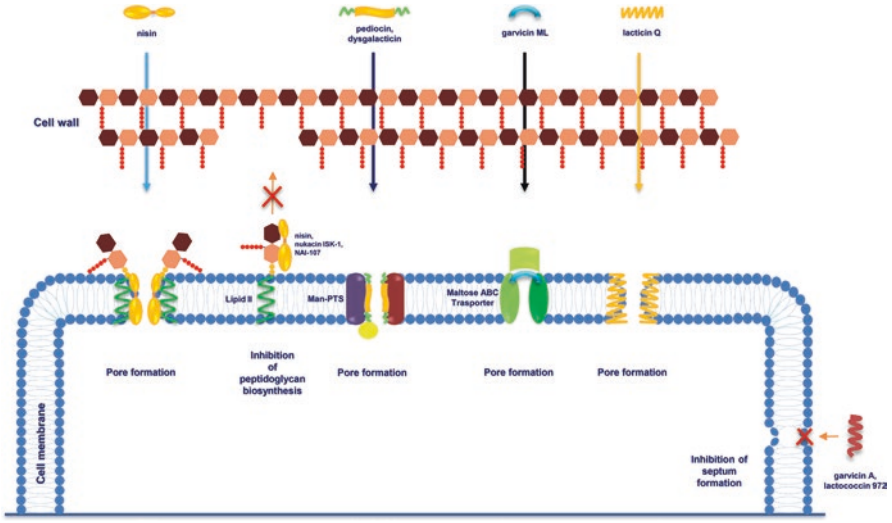

Fig. 1 Antimicrobial mechanism of action of bacteriocins produced by Gram positive bacteria

Bacteriocins that Inhibit Cell Wall Synthesis

The cell wall is a cellular component that is very important for the survival of any bacteria. Cell walls have vital functions, such as maintaining cellular integrity, creating cell morphology and regulating the osmotic pressure in the cell meaning that preventing cell wall biosynthesis is one of critical target for inhibition (Cavera et al. 2015).

Nisin A—produced by *Lactococcus lactis*—is a lantibiotic that targets cell wall biosynthesis. It inhibits the cell wall biosynthesis by attaching to the Lipid II molecule that carries peptidoglycan sub-units from the cytoplasm to the cell wall (Perez et al. 2015). Nisin attaches to phosphate groups in the Lipid II molecule (Guilhelmelli et al. 2013). Then, the nisin-Lipid II complex creates pores on the membrane of the bacterial cell in higher concentrations. Similarly, nukacin ISK-1—produced by *Staphylococcus warneri*—inhibits cell wall biosynthesis by attaching to the Lipid II molecule. Different concentrations of nukacin were reported to not create any pores on the cell membrane. This bacteriocin is reported to be effective against methicillin-resistant *Staphylococcus aureus* biofilms. The A-ring was found to be responsible for the attachment of the bacteriocin to the Lipid II molecule (Cavera et al. 2015). The bacteriocin NAI-107—a lantibiotic produced by the *Microbispora* sp. ATCC-PTA-5024 bacterium—has been found to inhibit the development of vancomycin-resistant enterococci and methicillin-resistant staphylococci by attaching to the Lipid II molecule on the cell membrane (Münch et al. 2014; Thomsen et al. 2016). Lacticin 481, on the other hand, which is a lantibiotic produced by *Lactococcus lactis*, inhibit the formation of peptidoglycan which is catalyzed by the

penicillin binding protein, even though it has a Lipid II attachment pattern (Dimov et al. 2005; Silva et al. 2018).

Bacteriocins that Destroy the Integrity of the Bacterial Membrane

Bacteriocins have two basic mechanisms affecting the membrane of the target cells: The first is inhibition through the destruction of the structure of the cell membrane due solely to the mass effect, without requiring a specific attachment point. This effect mechanism works when there is a high concentration of bacteriocins. The other effect mechanism that makes bacteriocins important is inhibition requiring a specific attachment point on the target cells, and is seen particularly in antilisterial bacteriocins.

Many bacteriocins that target membrane integrity use the Lipid II molecule as their attachment region, and the best known of these bacteriocins is nisin. After attaching to this molecule on the cell surface, nisin translocates to the cytoplasmic membrane. As a result of this antimicrobial effect by bacteriocins, the proton motive force disintegrates and the concentration of cellular electrolytes declines due to the loss of various ions. Amino acids, potassium ions and ATP leak from within the cell to the extracellular environment through the pores (Jack et al. 1995; Cuesta et al. 2000; Sullivan and Nord 2005; Guilhelmelli et al. 2013). Many bacteriocins other than nisin are included in this class, such as plantaricin, epidermin and geobacillin.

Bac-GM17 is a bacteriocin produced by *Bacillus clausii* GM17, having pH (pH 3–9) and heat stability. It displays bactericidal activity against many Gram-positive and Gram-negative bacteria and yeasts. Bacteriocin Pep5—produced by the *Staphylococcus epidermidis* 5 bacterium, and bacteriocin epidermin—produced by *Staphylococcus epidermidis* Tu3298, inhibit the development of *Staphylococcus epidermidis* in silicone catheters. Geobacillin I, produced by *Geobacillus thermodenitrificans* NG80-2, is a bacteriocin that is structurally and functionally similar to nisin. Similarly, plantaricins E, F J and K—as four different bacteriocins produced by *Lactobacillus plantarum*—display anti-Candida activity by attaching to the Lipid II molecule (Cavera et al. 2015).

Some bacteriocins are able to display different mode of action by destroying membrane integrity other than by attaching to the Lipid II. Dysgalacticin—produced by the *Streptococcus dysgalatiae* subsp. *equimilis* W2580—attaches to the man-PTS system and/or the glucose molecule on the membrane. By attaching to the man-PTS system, dysgalacticin destroys the cytoplasmic membrane structure and causes potassium ions to leak out, as a result of which the membrane loses functionality. Lactococcin A and B, pediocin and pediocin-like bacteriocins also act by attaching to the man-PTS system. As mentioned above, pediocins classified within Group IIa are cationic peptides and have similar primary structures. Pediocins have two important structural regions, being the highly protected N terminal containing the YGNGV consensus pattern and the C terminal that has less protection. Both regions directly affect the antimicrobial qualities of pediocins due to their characteristics. Positively charged residues in the structure of the pediocin are usually located

in the hydrophilic N terminal section, and so the electrostatic interaction of this region enables pediocins to attach to the phospholipid structure. C terminal of pediocins, on the other hand, are important for identifying the target cell (Chen et al. 1997; Miller et al. 1998; Uteng et al. 2003; Drider et al. 2006).

Some bacteriocins that display antimicrobial activity by creating pores within the cell membrane use mechanisms other than attachment to the Lipid II molecule or the man-PTS system. For instances, the bacteriocin lacticin Q—produced by *Lactococcus lactis* QU 5—creates toroidal pores by replacing lipids in the cytoplasmic membrane. As cellular proteins leak out through the created pores, cell death is achieved without requiring a specific receptor (Zendo 2013; Iwatani et al. 2016). Garvicin ML—produced by *Lactococcus garviea*e has been reported to interact highly with the maltose ABC transport system located on the cell surface and to be a target receptor site for this precursor. Garvicin ML, recognizes the maltose ABC carrier system as a receptor and acts by creating pores (Gabrielsen et al. 2014). Enterocin AS48—produced by *Enterococcus faecalis*—creates water soluble dimers on the membrane of the target cell, and the water-soluble dimers then attach to the membrane. The structure that forms as a result allows it to enter the cell. Enterocin AS48 requires no specific receptors in its pore creation mechanism, and so shows its effect by interacting directly with the lipid bilayer (Burgos et al. 2014). Carnosiklin A, which similarly creates ion-specific pores by directly interacting with the lipid bilayer, without requiring a specific receptor, is produced by *Carnobacterium maltaromaticum* UAL307 (Martin-Visscher et al. 2009).

Bacteriocins that Inhibit Septum Formation

Bacteriocins usually act on functions such as transcription, translation, replication and cell wall biosynthesis, which are vital for living cells (Oscariz and Pisabarro 2001). Discovering new targets for bacteriocins could make them more effective to combat against pathogens. Recent mode of actions are discovered for bacteriocins on an almost daily basis, and new cellular targets are identified. Garvicin A and lactococcin 972 show antimicrobial effects by inhibiting septum formation in closely related strains. Garvicin A is produced by *Lactococcus garvieae* and is effective against other *L. garvieae* strains, while lactococcin 972 is produced by *Lactococcus lactis*, and is only effective against closely related *Lactococcus* strains. The effect mechanism of lactococcin 972 is blocking the septum formation (Martínez et al. 2008; Madera et al. 2009; Maldonado-Barragán et al. 2013).

The Mode of Action of the Gram-Negative Bacteriocins

The effect mechanisms of Gram-negative bacteriocins differ to those of Gram-positive bacteriocins in terms of the cell components they target and the biochemical processes involved. While Gram-positive bacteriocins are divided into three

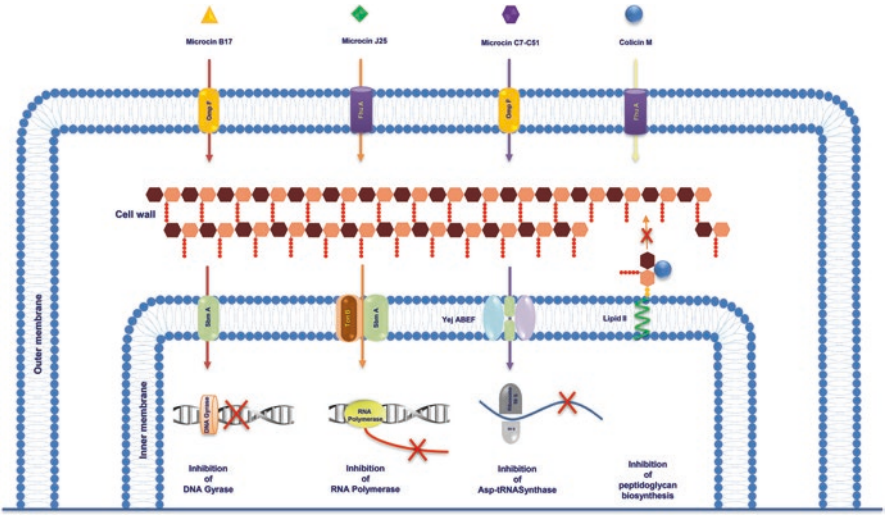

Fig. 2 Antimicrobial mechanism of action of bacteriocins produced by Gram negative bacteria

classes: inhibitors of cell wall synthesis, destroyers of the cell membrane structure and inhibitors of septum formation; Gram-negative bacteriocins target also DNA replication, transcription and protein synthesis. Thus, it is possible to divide Gram-negative bacteriocins into four groups, as inhibitors of cell wall synthesis, pore creators, inhibitors of DNA replication and transcription, and inhibitors of protein synthesis (Fig. 2).

Bacteriocins that Inhibit Cell Wall Synthesis

The cell wall has vital functions, such as maintaining cellular integrity, creating cell morphology and regulating osmotic pressure in the cell (Cavera et al. 2015), and so preventing cell wall biosynthesis emerges as a critical target. Among the Gram-negative bacteriocins, colicins show an antimicrobial effect by inhibiting peptidoglycan synthesis. Colicins prevent peptidoglycan synthesis by breaking down the precursor molecules (peptidoglycanase) that play a role in peptidogylcan synthesis, leading to cell death (Yang et al. 2014). Colicin M is the colicin with the smallest molecular mass (29.5 kDa), and the only colicin known to cause cell lysis by preventing peptidoglycan biosynthesis. Colicin M molecules inhibit peptidoglycan synthesis by entering the periplasmic space and attaching to the Lipid II molecule. They break down the Lipid II molecule, which serves as a carrier in peptidoglycan synthesis, by attaching to it via a phosphoester bond. As a consequence, peptidoglycan sub-units, which need to be carried outside the cell, disappear in the periplasmic space, peptidoglycan synthesis is inhibited, cell lysis takes place and the cell dies. Colicin M also inhibits the O-antigen of the lipopolysaccharide layer (Barreteau et al. 2012).

Bacteriocins that Creates Pores on the Cytoplasmic Membrane

Colicins, which are Gram-negative bacteriocins, also show antimicrobial effect by creating pores on the cytosplasmic membrane. Colicins that create pores or channels on the internal membrane cause vital cytoplasmic components in the cell to leak out, destroying the electrochemical gradient and leading to a loss of ions, ultimately resulting in cell death (Yang et al. 2014). Colicins A, B, E1, Ia, Ib, K and N are among those known to create pores. Microcin E492, a microcin produced by *Klebsiella pneumoniae*, is also a bacteriocin that creates pores.

The C terminal domain enables colicins to act by creating pores on the cytoplasmic membrane. The colicin molecule does not require a specific cell protein to attach to the lipid membrane, and then settle in the membrane and create pores or channels, as all of these events take place depending on voltage. As a result of electrostatic interaction, the ring of the positively charged side chain of the colicin molecule interacts with the phospholipid layer, which results in the formation of a pore structure in the form of an opening umbrella. The pore model that forms as a result is called an umbrella model (Mobius et al. 2005; Cascales et al. 2007; Padmavathi and Steinhoff 2008; Zamaroczy and Mora 2012).

Microcin E492, produced by *Klebsiella pneumoniae* RYC492 and included in the microcin family, is a bacteriocin with a small molecular mass that creates pores on the cytoplasmic membrane, and acts by creating cation-selective channels on the phospholipid layer. This bacteriocin needs the TonB protein to be carried from the external membrane, and the proton motive force for its antimicrobial activity (Lagos et al. 2009; Vasilchenko and Valyshev 2018).

Bacteriocins that Inhibit DNA Replication

Gram-negative nuclease-type bacteriocins, once reach inside the cell, target nucleic acids such as DNA and RNA, which are vital for the cell. The DNA molecule is an important target for some colicins, microcins and other bacteriocins (e.g. carocin S2) produced by other Gram-negative bacteria. These bacteriocins contain DNase, and show antimicrobial effect by breaking down the DNA molecule in a non-specific manner. Their antimicrobial effects are bactericidal (Cavera et al. 2015; Yang et al. 2014).

DNase colicins are defined as bacteriocins that break down the DNA molecule in random places, depending on metal ions. DNase colicins include colicin E2, which was the first to be identified, and colicins E7, E8 and E9. Colicins usually attach first to the BtuB receptor on the external membrane, and then move to the periplasmic space. They enter the cell by attaching to the Tol proteins on the internal membrane. DNase colicins bring about cell death by causing random damage to the bacterial genome, acting by creating nick on the double-stranded DNA. The E9 DNase colicin acts by attaching to both double-stranded and single-stranded DNA, but acts predominantly on double-stranded DNA. DNase colicins require nickel, cobalt, copper and particularly zinc metals to attach to DNA, but have been found to have

a higher affinity to zinc in their attachment to DNA (Cascales et al. 2007; Zamaroczy and Mora 2012; Papadakos et al. 2012; Kim et al. 2014).

Microcin B17, member of the microcin family, is a bacteriocin having decatenation mode of action. This bacteriocin acts as a DNA gyrase inhibitor. After passing through the external cell membrane via OmpF pores, microcin B17 then enters the cytoplasm via the Sbm A protein on the internal membrane. After reaching inside the cell, it targets the DNA gyrase and prevents the formation of the DNA supercoil structure. Microcin M17 is reported to obtain the energy required to separate the DNA-DNA gyrase complex from the ATP, as this reaction significantly increases in the presence of ATP (Duquesne et al. 2007; Collin et al. 2013; Cavera et al. 2015).

Bacteriocins that Inhibit Protein Synthesis

Protein synthesis is critical for all microorganisms, and is thus one of the most important target of bacteriocins inside the cell for showing inhibitory activity. Among the Gram-negative bacteriocins, members of both the colicin family and the microcin family target protein synthesis. Colicins display 16S rRNase and tRNase activity, while microcins also display RNase and tRNase activity. Aside from colicins and microcins, other bacteriocins produced by Gram-negative bacteria display RNase activity. Carocin S2, produced by *Petrobacterium carotovorum*, is a bacteriocin that has RNase as its effect mechanism (Yang et al. 2014; Cavera et al. 2015).

Colicins E3, E4, E6 and cloacin DF13 display 16S rRNase activity, whereas colicins D and E5 display tRNase activity and E group colicins display endonuclease activity. They require the BtuB protein to pass through the external membrane, and the Tol protein to pass through the internal membrane, and inhibit translation by breaking down the phosphodiester bonds on the 3′ end of the coding sequence of 16S rRNA. Colicin E3 acts specifically on the 30S subunit of the bacterial ribosome. Colicins D and E5, on the other hand, act by rapidly consuming the tRNAs in the cytoplasm, which have an important role in protein synthesis, displaying their effect by breaking down the phosphodiester bonds on the anticodon loop of tRNAs. The attachment receptor for Colicin D is the FepA protein on the external membrane, and it attaches to the Ton protein on the internal membrane (Masaki and Ogawa 2002; Papadakos et al. 2012; Zamaroczy and Mora 2012; Kim et al. 2014; Yang et al. 2014; Cavera et al. 2015).

Among the microcins, microcin J25 inhibits transcription, and microcin C7/C51 inhibits translation. Microcin J25 is transferred to the periplasmic space by interacting with the ferrichrome-iron receptor FhuA, and needs the TonB protein complex to pass through the internal membrane. It then enters the cell by interacting with the SbmA protein on the cytoplasmic membrane. Microcin J25 starts its inhibition by obstructing the second channel of the RNA polymerase, and then targets the mitochondria and even the respiratory system, depending on its increase in concentration in the cytoplasm. Microcin C7/C51 enters the cell by interacting with the OmpF molecule on the external membrane, and with the YejI molecule on the internal membrane. Once activated, it imitates the aspartyl adenylate molecule, and in

this way it is recognized by the tRNA synthase enzyme. As it is recognized by the aspartyl tRNA synthase, it prevents tRNA aminoacylation and thus inhibits protein synthesis (Bayro et al. 2003; Delgado et al. 2005; Duquesne et al. 2007; Rebuffat 2016).

Bacteriocin Applications

As a result of increasing consumer demand for minimally processed foods in recent years, bacteriocins are often preferred for food preservation. In addition to their antimicrobial activities, the popularity of bacteriocins is based also on their formation through natural processes, and the fact that they do not affect the taste, smell or texture of foods. Being in the form of peptides or proteins, they can be cleaved by proteolytic enzymes and stomach secretions, and are easily digested by the human body. In addition, some bacteriocins remain stable even under autoclave temperatures, which increases their usability in the many foods that are processed at high temperatures. As a result, bacteriocins can be used in many different types of foods, starting with meat and dairy products. Bacteriocins can be used in foods in different ways: They can be directly added to the food, or protective cultures that produce bacteriocins can be added to the food or immobilized on the protective packaging material (Zacharof and Lovitt 2012; O'Shea et al. 2013).

Applications of Gram-Positive Bacteriocins

Among the Gram-positive bacteriocins, those produced by LAB has attracted the most attention, in that they are used as the starter culture in LAB fermented foods, are generally recognized as safe (GRAS), have qualified presumption of safety (QPS) status, have bactericidal effects against many deterioration factors and pathogenic bacteria in the nanomolar range, and finally, do not affect the sensory qualities of food (Alvarez-Sieiro et al. 2016; Field et al. 2018). The LAB bacteriocins so far approved for use in foods are nisin and pediocin, although studies report that promising results have been obtained regarding the use of other LAB bacteriocins as biopreservatives in foods, and the various advantages they offer (Alvarez-Sieiro et al. 2016).

Bacteriocins can be used in food systems in different ways. They can be directly added to foods in purified form, for which pure or semi-pure nisin preparations produced under different commercial names are available in various countries. Bacteriocins are also used in the form of fermentates, obtained from the concentration of fermentation products containing the bacteriocin, and there are examples of pediocin-producing fermentation products have been used. Finally, bacteriocin-producing cultures can be added to foods as starter cultures, additional cultures or

protective cultures. Aside from these applications, bacteriocins can also be used by integrating them into the packaging material at the time of packaging.

Although many bacteriocins have been identified as being produced by LAB, nisin is the one with the widest application and approval for use in foods. Nisin was the first bacteriocin commercially produced from *Lactococcus lactis*, classified as a lantibiotic. As it has a very wide effect spectrum, this bacteriocin is used as a preservative by food industries, and as a therapeutic in the field of medicine. Nisin has been classified as GRAS (safe for human and animal consumption) by the FDA, and is licensed (E234) and approved for use by the EFSA (Simsek and Saris 2009; Field et al. 2015). Today, this bacteriocin is used for the preservation of many foods, including milk and dairy products, canned foods and instant soups in more than 50 countries, and is also used in a number of different health products, including toothpastes and gauzes (Delves-Broughton et al. 1996; Zou et al. 2018). Nisin is used in cheese to inhibit *Listeria monocytogenes*, and in meat products to prevent the development of *Clostridium botulinum*. In a study by Mitra et al. (2010), nisin was added to pasteurized milk, and it was found that the bacteria that remained alive after pasteurization and that caused the milk deterioration were inhibited to undetectable levels. In this way, it is possible to extend the shelf life of pasteurized milk for up to 2 months under refrigerated conditions. In a study by Dal Bello et al. (2012), bacteriocin-producing strains isolated from various Italian fermented foods were used, and the development of *Listeria monocytogenes* and other pathogen bacteria was reported to be controlled successfully with the addition of strains producing nisin A, nisin Z and lacticin 481 to cottage cheese as a starter culture. The wide antimicrobial spectrum of nisin also targets LAB used as starter cultures, and so the use of nisin in dairy products and other fermented foods is limited (Silva et al. 2018). This makes it very important to identify new bacteriocins using different isolation strategies that would be expected to be target specific and to have a narrow spectrum, to retain their stability in different food systems, not to act on LAB, and to be resistant to thermal processes.

Lacticins are bacteriocins produced by *Lactococcus lactic* strains, and there are two bacteriocins in this group: lacticin 3147 and lacticin 481. Lacticin 3147 has been added in powder form to baby formulae, cottage cheese and yogurt, and has been reported as successful in controlling *Listeria* and *Bacillus* bacteria. Lacticin 481 has a moderately wide antimicrobial spectrum, and is effective against *Clostridium tyrobutyricum* and *Listeria monocytogenes* pathogens. A semi-purified lacticin 481 preparation was found to lower the number of *Listeria monocytogenes* in fresh cheese kept under refrigerated conditions by 3 log over 3–7 days (Silva et al. 2018). In a study conducted by Sarika et al. (2012), a 1600 AU/ml solution of bacteriocin PSY2 produced by an *L. lactis* PSY2 strain was sprayed on fillets of *Epinephelus diacanthus* fish in the form of a 2 ml spray, which were then packaged and stored at 4 °C. The samples treated with the bacteriocin were found to have a storage life that was 7 days longer than the control samples. After 14 days of storage, the number of bacteria that caused spoilage were found to be 2.5 log lower than the control samples.

Similarly, pediocins produced by different *Pediococcus* species have been found to be effective against pathogens existed in foods. In particular, they are used to prevent problems caused by *Listeria monocytogenes* in cheese, meats, meat products and various fermented foods. Pediocins, referred to also as antilisterial or listeria-active bacteriocins, have been reported to be more effective than nisin against the food-based pathogens *Listeria monocytogenes* and *Staphylococcus aureus*, and Gram-negative bacteria *Pseudomonas* and *Esherichia coli*. Pediocin was found successful to inhibit the 10^2 CFU/g inoculated *Listeria monocytogenes* at the cottage cheese, creams and cheese sauces. Similarly, using the semi-purified pediocin produced in whey reduced the number of *Staphylococcus aureus* in the production of buffalo cheese and extended the shelf life (Silva et al. 2018).

Enterocins are bacteriocins that are produced by enterococci and are effective against different pathogens, including *Listeria monocytogenes*, *Clostridium* species, *E. coli*, *Vibrio cholerae*, *S. aureus*, and *B. cereus*, and against bacteria that cause food spoilage. Enterocin-producing *Enterococcus faecium* is used as a starter culture in fermented meats and meat products. *Enterococcus faecium* RZS C13 and *Enterococcus faecium* CCM 4231 are used as starter cultures in the production of Spanish-type sausages. Enterocin AS-48—produced by *Enterococcus faecalis*—is effective against enterotoxic *S. aureus*, *Bacillus* and *Clostridium* species (Bharti et al. 2015; Silva et al. 2018). In a study conducted by Yıldırım et al. (2016), different concentrations of enterocin KP produced by *Enterococcus faecalis* KP were added to non-fat, low-fat and whole-fat milk containing different concentrations of *Listeria monocytogenes*, and it was found to have a strong antilisterial effect, although the strong antilisterial effect was found to weaken as the fat ratio and the concentration of inoculated bacteria increased.

Many different strains of the *Lactobacillus plantarum* are known to produce various bacteriocins that are known as plantaricins, which have an antimicrobial effect against *Listeria monocytogenes*, *Listeria innocua* and *Listeria ivanovii* species. In a model system containing bacteriocin-producing *Lactobacillus plantarum* AMA-K and *Listeria innocua*, the number of *Listeria innocua* was reported to decline from 3.4×10^4 CFU/ml to 7×10^2 CFU/ml after 12 h, and to undetectable levels after 24 h (Todorov 2009). BacTN635, produced by *Lactobacillus plantarum* TN635, as another bacteriocin producer, was reported to kill the pathogen *Salmonella* species and even the pathogen yeast *Candida tropicalis* R2 CIP203 (Chalón et al. 2012).

In a study conducted by Nakamura et al. (2013), in which the authors added gassericin A—produced by *Lactobacillus gasseri* LA39—to thick cream, the *B. cereus* AK1124 and *L. lactis* subsp. *lactis* AK1155 strains were found to be completely inhibited in the sample that contained 0.5% glycine and 5% gassericin A.

Another use of bacteriocins in foods is the inoculation bacteriocin-producing bacteria to foods. In a study conducted to examine this approach, a bacteriocin-producing *L. plantarum* LMG P-26358 strain was found to inhibit the *L. innocua* species (Mills et al. 2011). Similarly, in another study conducted by De Vuyst and Leroy (2007), a bacteriocin-producing *L. curvatus* LTH 1174 strain was reported to inhibit the *Listeria innocua* LMG 13,568 species in a fermented sausage specific to Belgium.

Applications of Gram-Negative Bacteriocins

The antimicrobial spectrums of Gram-negative bacteriocins consist of closely related Gram-negative bacteria. These bacteriocins differ from the bacteriocins produced by Gram-positive bacteria in that they also affect vital nucleic acids inside the cell. Some Gram-negative bacteriocins, for example, act by targeting DNA and RNA molecules. They are not classified as GRAS by authorities, and are produced by coliform bacteria, which are factors that limit their use in foods (Chalón et al. 2012). Unlike bacteriocins produced by Gram-positive bacteria, and by LAB in particular, which are preferred for food preservation, these bacteriocins are more widely used in the clinical and medical fields.

Colicins and microcins produced by *Escherichia coli* are the majority of the bacteriocins produced by Gram-negative bacteria. Bacteriocins produced by more than 20 colicin-producing *E. coli* strains were reported to inhibit five different *E. coli* species (O26, O111, O126, O145 and O157: H7). Colicin E1 and colicin N have been found to be effective against enterotoxigenic pathogens *E. coli* F4 and F48 strains, while colicins have been found to act on cancer cells. Colicins A and E1, which create pores, have been found to prevent the development of human standard fibroblast line MRC5 and 11 human tumor cell lines. It has also been reported that colicins that are effective against cancer cells can be used as anti-tumor medicine or added to foods for consumption (Yang et al. 2014). Colicin-producing species are also used to inhibit the pathogens that form colonies in the digestion systems of poultry animals and bovine animals, and thus encourage the development of bacteria that are beneficial for the intestines. Colicin-producing bacteria are used to control pathogenic *Salmonella* species. In a study conducted to prevent the development of *E. coli* O157: H7 in beef carcasses, colicin E1 was found to be effective in inhibiting the target bacteria, and it was concluded that colicin E1 could be used to prevent *E. coli* O157: H7 contamination in beef carcasses (Chalón et al. 2012).

Microcins are bacteriocins that have a peptide structure and a smaller molecular mass, and that are also produced by *E. coli* strains. Microcin J25 is effective against *Salmonella* species, with *Salmonella newport* and *Salmonella enteridis* species, in particular, reported to be highly sensitive to this bacteriocin. In addition to being a promising food preservative, microcin J25 can be used effectively against *Salmonella* species in clinical applications (Chalón et al. 2012). Microcins can be consumed as probiotics in order to prevent *Salmonella* invasions in humans, as the *E. coli* that produces microcin 24 is reported to inhibit the development of pathogenic *Salmonella* and *E. coli* O157: H7 species. It has been reported that some microcins could potentially be used as anti-tumor agents. Microcin E492 has been found to induce the biochemical and morphological changes that emerge as a result of apoptosis in the human cell line. The heterologous production of microcin V by LAB has been achieved, making it possible to use bacteriocins produced by LAB with microcin V activity against Gram-negative bacteria that cause food spoilage (Duquesne et al. 2007; Chalón et al. 2012).

Bacteriocin Applications Together with Other Methods

Antimicrobial spectrums of bacteriocins cover only closely related species, Gram-positive bacteriocins act on Gram-positive bacteria, and Gram-negative bacteriocins act on Gram-negative bacteria. Thus, to widen their antimicrobial spectrum, bacteriocins are used together with the preservation methods that are preferred in hurdle technologies, such as organic acids, chemical additives, various thermal treatments and high pressure (Chalón et al. 2012; O'Connor et al. 2015; Silva et al. 2018). With the synergistic effect thus achieved, the antimicrobial effect becomes stronger, and can act on both Gram-positive and Gram-negative bacteria. Accordingly, the inclusion of bacteriocins among the hurdle technologies and the strengthening of their effects together with other components can lead to more effective and successful results.

The most preferred means of inhibiting deterioration or pathogen Gram-negative bacteria in foods is to use EDTA (Ethylenediaminetetraacetic acid) and bacteriocins together. EDTA increases the permeability of the external membrane by interacting with the lipopolysaccharide layer on the cell membrane. When used together with bacteriocins, it has a wide spectrum that includes Gram-negative bacteria *Salmonella*, *E. aerogenes*, *S. flexneri*, *C. freudii*, *E. coli*, *P. aeruginosa* and *A. butzleri* (Prudêncio et al. 2015; Silva et al. 2018). The carnocyclin A bacteriocin does not have antimicrobial effects against *E. coli*, *P. aeruginosa* or *S. typhimurium* bacteria, but when combined with 40 mM EDTA, it has been reported to inhibit *E. coli* and *P. aeruginosa* bacteria. Similarly, it has been found that antimicrobial activity could be strengthened by combining nisin with 40 mM EDTA (O'Connor et al. 2015).

High pressure applications aim to extend the shelf life of foodstuffs by inactivating the microorganism found in foods at room temperature. This application alone is not sufficient to inactivate all microorganisms, and is more effective when used together with other preservation methods, like bacteriocins, having an effect mechanism that is similar to that of EDTA. The application damages the structure of the external membranes of Gram-negative cells, in particular, and increases the effect of bacteriocins on these cells (Prudêncio et al. 2015). There have been many studies detailing the synergistic effect of a combination of high pressure and nisin. Microbiological quality is reported to improve with the use of bacteriocin-producing LAB in combination with moderately high pressure in cheese production (Silva et al. 2018).

Bacteriocins are also reported to display a synergistic effect when used together with heat applications, although bacteriocins are best known for their use as food additives following low-temperature applications. It has been found that following temperature applications, sublethal stress conditions emerge for bacteriocin-resistant bacteria, at which point nisin and pediocin applications become effective. This effect has been found to be pronounced in Gram-negative bacteria that are not sensitive to bacteriocins used in foods. Low-temperature applications cause changes to the external membrane of the cell, amending the permeability of the cell mem-

brane through which the applied nisin can pass and act against *S. typhimurium* and *E. coli* bacteria under refrigeration (Prudêncio et al. 2015; Silva et al. 2018).

One of the natural antimicrobial systems with which bacteriocins are combined is the lactoperoxidase system, which has antimicrobial effects and is found in raw milk. In a study examining its use in combination with nisin, it was found to decrease the number of *Listeria monocytogenes* in non-fat milk by 5.6 log when compared to a control sample (Silva et al. 2018). In another study, the Gram-negative pathogenic bacterium *Cronobacter sakazakii*, which is found in powdered baby milk and can lead to meningitis, septicemia and enterocolitis in babies, was found to be inhibited in 8 h when nisin or lacticin 3147 was combined with the lactoperoxidase system (O'Connor et al. 2015).

Conclusion

The studies conducted to date have shown that bacteriocins have a significant antimicrobial effect on many pathogenic bacteria, including *B. cereus*, *L. monocytogenes* and *S. aureus*, which are important in foods. However, this does not change the fact that the number of commercially used bacteriocins are much lower than the number of identified bacteriocins. Even though there are a large variety of bacteriocins produced by many bacteria, LAB (GRAS) being first among them, there are various problems that hinder their antimicrobial performance and their effective use in food systems. The greatest of these problems is the fact that bacteriocins have a narrow antimicrobial effect spectrum due to their specific inhibition characteristics. Accordingly, there is a need to identify target-specific bacteriocins with a wide spectrum, to characterize them in detail, and commercialize them using a new-generation strategies.

There are many bacteriocins that are effective against pathogenic and food-spoiling Gram-positive bacteria, whereas more bacteriocins are needed that can effectively target Gram-negative bacteria, with potential application in food systems. To this end, LAB can be made to produce Gram-negative bacteriocins, or LAB bacteriocins, which usually target Gram-positive bacteria and are used in combination with other preservation methods, which many studies have shown to be more effective.

Future Work

The broad use as food preservatives and their potential medical and clinical applications, there are many studies currently being conducted on bacteriocins, and this trend is likely to continue in the future.

For bacteriocins to be used more effectively, especially in food systems, the current limitations need to be removed, the most significant of which is that their effect mechanisms are narrow. As a further limitation, the effect mechanisms of bacteriocins have yet to be fully understood. Existing applications need to be supplemented with new strategies developed for bacteriocins related to their use against Gram-negative pathogens and food-spoiling bacteria in foods.

Future studies on bacteriocins can be expected to focus on gaining a better understanding of their effect mechanisms, with the aim of identifying specific targets for currently identified bacteriocins, and bacteriocins that are yet to be discovered. In this way, the microorganisms that are targeted by bacteriocins would be identified, and possible to use them more effectively and in a more target-specific manner.

Bacteriocins that target Gram-negative bacteria are produced by enterococci and other Gram-negative bacteria, which limits their uses in food systems. However, many studies showed that Gram-negative pathogenic and food-spoiling bacteria can be targeted when LAB are used in combination with other preservation methods. Accordingly, these limitations can be removed through studies focusing on the combined use of bacteriocins.

Strategically identifying pathogen-specific or target-specific bacteriocins can be an important way out, although gaining a better understanding of the effect mechanism would make it easier to use bacteriocins in a target-specific manner. When bacteriocins are being selected, those that are effective against all strains of a bacterium species found in foods would be preferred so as to kill all pathogenic or food-spoiling strains. Using this strategy, target-specific bacteriocins would be obtained and cocktails would be created depending on the types of microorganisms that threaten certain foods, and these wide-spectrum, target-specific bacteriocin cocktails would be offered for use to the food sector.

New developments in conventional microbiological techniques, mass spectrometry, molecular techniques and bioinformatic studies, together with newly developed scanning methods, would allow the identification of new-generation bacteriocins.

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