

Microbiological Contamination in Foods and Beverages: Consequences and Alternatives in the Era of Microbial Resistance

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1 Introduction

Most foods constitute a rich source of nutrients for microbial development. Besides microorganisms, metabolic compounds, such as enzymes, lead to contamination, spoilage, and degradation. Antimicrobials have been widely used to combat spoilage and pathogenic microorganism in several areas that involve human and animal health, including the food chain. Usually, problems in the supply and processing chain could change the environmental conditions of food and promote the growth of these microbial agents. Besides the food changes, some microbial agents can produce toxins and cause several illnesses to consumers. Several techniques are used in the food industry to detect the food microbial contamination. Moreover, new methodologies are being developed to prevent and control these contaminants and their resistance to antimicrobials. This chapter discusses the biochemistry, nutritional, and sensorial effects caused by the microbial contamination of beverages, dairy, meat, and egg products, fruits and vegetables, canned foods, and others. In addition, we discuss the current and the future technologies used to prevent, detect, and control the food contamination and degradation.

2 Deterioration and Contamination in Food Products

2.1 Commodities of Animal Origin

Foods of animal origin provide essential nutrients, constituting a major fraction of the diet of people worldwide. Due to their rich compounds that include proteins, carbohydrates, and fats, their chemistry, structure, and properties, these products are sensitive to deterioration by microorganism or be possible vehicles of microorganisms or toxins that affect human health (Table 3.1). In this topic we discuss the most common microbiological contamination in this kind of product.

Table 3.1: Main changes that microorganism caused in food products of animal origin.

Products	Microbe Types	Main Microorganisms Genera Present	Changes in Food Due to Spoilage	Barriers That Prevent Microbial Growth
Eggs	Bacteria Molds Yeasts	<i>Proteus, Aeromonas, Serratia, Enterobacter, Acinetobacter, Alcaligenes, Pseudomonas, Salmonella, Campylobacter, Listeria, and Staphylococcus</i> <i>Cladosporium, Alternaria, Thamnidium, and Mucor, Aspergillus</i> <i>Candida, Debaryomyces, and Hyphopichia</i>	Yolk blackening; obnoxious odor (putrescine, cadaverine, phenylethylamine, spermidine, spermine, and others); black, red, custard, green, blue, and colorless rot; changes in the shell pigmentation	Eggshell protection (cuticle, inner membranes, and pores); interior protection (lysozymes, conalbumin, and avidin)
Raw milk and dairy products in general	Bacteria Fungi	<i>Pseudomonas, BAL, E. coli O157:H7, Salmonella, Staphylococcus, Listeria, Campylobacter, Brucella, Yersinia, Shigella, and Tuberculosis</i> <i>Cladosporium, Geotrichum, Mucor, Trichoderma, Penicillium, and Aspergillus</i>	Proteolysis and lipolysis; increase of pH; production of volatile off-flavor compounds; green, blue, and yellow pigmentation	Absence of significant amounts of amino acids (lysine, arginine, isoleucine, and glutamic acid); storage under refrigeration; heat treatments
Cheese	Bacteria	<i>Pseudomonas, BAL, E. coli, and Listeria</i>		
Yogurt	Bacteria	<i>Pseudomonas, BAL, Salmonella, and Shigella</i>		
Buttermilk	Bacteria	<i>Pseudomonas, Listeria, Brucella, Mycobacterium, and Salmonella</i>		
Meat, poultry, and seafood and its derivatives	Bacteria	<i>Pseudomonas, Brochothrix, Shewanella, Photobacterium, BAL, Salmonella, Clostridium, E. coli, Campylobacter, Staphylococcus, and Listeria</i>	Proteolysis and lipolysis; off-flavor and off-odor volatile compounds; discoloration or production of different pigments (green and blue); slime	Lower pH values in fermented products; aromatic compounds in smoked products

2.1.1 Table eggs

The chicken egg is one of the most common foods consumed worldwide. The global production was 69.77 Mt. corresponding of approximately 1.200 billion of produced eggs (FAOSTAT, 2016). The US per capita consumption was 263.3 in 2014 and the USDA projects an increase in 2016 to 265.8 eggs per person (AEB, 2016; USDA, 2016). This increase in production and consumption is also notable in developing countries where changing diets have made people start to consume a bigger amount of calories from protein sources (Zaheer, 2015). Moreover, eggs offer the finest nutritional composition when compared to the other sources of protein, at a low cost. They are an excellent source of proteins of high

biological value, high ratio of unsaturated fatty acids to saturated fatty acids, and also a great source of minerals and vitamins (Bertechini and Mazzuco, 2013; Figueiredo et al., 2014).

Although there are advantages of egg consumption in the diet, there are also a variety of microbiological hazards that are associated with eggs (Arnold et al., 2014; EFSA, 2014). Several microorganisms, including bacteria, yeasts, and fungi can contaminate the egg, increasing the risk of deterioration and causing food-borne illnesses in humans. The freshly laid eggs have a fragile, sponge-like, and moist structure, and, generally, are free from microorganisms. The egg microflora will be created after the oviposition, as soon it comes in contact with the cloaca of the hen, the materials of the nest that may include dust, soil, and feces. These elements are the primary source of egg microflora. The microbial population present in the eggshell will depend on several factors, such as the temperature, the humidity, and the hygienic conditions of the environment. Any microorganism in contact will adhere to the eggshell. These microflora include a diverse genera of bacteria, molds, and yeasts, which can contaminate the eggshell, penetrate through its membranes, and access the egg content, resulting in deterioration (Musgrove, 2011; Shebuski and Freier, 2009; Sparks, 2014; Whiley and Ross, 2015).

To get access to egg content, the microorganisms should overcome its several defense mechanisms (Adil, 2016). The first line of defense includes the cuticle, the inner membranes, and the thickness porosity present in the eggshell. Usually, it is hard for the microorganism to access the content through the normal pores.

However, after egg washing the cuticle will be removed and the pores will be filled with water, allowing the microorganism migration (D'Alba et al., 2014; Gole et al., 2014; Mikšík et al., 2014).

After the invasion, the microorganisms need to overcome the defenses of the albumen. Some proteins like lysozymes, which have antimicrobial effects against Gram-positive bacteria; conalbumin, which binds iron, which is necessary for bacterial growth; and avidin, which binds biotin, another important compound for bacterial growth. Moreover, they need to overcome the high pH and the viscosity of the albumen, which also retards microbial growth (Adil, 2016; Baron et al., 2016; Du et al., 2015; EFSA, 2014). All these defense mechanisms are influenced by egg quality, which is directly related to the nutrition, management, genetics, and age of the laying hen and also related to the storage time of the egg (Menezes et al., 2012; Musgrove, 2011; Spada et al., 2012; Tang et al., 2015). These microbial contaminations also can occur on all links of the egg production chain, from the laying hens on the farm to the processing steps of the egg industry (Cader et al., 2014). On the farm, the egg can be contaminated during its formation in the reproductive tract of the laying hens. Some bacteria are capable of colonizing the avian gut, migrating by the bloodstream to the ovary and infecting the yolk, contaminating the egg before the oviposition (Gantois et al., 2009; Gross et al., 2015). Inside the yolk the bacteria can multiply and start the deterioration process. The

production system is another potential source of microbial contaminations. Despite some small farms, where the eggs are manually collected and transported, to be processed “offline,” majority of the egg’s facilities are semiautomated and the egg is processed “inline.” In line facilities, keep the laying hen in separated cages, and the eggs roll under by gravity from the cages to troughs where they are washed, cleaned, and sanitized before packaging (Janczak and Riber, 2015; Jones et al., 2015; Musgrove, 2011). The washing procedures decrease the microbial load, limiting the chance of contamination of the egg’s internal contents. However, this procediment removes the cuticle, exposing the pores and making the egg more vulnerable to posterior contaminations (Hannah et al., 2011; Gole et al., 2014; Leleu et al., 2011; Whiley and Ross, 2015). If processing and packing are executed on contaminated equipment or in an unhygienic environment, the advantage of processing may be lost due to recontamination (Musgrove, 2011).

After packaging, the storage time and temperature also influence the egg deterioration (Chung and Lee, 2014; Jones and Musgrove, 2005). During storage, the egg starts to lose CO₂ through the shell, increasing the inside pH. Higher storage temperatures can favor CO₂ loss. In a few days the pH increases from pH 7.6 in a freshly laid egg to between 9.0 and 9.7. This change in pH also results in lower viscosity of the albumen (Banerjee et al., 2011; EFSA, 2014; Eke et al., 2013; Figueiredo et al., 2014; Jin et al., 2011). With lower viscosity the chalazae detach from the membrane allowing the yolk to move freely within the egg. The unstable yolk becomes enlarged and flattened. Together with these changes the air cell starts to enlarge due to the water evaporation from the liquid content through the shell pores and getting replaced by air. All these changes make the egg suitable for invasion by several microorganisms (Cader et al., 2014; EFSA, 2014; Whiley and Ross, 2015; Zaheer, 2015).

Some bacteria are capable of penetrating inside the eggshell, resisting the protective mechanisms, metabolizing components of the egg content, and producing several deterioration characteristics (Al-Bahry et al., 2012; D’Aoust, 2009). Some microorganisms must produce siderophores to scavenge iron from the tight iron proteins to grow inside of the egg. Proteolytic bacteria digest the albumen, blackening the yolk and producing an obnoxious odor due the lysine decarboxylation, which produce biogenic amines, such as putrescine, cadaverine, phenylethylamine, spermidine, spermine, and others (Figueiredo et al., 2014; Fung, 2009; Gram et al., 2002; ICMSF, 2005).

The deterioration microorganisms commonly associated with black rots are *Proteus* spp. and *Aeromonas liquefaciens*. Other bacteria are associated with other rot colors, such as *Serratia* spp., responsible for the red rots, *Enterobacter* spp. responsible for the custard rots, *Acinetobacter* spp., *Alcaligenes* spp., coliforms, and others that are responsible for the colorless rots and certain species of *Pseudomonas* spp. are responsible for green, blue, or pink rots. Some microorganisms such *Flavobacterium* spp. and *Cytophaga* spp. can change the pigmentation color of the shell membrane to yellow. Off-flavors can be caused by several bacteria; *Enterobacter* spp. causes a hay odor and *Escherichia coli* a fish flavor due

to the formation of trimethylamine (Baylis, 2006; Corry, 2007; D'Aoust, 2009; Fung, 2009; Shebuski and Freier, 2009). *Salmonella* spp., which do not change the color, smell, and consistency of the egg content, but imply a serious threat to human health. Other pathogens associated with egg-borne illness by consumption are *Campylobacter* spp., *Listeria* spp., and *Staphylococcus* spp. (Arnold et al., 2014; EFSA, 2014; Sparks, 2014).

Other microorganisms related to egg's deterioration includes *Acinetobacter* spp., *Aerobacter* spp., *Aeromonas* spp., *Alcaligenes* spp., *Cedecea davisae*, *Chryseomonas luteola*, *Citrobacter* spp., *Cytophaga* spp., *Enterobacter* spp., *Erwinia* spp., *Escherichia* spp., *Flavobacterium cytophaga*, *Hafnia alvei*, *Klebsiella* spp., *Kluyvera* spp., *Leclercia adecarboxylata*, *Listonella damsela*, *Morganella* spp., *Proteus* spp., *Providencia* spp., *Pseudomonas* spp., *Rahnella* spp., *Serratia marcescens*, *Sphingobacterium* spp., *Vibrio* spp., and *Xanthomonas maltophilia* (D'Aoust, 2009; Jones and Musgrove, 2008; Musgrove et al., 2008a; Shebuski and Freier, 2009; Sparks, 2014).

Although being studied extensively from the bacterial point of view, the egg microbial deterioration is also related to molds and yeasts. Molds can grow on eggs when they are stored under refrigeration with high room humidity (Cader et al., 2014). The hypha can penetrate the shell's pores and membranes, spreading throughout the interior of the egg and initiating deterioration. The presence of grown molds on the eggshell also give musty odors and tastes. Generally, molds multiply in the air sacs, because they are obligate aerobe. This deterioration is referred to spots due to the formation of mycelial growth on the eggs. Green, blue, and yellow spots are caused by *Penicillium* spp., black spots by *Cladosporium* spp. and pink spots *Sporotrichum*. Other molds related to deterioration are *Aspergillus* spp., *Alternaria* spp., *Botrytis* spp., *Thamnidium* spp., and *Mucor* spp. (Folorunsho and Charles, 2013; Pitt and Hocking, 2009; Sparks, 2014). The presence of *Aspergillus* spp. and *Penicillium* spp. are a public health concern, because this microorganism can produce mycotoxins, teratogenic, and carcinogenic toxins (Pitt and Hocking, 2009).

In addition, very little information is available on the literature about the yeasts associated with eggs deterioration. The most commonly identified yeast isolates on the eggshells; egg processing facility and storage are *Candida* spp., *Candida famata* an anamorph of *Debaryomyces hansenii*, *Candida lusitanae*, and *Hyphopichia burtonii*. (Deak, 2008; Musgrove et al., 2008b).

2.1.2 Milk and dairy products

Milk from cows, buffalo, goats, and sheep are used for human consumption either as heat-treated milk or as dairy products (Walstra et al., 2006). Besides food poisoning and/or spoilage due microbial activity, milk is subjected to chemical changes, including fat oxidation and hydrolysis in full-cream milk (Walstra et al., 2006). Milk and dairy products are susceptible to microbial contamination at any point in the food processing chain (Fig. 3.1). Contamination may occur in milking, after pasteurization, during packaging, or because

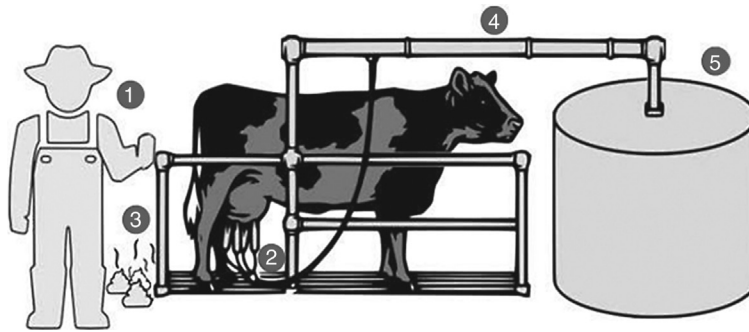


Figure 3.1: Some Possible Points of Contamination in Milk Production.

1: Cross contamination by handling; 2,4,5: inadequate cleaning of milking equipment; 3: animal feces; 5: oscillation in temperature of refrigeration tank.

of the addition of contaminated additives (Beletsiotis et al., 2011). The microbiological quality of milk and its derivatives is influenced by the initial flora of raw milk, the processing conditions, and conservative treatments (Varga, 2007). The presence of spoilage bacteria depends on their ability to resist processing and storage conditions. To ensure safety and quality, the concentration of these microbes should be as minimal as possible (Losito et al., 2014; Remenant et al., 2015). Microbial contamination causes approximately 25% of the world's food supply loss (Losito et al., 2014).

Contamination, spoilage, and degradation characteristics are determined by sensorial analyses and/or by measurement of physicochemical parameters, such as the amount or nature of metabolites or gases, color, pH, which are related to the presence of spoilage microorganisms (Jaffrès et al., 2011; Macé et al., 2013; Remenant et al., 2015).

Dairy products are highly perishable as they are a growth medium for many types of microbes, especially fungi and bacteria. Therefore, it is important to monitor the microbiological quality of dairy products and, in particular, the total viable count of *E. coli*, as they are indicators of the hygienic state of these products. Also, in fermented products it is important to monitor the concentration of lactic acid bacteria (LAB), as they are the major components of starter cultures used in the production of these products (Jana and Mandal, 2011; Losito et al., 2014; Ruegg, 2003; Varga, 2007).

Bacteria are the main microorganisms associated with spoilage of milk and dairy products. Temperature and other factors, such as pH, oxygen pressure, and nutrients are important factors for growth and multiplication of bacterial species. Decreasing temperature retards the metabolic and growth rate of most mesophilic bacteria (Walstra et al., 2006).

Milk is susceptible to microbial growth for many microorganisms due its high water content, near-neutral pH, and availability of different nutrients. However, the absence of significant amounts of some amino acids (lysine, arginine, isoleucine, and glutamic acid) restricts the

range of contaminant microbes. Under refrigeration temperatures, psychotrophic bacteria have a higher growth rate and produce lipolytic and proteolytic enzymes. These enzymes have major effects on the quality of milk and dairy products due their ability to resist thermal treatments (Nsofor and Frank, 2013).

During the cheese-manufacturing process, the pH of curd decreases to 4.5 due to the lactic acid production by LAB, this low pH inhibits the growth of some pathogenic and spoilage bacteria (Lee et al., 2012). During storage, the proteolytic activity of starter molds raises the pH of the cheese to approximately pH 7.0 (Gripson, 2001; Price et al., 2004). To understand the behavior of pathogenic *E. coli*, several studies, including microbial interactions, have been done in cheese model systems (Frank et al., 1977; Hussein and Sakuma, 2005; Marek et al., 2004; Montet et al., 2009; Rash and Kosikowski, 1982; Rodriguez et al., 2005; Saad et al., 2001). However, microbial interactions between molds and pathogenic *E. coli* in cheese are poorly understood. Although it is thought that the pH increase due to proteolytic activity of molds has a stimulatory role to the growth of food-borne pathogens (Bevilacqua et al., 2008; Cibelli et al., 2008; Richards and Beuchat, 2005), most of the findings have not excluded the effect of natural microflora.

In fresh products, certain metabiotic associations between foodborne molds and pathogenic bacteria have been reported. Proteolytic activity of various molds alkalizes inoculated samples, and thus, the pH increases and it is likely to enhance the growth of *E. coli* O157:H7 and *Salmonella* Infantis (Cibelli et al., 2008; Riordan et al., 2000). The biomass of molds is much higher in mold-ripened cheeses than in damaged fresh produce, the molds in these cheeses might have a broader and more intense effect on food-borne pathogens. In a model system using whey, the higher growth rate of *L. monocytogenes* in coculture with *Penicillium camemberti* at various pH. Therefore, it is important to elucidate the mold effect on the behavior of these microorganisms (Ryser and Marth, 1988).

The range of dairy products can also be contaminated by mycelial fungi, including the genera *Penicillium*, *Aspergillus*, *Cladosporium*, *Geotrichum*, *Mucor*, and *Trichoderma*. Contamination of foodstuffs with *Penicillium* and *Aspergillus* genera can lead to production of mycotoxins. These compounds possess carcinogenic, teratogenic, and other properties, which can pose a real threat for consumer health (Kozlovsky et al., 2014; Sengun et al., 2008). Buttermilk is considered as a low-value product due its high variation in the biochemical composition, elevated microbial load, and limited functional properties. In fact, buttermilk represents a potential source of spoilage and pathogenic microorganisms, such as *Listeria*, *Brucella*, *Mycobacterium*, or *Salmonella* (Fauquant et al., 2014).

Consumption of raw or inadequately pasteurized milk and dairy products has played significant roles in food-borne outbreaks including salmonellosis, listeriosis, hemolytic uremic syndrome associated with *E. coli* O157:H7, staphylococcal enterotoxin poisoning, campylobacteriosis, brucellosis, yersinioses, and tuberculosis (LeJeune and

Rajala-Schultz, 2009). Pathogens, such as *L. monocytogenes*, *Salmonella* species, and *E. coli* O157:H7 have been found in raw milk and milk products and the illnesses associated with these pathogens are severe. For example, invasive listeriosis typically has high fatality rates for susceptible populations (Karns et al., 2007). *Salmonella* typhi and *Shigella* outbreaks have been associated with consumption of yogurt, milk, and milk products. These species have the ability to adapt and survive during long-term storage in food matrices. Thus, the higher susceptibility in young children of contracting Shigellosis and typhoid fever in addition to the high prevalence of *Salmonella* and *Shigella* show a great concern (Day et al., 2011). Soft cheeses show another concern to the dairy industry as they are a suitable environment to multiplication of *L. monocytogenes* (Melo et al., 2015).

Poor-quality silage is commonly contaminated with *L. monocytogenes* and ruminants can transmit the pathogen, either as asymptomatic carriers or by shedding it in their milk as a consequence of clinical or subclinical mastitis (Melo et al., 2015). Consequently, raw milk can be contaminated during milking by poor cleaning of equipment, during storage or during transport for cheese processing units, which can also be an inadequate cleaning (Almeida et al., 2013; Latorre et al., 2010). *Pseudomonas* genus, specially *Pseudomonas fluorescens*, *P. putida*, and *P. fragi*, also contribute to a large extent to the spoilage of proteinaceous raw foods stored under aerobic refrigerated (Quigley et al., 2013; Remenant et al., 2015). *Pseudomonas* spp. are involved in the spoilage of fresh-food products, as they can produce extracellular enzymes that are heat-stable and can remain viable during pasteurization or ultrahigh-temperature treatments (De Jonghe et al., 2011; Remenant et al., 2015). *Pseudomonas* genus can lead to color defects as a result of the ability of some species/strains to produce pigments on food like pyocyanin, a blue-green phenazine derivative, and yellow-green fluorescent pigments (Martin et al., 2011; Moore et al., 2006). Besides that, volatile compounds produced in different metabolic pathways of bacteria from *Pseudomonas* genera can cause off-flavor deterioration (Remenant et al., 2015).

Outbreaks of pathogenic *E. coli* have been epidemiologically linked with the consumption of various kinds of cheese (ICMSF, 2005). *E. coli* STEC is one of the most important pathogens in the microbiological safety of cheese due to its relatively high acid tolerance and the low infectious dose (Lee et al., 2012).

2.1.3 Meat, poultry, and seafood

For their intrinsic characteristics, such as chemical composition, high water content, and pH close to neutrality, meat, poultry, and seafood are a great medium for the multiplication of microorganisms. Multiple factors, including storage temperature, nearly neutral pH and nutrients are responsible for this category contamination (Gouvêa et al., 2016; Murmann et al., 2005). Meat and poultry, as well as other food products from animal origin, contain liquids and juices that escape from the internal meat tissues. These liquids, which are rich in nutrients, provide a medium for bacterial multiplication, causing spoilage, food-borne

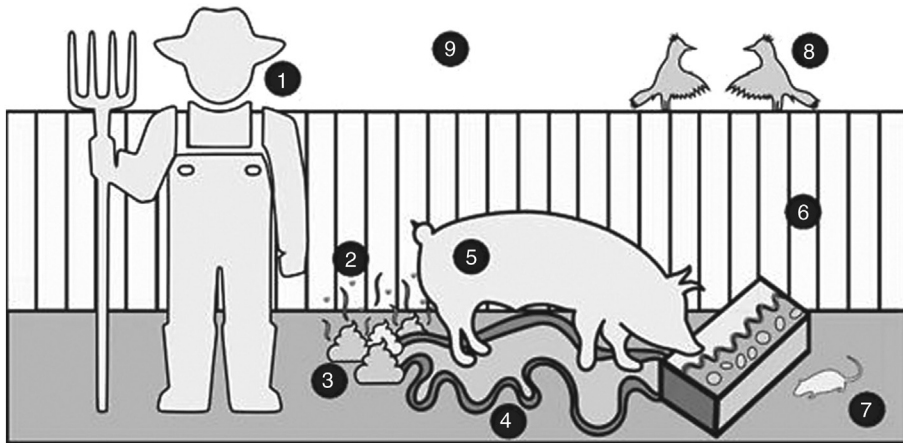


Figure 3.2: Principal Sources of Contamination of Animals Before and After Slaughter.

1: Cross contamination by handling; 2: animal feces; 3: insects; 4: residual water and cleaning of floors; 5: animal gut microbiota; 6: feed and water; 7: rodents; 8: birds; 9: wind.

illness, as well as products of fermentation (Gouvêa et al., 2016; Sofos et al., 2013). Except for the digestive tract, nasopharyngeal cavity and the end portion of the urogenital tract, tissue of healthy animals may be considered sterile, being contaminated during handling and processing.

Contamination originates from the external animal surface (Fig. 3.2), including the gastrointestinal tract, feces, as well as the environmental factors, such as air, soil, water, equipment surfaces, and humans (Lawrie, 2005; Mendonça et al., 2012; Sofos et al., 2013). Besides microbes, chemical and enzymatic activities can cause food loss. If pathogens are absent in a spoilage product, they are not necessarily harmful for the consumer, however, spoilage is considered as an economical loss (Sofos et al., 2013).

Competition for nutrients and ions, metabiosis or antibiosis, and cell-to-cell communication (like quorum sensing) can contribute to the selection of the dominant flora. Hence, different microbial species dominate in different environments and contribute to muscle food spoilage as they release different volatile compounds (Nychas et al., 2007).

The inactivation or removal of physical barriers and natural defense mechanisms of the animal during skinning and slaughter allows microbes to attach and multiply in the product. Metabolites associated with spoilage animal origin products result from substrate modifications by microbial contamination, enzymes, and other chemical reactions in the muscle, and it leads to the formation of off-flavors and off-odors compounds, discoloration, slime, or other physical–chemical characteristics of texture, which makes the food unacceptable for consumption. Chemical changes may be the result of competition among aerobic Gram-negative species or facultatively anaerobic Gram-positive bacteria (Gram et al., 2002; Nychas et al., 2007; Sofos et al., 2013).

Many types of bacterial strains, especially mesophilic and some psychrotrophic bacteria, can be isolated from refrigerated meat product. *Salmonella* sp., *Clostridium botulinum*, *C. perfringens*, *Campylobacter* sp., *E. coli*, and even *L. monocytogenes* are the most commonly found (Gouvêa et al., 2016). The bacterial species linked to spoilage of meat, poultry, and seafood products are from the genera *Pseudomonas*, as well as *Brochothrix thermosphacta* and LAB. With foods stored aerobically at cold temperatures, spoilage is caused through oxidative metabolism by aerobic or facultative anaerobic Gram-negative bacteria, such as *Pseudomonas* (*P. fragi*, *P. fluorescens*, *P. putida*, and *P. lundensis*), *Shewanella putrefaciens*, and *Photobacterium phosphoreum* (Gram and Huss, 2000; Stanbridge and Davis, 1998). Meat-fermented products, with lower pH values, combined with storage, may allow development of yeasts (Sofos et al., 2013). Spoilage differs among cooked, cured, heat-processed, fermented, or dried products of varying water activity and pH (ICMSF, 2005). Spoilage during storage of perishable, processed, or cooked, uncured meats is due to surviving or postcooking microbial contaminants, such as micrococci, streptococci, lactobacilli, and *B. thermosphacta* (Ingram and Simonsen, 1980).

In aerobic conditions, the cooled poultry is susceptible to deterioration by microorganisms that predominate on the product surface (Pardi et al., 2006). The main microorganisms involved in the process of deterioration of chilled poultry meat stored in the presence of oxygen, belong to the genus *Pseudomonas*, a Gram-negative bacteria. This genus is characterized as a major spoilage microorganism due to the production and extracellular proteolytic and lipolytic enzymes. Among the various species, *P. fluorescens* produces a green pigment and is often associated with the deterioration of fresh beef (Buňková et al., 2010; Lopez et al., 2015).

Salmonella spp. may be present in all types of animal and plant products and poultry products have this agent strongly linked to their image. This microorganism has become an important sanitary barrier in chicken meat exports. *Salmonella* spp. is one of the major causes of food-borne diseases and a problem for the poultry industry (Hungaro et al., 2013). Most infections by *Salmonella* spp. are associated with food consumption of animal origin, and poultry meat and by-products are considered to be the major vehicles of *Salmonella* spp. to humans (Hungaro et al., 2013; Marin and Lainez, 2009). The contamination may occur throughout the poultry production chain, and processing steps, such as head pulling and evisceration are considered potential risk factors that contribute to high incidence of *Salmonella* spp. in chicken carcasses. The infection by *Salmonella* genus in pigs is a great concern in the European Union (EU), being the second most important cause of salmonellosis in humans (De Ridder et al., 2013; Hauser et al., 2010; Van Parys et al., 2013). The incidence of salmonellosis has increased significantly in many parts of the world. Most serovars cause gastroenteritis without complications and without treatment. In cases of salmonellosis the food source for the infective dose may vary depending on the serovar.

Undercooked hamburgers from the same fast-food restaurant chain were identified as the vehicle, and *E. coli* O157:H7 was isolated from patients and a frozen ground beef patty. Outbreaks of *E. coli* O157:H7 infections have been primarily associated with eating undercooked ground beef, but a variety of other foods have also been implicated as vehicles (CDC, 2009; Mendonça et al., 2012). Generally, in raw fermented sausages produced under proper and controlled conditions, microbial food-borne pathogens can survive. Microbial safety concerns associated with fermented sausages relate to a bacterial food-borne pathogens originating from raw materials used for sausage production and to a lesser extent, toxic biogenic amines (BA), such as histamine and tyramine produced by background microbiota (Blagojevic et al., 2015). A number of outbreaks of toxicoinfections by verocytotoxigenic *E. coli*, *Staphylococcus aureus*, *Salmonella*, or *L. monocytogenes* is also associated with consumption of fermented sausages, raw fish, chicken, and beef (Al-Holy and Rasco, 2015; Holck et al., 2011; WHO, 2004). Abusive temperatures, inadequate conditions of slaughter and evisceration, extensive handling, and equipment and utensils used in food processing can enable the production of *S. aureus* toxins causing food poisoning. *S. aureus* is able to proliferate at temperatures ranging from 7 to 48°C. Although the temperature is sufficient to eliminate the pathogen, it is not sufficient to inactivate enterotoxins (Baeza et al., 2009; Jofré et al., 2008; Rode et al., 2007).

2.2 Commodities of Plant Origin

Food products of plant origin constitute the essential diet for people around the world and the healthy alternative for vegetarians and vegans. Plants provide several nutrients, including proteins, carbohydrates, and fibers. Cereal grains, fresh fruit, and vegetables, legumes, beverages, and nuts also offer a rich environment for development of spoilage or pathogenic microorganisms (Table 3.2), affecting the nutritional composition and safety of these products.

2.2.1 Fruits and vegetables

Despite the short rise in 2009, the consumption of fruits and vegetables decrease 7% in 2014. However, the expectation of consumption is 4% increase until 2020 among with the population growth (PBHF, 2015). The combination between the boost of consumption and the growth of foodstuff exportation associated with the different steps in the food-processing chain in which can occur microbiological contamination changed the food safety overview.

Fruits and vegetables have a high water content (approx. 95%), which contributed to fragility by exposition to microbial contamination and perishability (Carlin, 2013). Due to the environmental development and the abundance of nutrients, spoilage, or pathogen bacteria can be present in fruits and vegetables during growing, harvest, and processing (Carlin, 2013; Iqbal et al., 2015).

Table 3.2: Main microbial contamination in food products of vegetal origin and others food products.

Products	Microbe Types	Main Microorganisms Genera Present	Changes in Food Due to Spoilage	Barriers That Prevent Microbial Growth
Fruits and vegetables	Fungi Bacteria	<i>Alternaria</i> , <i>Fusarium</i> , <i>Penicillium</i> , <i>Aspergillus</i> , and <i>Basidiomycetous</i> <i>L. monocytogenes</i> , <i>E. coli</i> , <i>Clostridium</i> , <i>C. jejuni</i> , <i>Shigella</i> , <i>BAL</i> , <i>Pectobacterium</i> , <i>Erwinia</i> , <i>Xanthomonas</i> , <i>Pseudomonas</i> , <i>Acidovorax</i> , and <i>Bacillus</i>	Softening of tissue, dark lesions, dark spots, gangrene, sour and soft roots, exopolysaccharides production	pH, cuticle, phenolic, tannins, sulfur-derived and terpenoids compounds
Fermented vegetables	Bacteria	<i>Lactobacillus plantarum</i> , <i>BAL</i> , and <i>Enterobacter</i> species	Degradation of vegetable material by enzymes	pH, salt, or sugar concentrations
Cereals, legumes, and nuts	Bacteria Molds	<i>Erwinia carotova</i> , <i>B. cereus</i> , <i>Clostridium</i> , <i>C. sakasakii</i> , <i>Salmonella</i> , <i>E. coli</i> , and <i>L. monocytogenes</i> <i>Alternaria</i> , <i>Cladosporium</i> , <i>Fusarium</i> , <i>Helminthosporium</i> , <i>Aspergillus</i> , <i>Rhizopus</i> , <i>Penicillium</i> , <i>Bipolaris</i> , <i>Acremonium</i> , <i>Epicoccum</i> , <i>Nigrospora</i> , <i>Mucor</i> , and <i>Wallemia</i>	Softening of tissues; oxidative rancidity of fats	Low water activity, thick shells, tannin content; amino acid composition, waxy content, permeability, and cell structure arrangement
Spices and herbs and sauces	Bacteria Fungi	<i>E. coli</i> , <i>L. monocytogenes</i> , <i>Salmonella</i> , <i>B. cereus</i> , <i>C. perfringens</i> , <i>Cronobacter</i> spp., <i>S. aureus</i> , <i>BAL</i> , such as <i>Weissella</i> spp., <i>Pediococcus</i> spp., <i>Enterococcus</i> spp., <i>Leuconostoc</i> spp., <i>Lactobacillus</i> spp., and <i>Z. bailii</i> <i>Aspergillus</i> spp., <i>Penicillium</i> spp., <i>Alternaria</i> spp., and <i>Fusarium</i>	Not reported yet	Presence of eugenol, cinnamic aldehyde, carvacrol, thymol, borneol, pinene, and other compounds
Canned foods	Bacteria	<i>C. botulinu</i> , <i>Geobacillus</i> , <i>Alicyclobacillus</i> , <i>Thermanaerobacterium</i> , <i>Staphylococcus</i> , <i>Klebsiella</i> , and <i>Bacillus</i> spp.	Stuffing, increase of acidity, sour flavor, and rancidity, egg, putrid, and butyric odor, fermented, blackened appearance, discoloration, and coagulation	Thermal process
Beverages	Bacteria Parasite Fungi Yeast Molds	<i>Alicyclobacillus</i> , <i>E. coli</i> , <i>Samonella</i> , <i>Propionibacterium cyclohexanicum</i> , <i>Clostridium</i> , <i>Erwinia</i> , <i>LAB</i> , and <i>Acetic bacteria</i> <i>Cryptosporidium</i> <i>B. fulva</i> , <i>B. nivea</i> , <i>N. fischeri</i> , and <i>Talaromyces</i> <i>Pichia</i> , <i>Candida</i> , <i>Saccharomyces</i> , and <i>Rhodotula</i> <i>Penicillium</i> , <i>Cladosporium</i> , <i>Aspergillus</i> , <i>Botrytis</i> , and <i>A. pullulans</i>	Production of CO ₂ and alcohol in fruit juice, turbidity, flocculation, pellicles, fermented flavor, mycelial mats, musty, and off-flavors	Polyphenols, pH, solid content

The microbial load of fruits and vegetables can increase by contact with air, soil, water, insects, and other animals, humans (especially, postharvest handling), storage and transport containers, and processing equipment (Fig. 3.1). Losses in the food supply is due to the contamination of spoilage bacteria. Therefore, the association of good agricultural practices and good manufacturing practices are efficient to reduce contamination by bacteria and fungi (Baudoïn et al., 2013; Martínez-Vaz et al., 2014).

The main factor that may prevent bacterial growth in fruits is pH, in some citrus fruits it can be around 2.0. Other compounds, such as phenolic, tannins, sulfur-derived, terpenoids, and external polymers (as cuticles) can inhibit bacterial growth in fruits and vegetables (Carlin, 2013). Deterioration of fruits and vegetables with low pH is restricted to molds and yeast (Carlin, 2013). A few vegetables have a pH close to neutrality, which can contribute to a larger contamination, especially gram-negative bacteria (Barth et al., 2009; Carlin, 2013). Pathogens or spoilage microorganisms can cause several biochemical changes in physiology and composition of fruits and vegetables. Pectolytic genus of fungi (e.g., *Alternaria*, *Fusarium*, *Penicillium*, *Aspergillus*) and bacteria (e.g., *Clostridium*, *Pseudomonas*, *Erwinia*, and *Bacillus*) are responsible for the pectin hydrolysis, generating softening and loss stiffness of tissues, and nonreversible damages of these products.

Soft areas in fruits and vegetables may suffer a second contamination by fungi (Carlin, 2013; Blasco et al., 2011). Also, bacteria can be established on the surface of fruits and vegetables forming biofilms and, for instance, producing viscous exopolysaccharides that causes deterioration. Furthermore, fungi (especially, *Basidiomycetous*) can cause dark lesions, dark spots, gangrene, sour and soft roots, and produce cutinase that is responsible for cuticle degradation of several fresh products (Carlin, 2013).

Shelf life and biodeterioration depends on microbial colonization, growth time, and conditions, such as nutrients and food conservation processes. Beyond the spoilage microbiota present in fruits and vegetables, some cases of cross-contamination can compromise the microbiological safety in fruits and vegetables. The concern about the presence of microbial pathogens, especially in ready-to-eat products and their relation with the most important outbreaks revealed *Salmonella* spp. (Martínez-Vaz et al., 2014; Talaro and Chess, 2015), *E. coli* O157:H7 (Talaro and Chess, 2015), *Campylobacter jejuni*, *Shigella* (Talaro and Chess, 2015), and *L. monocytogenes* (CDC, 2016), as well, helminths acquired and mycotoxins producers in these raw materials and may cause serious gastroenteritis and are a potential risks for humans (Hayunga, 2013).

In relation to fermented vegetables that were stored for 1 year or more without refrigeration, yeast, molds, *Pseudomonas*, *Erwinia*, and *Enterobacter* species are responsible for deterioration of the vegetable material due to expression of enzymes, such as proteases, lipases, amylases, nucleases, and others. However, due to LAB characteristics, these bacteria dominate the growth in these kinds of products.

2.2.2 Cereals, seeds, nuts and other products with low moisture

Cereals are one of the basis of worldwide feeding, they are important sources of micronutrients (vitamins and minerals) and macronutrients, being starch, proteins, and lipids the most significant ones (Sarwar et al., 2013). Seeds and nuts are an important source of carbohydrates and proteins, as well as oils, especially unsaturated fatty acids, and, in minor amounts, minerals, and vitamins (Freitas et al., 2012). This group of raw materials and products includes foods with low water activity that contributes for microbiological stability (Brar et al., 2016). The multiplication of molds and yeasts is frequent, however, there is often the presence of bacteria. Bacterial contamination is related to contact of product with insects, rodents, soil, water, foreign material, and ripeness, form of drying, and integrity of product. Animal feces also can be a vehicle of bacterial contamination in crops, especially if nuts fall down in the harvest process (Harris et al., 2013).

The spoilage of grains, seeds, and nuts occur preferentially when the crop is injured during harvesting or posterior steps, giving access to internal tissues. The proliferation causes softening of the plant tissues and leads to further degradation. The main bacteria that causes damages to grains is *Erwinia carotovora*, but *Bacillus* spp. and *Clostridium* spp. are often found in these products and they are responsible for the tissue softening by the production of pectolytic enzymes and toxin production (Harris et al., 2013; Montville and Matthews, 2007).

Cereal grains have a rich and varied microbiota in all production stages (Fig. 3.3). Molds, such as *Alternaria*, *Cladosporium*, *Fusarium* (Harris et al., 2013; Pavón et al., 2011) and *Helminthosporium* may contaminate cereal grains before harvest if climatic conditions are favorable mainly in wheat and barley crops. These groups of fungi can reduce the quality by damage in grain and produce mycotoxins. During storage another kind of contamination may occur. Postharvest contamination is the responsibility of molds, such as *Aspergillus*, *Rhizopus*, *Penicilium*, *Mucor*, and *Wallemia* when drying of grains is inadequate and the levels of mycotoxins can be increased in this stage of production (Aneja et al., 2014; Harris et al., 2013). Bacteria also can be present in cereal grains: LAB, micrococci, bacilli and enteric bacteria are kinds of microorganisms (Harris et al., 2013) (Fig. 3.4).

S. aureus can grow in cereal products like bread and pasta and derivatives by improper handling or lack in production, causing illness due to their ability to produce toxins (Harris et al., 2013). In the other hand, *Bacillus cereus*, *C. botulinum*, and *Cronobacter sakazakii* are the main bacteria involved in outbreaks related to infant cereals, milk powders, and rice seeds (Duc et al., 2004; Fakruddin et al., 2014; Molloy et al., 2009; Singh et al., 2015a). Few spores are enough to cause infant botulism (Sugiyama, 1979).

In relation to nuts and products the presence of poreless thick shells gives an important barrier to microbial entry. Oxidative rancidity of fats by fungi enzymes is the main cause of spoilage in nuts (Harris et al., 2013). The genera more usually found in these products are *Alternaria*, *Bipolaris*, *Acremonium*, *Cladosporium*, *Epicoccum*, *Helminthosporium*, *Nigrospora*, and

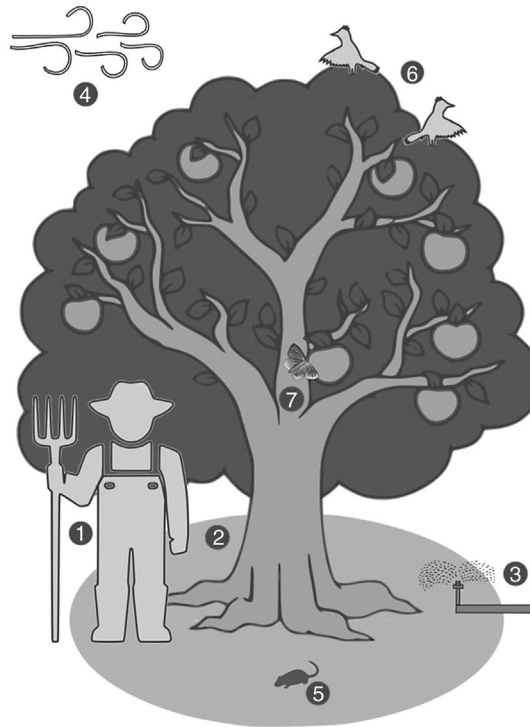


Figure 3.3: Some Sources of Contamination in Fruits and Vegetables.
 1: Handling; 2: soil; 3: irrigation water; 4: wind; 5: rodents; 6: birds; 7: insects.

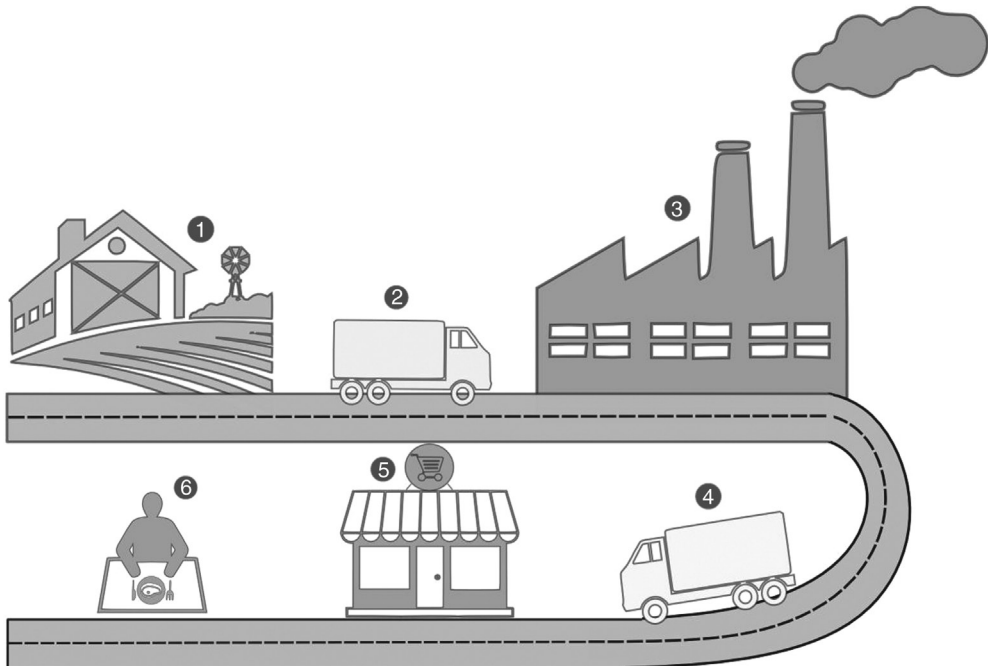


Figure 3.4: Critical Points of Contamination in Fruits, Vegetables, or Grain Production.
 1: Farm; 2: transport at industrial sector; 3: processing; 4: transport and distribution;
 5: commercialization; 6: home handling.

others (Harris et al., 2013; Jain et al., 2008). However, greater concerns about fungi are *Fusarium* spp., *Penicillium* spp., and *Aspergillus flavus*, the main fungi connected with the production of mycotoxins (Pinotti et al., 2016). Some factors can provide resistance to *A. flavus* in this kind of product include increased tannin content; amino acid composition, waxy content, permeability and cell structure arrangement (Harris et al., 2013).

Low activity water foods, such as seeds, nuts, chocolate, powdered milk, cereals have been identified as *Salmonella* vehicle (Bedard et al., 2014). *Salmonella* is the bacterial food-borne pathogen most prevalent in nuts and nut products and their survival has been reported for at least 1 year under frozen and refrigeration conditions (Bedard et al., 2014; Brar et al., 2015, 2016). However, the most concerning genera of bacteria in the context of inadequate food processing is *Bacillus* and *Clostridium*. These bacteria have the ability to form endospores, which can survive during thermal treatments and germinate in derived products (Daczowska-Kozon et al., 2009). *E. coli* O157:H7 has been associated with the main outbreak by consumption of in-shell hazelnuts and can survive in nuts for several months to years (Harris et al., 2013; Miller et al., 2012). *L. monocytogenes* also has been isolated of products, such as walnuts, peanut butter, sunflower seeds, snack mixes, and popcorn (Brar et al., 2015).

2.2.3 Spices, herbs, and sauces

Dehydrated or fresh parts of plants and leaves, roots, bark, buds, or fruit of aromatic plants are used in most of processed foods due to their ability to provide different flavors, colors, and scents to the final product (Davidson et al., 2013). Besides that, spices and herbs have a potential use as natural antimicrobial compounds against food-borne bacteria, being considered as natural preservatives (Davidson et al., 2013; Witkowska et al., 2013). Although spices, herbs, and dried foods have been associated as vehicles of different microorganisms to the food products.

When the subject is fungi, usually aflatoxins producers, these condiments suffer spoilage if postharvesting transport and storage are not controlled. The multiplication of fungi requires a water activity around 0.85, which can be reached especially during storage under elevated humidity (Tomar et al., 2013). Different species of fungi are aided by spices, being *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., and *Fusarium* spp. the most frequent genera (Ibrahim et al., 2014).

The main problem about spices and herbs is that they are added in the food without or with minimum preliminary processing, showing a potential risk of cross-contamination (Säde et al., 2016; Vitullo et al., 2011; Wójcik-Stopczyńska et al., 2009). In addition, resident enteropathogens are a big concern in products containing these additives because they have the ability to multiply and produce enterotoxins in the human gastrointestinal tract (Jay, 2005). For these reasons these raw materials and products should be sterilized to avoid colonization by pathogens and wild fermentations. Also, plants and vegetables are a natural environment for BAL, these bacteria become a concern for food spoilage for those products

that use spices as ingredients in the food-processing chain. The principal BAL found in these kinds of products are *Weisella* spp., *Pediococcus* spp., *Enterococcus* spp., *Leuconostoc* spp., and *Lactobacillus* spp. (Säde et al., 2016)

The bacterial genera widely found in spices and herbs are *E. coli*, *Salmonella* spp., *L. monocytogenes*, and *S. aureus* (Vitulo et al., 2011). *B. cereus* and *C. perfringens* and their spores are also found in these products and its derivatives; these two species have an extra technological barrier due their ability to form endospores and to resist thermal treatments (Addis and Sisay, 2015; Wójcik-Stopczyńska et al., 2009). *Cronobacter* and former spores also have been reported in dry food ingredients, such as herbs and spices (Setlow and Johnson, 2013).

Sauces are known by their complex microstructure and rheological characteristics. These products include emulsions, such as mayonnaise and concentrated suspensions, such as ketchup. Due to their characteristics, these products have a low pH and low water content and their biodeterioration is caused by yeast and BAL. *Zygosaccharomyces bailii* is a main problem in these products, because it has an osmophilic behavior and is resistant to organic acids used as preservatives (Dang et al., 2010).

3 Other Food Products

3.1 Canned Foods

The process of canning foods was originated in order to prevent the proliferation of aerobic microorganisms and to ensure longer shelf life to unstable foods, such as meat products, seafood, dairy, certain vegetables and fruits. The thermal treatment required depends on the acidity of the final product. Low-acid canned foods (pH > 4.5) should be “commercially sterile” to guaranteed acceptable levels of bacteria and any thermoresistant spores, especially of *C. botulinum*. High acid canned food (pH < 4.5) pasteurization is enough to prevent spoilage, because most species of spore-forming bacteria do not grow in high acidity environments and processes severe like commercially sterilization lead to loss of nutrients and quality food (Jay, 2005; Setlow and Johnson, 2013).

When the thermal process is incomplete or inefficient or in cases of damage to the cans during packing, transport, and storage can lead to microbial contamination of canned products (Oranusi et al., 2012). The contamination is restricted to Gram-positive bacteria, such as *Clostridium* spp., *Geobacillus*, *Alycyclobacillus*, *Thermanaerobacterium*, and *Bacillus* spp. This forming of spores species that generate food spoilage or toxin production are a particular concern in the food-canning industry. Rarely, some species of *Staphylococcus* and *Klebsiella* can be present. (Addis and Sisay, 2015; Oranusi et al., 2012).

Spoilage in these products is associated with thermophilic flat-sour organisms, thermophilic anaerobes not producing and forming hydrogen sulfide, putrefactive anaerobes, facultative

Bacillus mesophiles, butyric clostridia, lactobacilli, and heat-resistant molds and yeast (Setlow and Johnson, 2013). Spoilage in canned products can be observed by stuffing due to the fermentation of carbohydrates and gas production. The main characteristic observed is the increase of acidity, sour flavor, and rancidity, egg, putrid and butyric odor, fermented, blackened appearance, discoloration and coagulation (especially in canned milks) (Oranusi et al., 2012; Setlow and Johnson, 2013).

3.2 Beverages

Beverage spoilage has been attributed mainly to lactic acid and osmophilic bacteria, yeast and fungi that produce heat-resistant ascospores (Jay, 2005; Setlow and Johnson, 2013). The concern for beverages is less, because most beverages have a pasteurization process that aims for inactivate spoilage and pathogens bacteria and additionally the beverage conservation chain includes refrigeration. Fruit juices can be vehicle of pathogens, such as *E. coli* O157, *Salmonella*, and *Cryptosporidium* and several spoilage bacteria, such as *Propionibacterium cyclohexanicum* and heat-resistant fungi, such as *Byssochlamys fulva*, *B. nivea*, *Neosartorya fischeri*, and some species of *Talaromyces*. Yeast, such as *Pichia*, *Candida*, *Saccharomyces*, and *Rhodotula* may produce CO₂ and alcohol in fruit juice, turbidity, flocculation, pellicles, and fermented flavor. Molds, such as *Penicillium*, *Cladosporium*, *Aspergillus*, *Botrytis*, and *Aureobasidium pullulans* may produce mycelial mats, musty and off-flavors in fruits juice and adhere to the inside of the material package (Aneja et al., 2014).

In fruits, juices have been possible to find acid tolerant bacteria as *Erwinia*, *Enterobacter*, *Clostridium*, *Alicyclobacillus*, *Propionibacterium*, *Pseudomonas*, and *Bacillus* as spoilage bacteria (Aneja et al., 2014). Some spoilage bacteria able to resist temperature–time and pH combination as *Alicyclobacillus acidoterrestris* and their endospores can be present in beverages after pasteurization process, germinating and leading to deterioration product (Hünniger et al., 2015; Smit et al., 2011). *Alicyclobacillus* bacteria is not pathogenic and can be inhibited by the high soluble solid content in juices; concentrated juices are less prone to contain this kind of bacteria, however, after dilution endospores can germinate (Smit et al., 2001; Steyn et al., 2011).

Alicyclobacillus deterioration in beverages, mainly apple juice, cause undesirable organoleptic characteristics, such as off-flavor and reek (due to guaiacol or halophenols compounds), solid settling, increase in turbidity and color loss. The most of the production-related spoilage problems with *Alicyclobacillus* mainly in spring or summer (Hünniger et al., 2015; Smit et al., 2011). Deterioration from *Alicyclobacillus* is visually imperceptible, because spoilage is not associated with gas or acid production (Steyn et al., 2011).

Heterofermentative LAB, such as *Lactobacillus*, *Pediococcus* spp., and other LABs are responsible by spoilage of beers and fruit juices. These microorganisms produce lactic acids and to a lesser amount can produce other substances, such as acetic and gluconic

acids, ethanol, and CO₂ (Aneja et al., 2014; Steyn et al., 2011). Acetic acid bacteria, such as *Acetobacter*, *Gluconobacter*, and *Gluconacetobacter* are capable of growing in low nutrient and low pH conditions, producing sour and vinegar flavor in fruit juices. Pathogenic bacteria, such as some strains of *E. coli*, *Shigella*, *S. aureus*, *C. botulinum*, *Vibrio cholerae*, *Salmonella*, and *Cronobacter* also has been isolated from acidic beverages and some strains may remain viable until several weeks due to their regulation of internal pH (Aneja et al., 2014).

4 Development of Microbial Resistant in Food Industry

Food products can represent a complex environment for bacteria. Several factors, such as pH, osmolarity, temperature, oxidation, and preservation methods can contribute to bacterial stresses. Although modern strategies are used for food preservation, bacterial cells have the ability to evolve or adapt to stresses, host defense or several situations that can cause damages on cellular complex (Alvarez-Ordóñez et al., 2015). Bacterial adaptation can proceed acquisition of stochastic mutations, changes in the pattern of gene expression, extreme regulation of genes in order to maintain the viability and adaptive response (Alvarez-Ordóñez et al., 2015). Bacterial adaptation is reflected in bacterial physiology, thereby, some changes can occur in several levels of expression, such as changes in DNA conformation, lipids, proteins, modulation of cellular envelope, and other structures with the aim of keeping the bacterial homeostasis. Bacteria can use several mechanisms to obtain resistance. Transference horizontal of genes, changes in the cell envelope (Bikard and Marraffini, 2012; Pereira et al., 2016), modification/restriction systems (Samson et al., 2013), production of shock proteins and chaperons and efflux pump are the main mechanism resistance involved in bacterial cells.

These mechanisms of bacterial resistant achieved in the food industry are more complex to explain and quantify than occur with antibiotics. Methods for characterizing microbial resistance in the food industry have not development yet (Alvarez-Ordóñez et al., 2015; Zhang et al., 2013). Resistance mechanisms have been widely studied through in vitro systems (Zhang et al., 2013), but it is very difficult to estimate if the same behavior will succeed in food industrial environment.

5 Control of Pathogen and Spoilage Bacteria in Food Industry

Chemical and physical technologies and treatments, disinfectants, and sanitizers are used in all stages of the food industry to guarantee microbiological quality of raw material and products, due to their direct contact with contaminated sources: human, soil, water, among others. Ready-to-eat products preserved by use of combinations environmental factors, such as temperature, atmosphere gas content, water activity (a_w), have less chance of being colonized by demanding spoilage and pathogenic bacteria, keeping the shelf life for a longer

time (Carlin, 2013). In relation to preservation using temperature, heat, and frozen have been used since early times for food modification (textures, flavors, or novel foods) and food preservation. Even so, food preservation or food processes by heat has been related with allergenicity of foods, mainly when the product was treated by heat or has a process evolving Maillard reaction (Jiménez-Saiz et al., 2014).

On the other hand, when foods have been preserved by drying, salting, smoking, fermenting, or others, there exists a concern about safety. The concern is related to the facts that, as described, bacteria can obtain resistant to these mechanism of control, the risk that some of these methods may offer to health and the fact that these traditional methods were development before the materialization of refrigeration (Motarjemi et al., 2014). Nowadays, it is known that thermal treatments are not applicable to all products and sophisticated and more controlled technologies are disposal and being developed to outwit bacterial resistance in the food industry. Novel technologies seek to ensure microbiological quality and prevail the integrity of macro- and micronutrients present in food products. High hydrostatic pressure (HHP), irradiation, electric pulsed field (EPF), biological control, and others are the most emergent and novel techniques used in food preservation. For example, milk has been subjected to conservation treatments, including pasteurization, heat sterilization, fermentation, dehydration, refrigeration, thermization, pulsed electric field (PEF), high-pressure processing, bacto-fugation, biopreservation, and freezing as preservation treatments. The combination of these processes with separation techniques, such as membrane filtration enables the consumer a variety of safe dairy products and/or with higher shelf life (Nsofor and Frank, 2013). In relation to egg products several other processing interventions (thermal and nonthermal) have been applied to reduce the microbial load on the shell and avoid egg' deterioration. Nonthermal methods are under development to decontaminate the shell egg, such as microwave pasteurization, ultrasounds, gas plasma, ozone, irradiation, ultraviolet light technology (Alamprese, 2015; Galiş et al., 2013; Wu, 2014).

Microwave pasteurization is considered a superior intervention to the water bath pasteurization method in terms of microbial reduction efficiency (Berardinelli et al., 2011; Lakins et al., 2008) The application of ultrasonic vibration during the heat treatment reduces drastically the microbial load of some products including eggs. Also, this application shows a minimal impact on their sensory characteristics, keeping the integrity of the inner content in eggs (Aygün and Sert, 2012).

The gas plasma and ozone technology are similar nonthermal proceedings to reduce microbial load on the egg surface. The gas plasma is produced at atmospheric pressure, and is characterized by low levels of electronic density and gas temperature (Moreau et al., 2008). The microbicide effect is due to the presence of reactive oxygen and nitrogen species, such as OH and NO radicals, atomic oxygen, ozone, and NO₂. This compound affects the microbial membrane (protein and lipids), involving an oxidative process resulting in cell lysis. This treatment involves the application of the gases or just ozone in a sealed chamber filled

with eggs. Since the application does not result in apparent changes in the egg's physical and chemical properties, and the microbial killing effect is not limited to the shell, but also extends to a degree into the egg pore, this methodology has a potential to be implemented by the egg industry (Berardinelli et al., 2011; Ragni et al., 2010; Yüceer et al., 2016). Ozone is one of the most potent biocide and sanitizing treatments approved by FDA. Ozone is able to oxidize several structures, leading the bacterial cell to death. Ozone treatments need short times for contact to be efficient and can be used in in vivo systems (Ronholm et al., 2016).

Irradiation is a treatment that consists in the food exposure to ionizing radiation from electromagnetic waves or high-energy electrons, ray (alfa, beta, gamma, X, and cosmic) (Jiménez-Saiz et al., 2015; Pasha et al., 2014). Irradiation has been used to kill bacterial pathogens, insects and worms in several food matrixes, such as flour to pork, ground beef, eggs, fruits, and vegetables. The irradiation was declared safe by the FDA, the US Department of Agriculture (USDA), the Centers for Disease Control and Prevention (CDC), and World Health Organization (WHO) for treatment of beef, pork, poultry, fish, eggs, spices, grains, and fruits and vegetables. However, there is concern by the loss of nutrients, organoleptic characteristics, consumer acceptance, and any other consequence of ionizing radiation (Li et al., 2013; Säde et al., 2016; Talaro and Chess, 2015). The protein exposure to radiation causes denaturation due to the generation of several radicals from radiolysis. Radiation induces the fragmentation and protein conglomerates by strong interactions like electrostatic. Irradiation can be considered a nonthermal process if the exposure is short, however, when the process is made for long periods, an increase in temperature may be reached and consequently the start of food water evaporation (Jiménez-Saiz et al., 2015).

UV light is a powerful bactericidal agent that inactivate bacteria and viruses by DNA damages due to pyrimidine dimers generation that avoid DNA replication (Aneja et al., 2014; Cebrián et al., 2016; Jay, 2005). Low doses of irradiation cause fewer changes in most fresh fruits and vegetables, but appearance, flavor, color, and texture may be affected in others products (Abida et al., 2014; Chen et al., 2010; Lasagabaster et al., 2011; Pasha et al., 2014). The irradiation efficiency depends on microorganism resistant, food composition, food moisture, temperature during irradiation, oxygen concentration, and food status: fresh or frozen (Mohácsi-Farkas et al., 2014). UV light has been used for decontamination of air, water, and surfaces. Recently, the food industry has been showing interest in the use of UV light for decontamination of liquid or solid foods (Cebrián et al., 2016).

High hydrostatic pressure processing (HPP) or pascalization is used in food industry for reduce or inactivate the food microbiota and enzymes, contributing to an increase of shelf life and keeping organoleptics and nutritional characteristics (Aneja et al., 2014; Jiménez-Saiz et al. 2015; PASHA et al., 2014). HPP procedures based in the application of up to 15,000 MPa pressures to food (Cebrián et al., 2016). HPP technologies with or without heat inactivating spoilage and pathogenic bacteria, yeast, molds, viruses, and spores (Aneja et al., 2014; Balasubramaniam et al., 2015). The HPP protocol is a combination of several

reactions, mainly, cellular membrane permeability and disintegration of covalent bonds (Pasha et al., 2014). Spores are inactivated using approximately 600 MPa and enzymes can be inactivated to 300 MPa (Cebrián et al., 2016; Pasha et al., 2014). During HPP process, the pressure is applied uniformly and instantly using a low compressibility liquid on food, independent of variables, such as mass, form, and composition (Patterson, 2005; Argyri et al., 2014). In the process, the adiabatic heat results in the raise of temperature from 3 to 6°C for each increase of 100 MPa. The increase in temperature depends on the system, nature of the product, process temperature, and applied pressure (Pereira and Vicente, 2010; Rajkovic et al., 2010). HPP's treatments have a lethal effect on microorganisms, affecting the cytoplasmic or membrane enzymes and inactivating some key enzymes for DNA replication processes and events of transcription (Cebrián et al., 2016; Pasha et al., 2014). In addition, HPP can cause disturbances in cellular metabolism and induce the endogenous reactive oxygen species (ROS) production (Cebrián et al., 2016).

PEF is a nonthermal technology of preservation that uses short electric blasts for microbial inactivation without affecting sensorial and nutritional properties of several foods (Cebrián et al., 2016; Pasha et al., 2014; Singh et al., 2015b). This technology is able to fluid foods in batch or continuous flow are placed between two electrodes and due to electroporation phenomena, affecting the cell membrane of microorganism and lead to shedding cytoplasmic content (Aneja et al., 2014). Permeation is reversible when the electric field is below critical value in a short time; however, if the process is made with high intensity, the results are lost turgescence, leakage of cytoplasmic contents, and cellular lysis (Jaeger et al., 2009). PEF applications cause alterations in electrical potential of cell membranes resulting in charge separation (Binoti et al., 2012; Singh et al., 2015b). This kind of treatment represents a promising alternative of food preservation, due to some advantages, such as few alterations of flavor and nutrients, microbial elimination, and inactivation of enzymes. The most important factor that justifies their application is the possibility of offer to consumer's healthy products with fresh appearance (Buckow et al., 2013; Cebrián et al., 2016; Pasha et al., 2014).

5.1 Bacteriophages as Potential Tool of Food Preservation

Bacteriophages are intracellular entities that infect prokaryotes helping to keep microbial balance in the Earth (Samson et al., 2013; Sulakvelidze, 2013; Wittebole et al., 2014). Phage therapy precedes the use of antibiotics and surrounds the use of bacteriophages as antibacterial agents (Levin and Bull, 2004). The bacteriophages were first described approximately 100 years ago by Frederick Twort. In 1915, Twort reported lysis in cell culture colonies of *S. aureus*. It was only 2 years later that Felix d'Herelle observed similar lysis and the "entities" that caused them were filtrated, tested, and named bacteriophages or phages (Ackermann, 2012; Taylor, 2014). In 1919, the phages were used to successfully treat hospitalized patients with dysentery. It was only in 1930 that the first product containing bacteriophages by the Institute of Vaccine and Sera, in Tbilisi, Georgia (Chanishvili, 2012;

Verbeken et al., 2014). Since bacteriophage discovery, Russia, Europe, and the United States have been used bacteriophages as alternatives to antibiotics (www.2015phage.org).

With the discovery of antibiotics in 1940, the researches with bacteriophages to control bacteria decreased (Guimarães et al., 2010; Hungaro et al., 2014; Taylor, 2014). The indiscriminate use of these chemicals was the mainly the agent to the development of resistant bacteria, responsible for thousands of deaths annually. Nowadays, the research and discovery of new and more powerful antibiotics is stagnated, forcing the development of new mechanisms for microbial control. In this way, the researches using bacteriophages were reinitiated in many fields, from public health to food safety and the knowledge about phages has been increased allowing the possibility of use in several areas (Golkar et al., 2014; Nilsson, 2014; Serwer et al., 2014; Verbeken et al., 2014; Wittebole et al., 2014).

The proposal of phage therapy is the use of natural lytic phages for the treatment of bacterial infections, and seems a good idea, because phages are abundant in nature (Wittebole et al., 2014), are easily isolable, are selective or not (Hyman and Abedon, 2012; Pereira et al., 2016), they can effectively kill bacteria at least in controlled laboratory conditions and bacteriophages have the same efficiency of antibiotics and sanitizers (Hungaro et al., 2013; Nilsson, 2014; Pereira et al., 2016).

Bacteriophages can infect only specific species of bacteria or even only strains of a species (Hyman and Abedon, 2012). The specificity is mediated by the proteins associated with the tail, which can attach in the bacterial cell surface. After the adhesion process, the lytic phage injects their genetic material in the host and the use of the host metabolic resources to multiply and generate new viral particles, causing the cell death by a process named lysis (Ackermann, 2012; Hyman and Abedon, 2012; Wittebole et al., 2014). The bacteriophages, which develop in a lytic cycle, show the potential to the biocontrol of food-borne pathogens and spoilage bacteria, enhancing food microbiological safety (Endersen et al., 2014). Besides that, they can be used at any part of the production chain, from the field to the consumer. For example, they can be used to control pathogens in live animals, to decontaminate carcass, equipment, and surfaces of contact in the food industry to extend the shelf life of perishable products (Coffey et al., 2010; Endersen et al., 2014; Garcia et al., 2008; Hungaro et al., 2014; Nobrega et al., 2015; Ronholm et al., 2016; Spricigo et al., 2013).

The potential use of phages and their lytic enzymes as alternatives to biocontrol was discussed in recent revisions, for the control of *Salmonella* have already been tested in swine before slaughtering (Albino et al., 2014; Callaway et al., 2010), chickens before slaughtering (Wong et al., 2014), poultry, egg shells, and lettuce (Spricigo et al., 2013), sausage, turkey, and seafood (Guenther et al., 2012) and also packages (Gouvêa et al., 2015). Many characteristics of the bacteriophages can favor its utilization in the food industry. Among them, unlike the antibiotics, the bacteriophages can evolve to outline bacterial resistance

Table 3.3: Main companies of bacteriophages products in the world.

Source: Website of companies. Company/Country	Website	Products Available in Market
AmpliPhi Biosciences (EUA)	http://www.ampliphio.com/our-science.html	ListShield; SalmoFresh; EcoShield; ShigActive; INT-401
Enbiotix (EUA)	http://enbiotix.com/	
Fixed Phage (UK)	http://www.fixed-phage.com/	
InnoPhage (PT)	http://www.innophage.com/	
Intralytix (EUA)	http://www.intralytix.com/	
Novolytics (UK)	http://www.novolytics.co.uk/	
Pherecydes Pharma (FR)	http://www.pherecydes-pharma.com/	
Sarum Biosciences (UK)	http://www.sarumbiosciences.co.uk/projects.htm	
Synthetic Genomics (EUA)	http://www.syntheticgenomics.com/	
Technophage (PT)	http://www.technophage.pt/	
Enbiotix (EUA)	http://enbiotix.com/	
InnoPhage (PT):	http://www.innophage.com/	
Epibiome (EUA)	http://epibiome.com/	
BioPhage (CA)	http://www.biophagepharma.net/index.php/en/	
PhageLux (CN)	http://www.phagelux.com/Index-English.htm	
APS Biocontrol (UK)	http://apsbiocontrol.com/	
Micreos Food Safety (NL)	http://www.micreos.com/	
Omnilytics (EUA)	http://www.omnilytics.com/home3.html	
JSC BIOCHIMPHARM	http://www.biochimpharm.ge/eng/indexeng.html	Biolyse Listex; Salmonalex; Staphfekt TM AGRIPHAGE Phagesti; Phagyo; Phagetyph; Phagestaph; Phagesal; Phagepy Staphage Lysate (SPL) BIG-LACTO α StaphTAME Lysando molecules; ARTILYSIN Sample6 phage DETECT/L Solu1 on Kit
Delmont (EUA)	http://www.delmontlabs.com/	
Biogenoci Co. Ltda (KR)	http://www.biogenicgroup.com/index_en.php	
GangaGen (EUA, IN)	http://www.gangagen.com/index.html	
Lysando GmbH (DE)	http://lysando.com/	
New Horizons Diagnostic (EUA)	http://www.nhdiag.com/index.htm	
Sample6 (EUA)	http://sample6.com/	
Jafral (SVN)	http://www.jafral.com/en/	

(Bikard and Marraffini, 2012; Jassim and Limoges, 2014; Labrie et al., 2010; Nilsson, 2014; Pereira et al., 2016).

A distinguished amount of enterprises produce and commercialize products with bacteriophages and their products (Table 3.3) considered by FDA as completely natural, safe, and with efficiency to reduce bacterial contamination in different food products (FDA, 2016). In according to companies related in Table 3.3, the use of bacteriophages

commercialized products have been used for several applications and objectives, including prevention and treatment and prevention of infection illness that affect humans and animals, phytopathogens control (Endersen et al., 2014; Sulakvelidze, 2013; Żaczek et al., 2015), biocontrol and detection of pathogens microorganisms related to foods, bacterial biofilms reductions, applications of phage lysins as medicines and many others applications (Hungaro et al., 2014).

In relation to Latin-American countries, there does not exist companies that produce and commercialize phages and derivative products. The main problem with this is the regimentation (<http://www.listex.eu/regulatory/>; Sulakvelidze, 2013).

6 Final Considerations

The food industry is sensitive to contamination and although novel strategies are in development, bacteria can evolve and overcome several barriers of preservation. The knowledge of the microenvironment of each critical point of the production chain is necessary to avoid spoiling and contamination of the products and constitute an important part of HACCP plan (hazard analysis critical control point). The microbiological analyses give information about the diversity and taxonomic identity of the species that could contaminate the food at every stage of production.

Continuous study about the behavior of bacteria in different environments found in food industry are necessary for understanding resistant mechanisms and improve every day the developed technologies used in the food industry. In relation to novel processing discussed in this chapter, interventions should be studied more and evaluated before being applied in the food industry because the equipment' and its implementation have a high cost.

On the other hand, bacteriophages seem an incredible tool for application in the food industry due to their advantages. However, more studies about their side effects are necessary for implementing adequate regulations of use. Additionally, the researchers in food preservation must continue the search for natural and clean alternatives to combat antimicrobial resistance.

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