Food Microbiology

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Introduction

Importance of Microorganisms in Foods

Every food harbors its own microbiota that may be specific and characteristic of a given point in the production process and of storage conditions. In processed foods, the microbiota consists of microorganisms from the raw materials that survived the processing, preservation, and storage conditions as well as microorganisms that contaminated the food during handling and processing. In raw foods, their characteristics, handling, and environmental conditions as well as farming practices will dictate the predominating microbiota.

Some microorganisms can be considered useful, if the changes due to their growth in foods are deemed beneficial, for example, in terms of flavor, texture, and appearance. But microorganisms can also be able to spoil foods. From the point of view of sensory rejection (deterioration), the spoilage microbiota consists of microorganisms that can grow on a food, causing undesirable changes. The potential of microorganisms to spoil food rests on their ability to produce metabolites that are associated with spoilage and that will lead to rejection of foods by consumers. In general, many microorganisms in a food are capable of producing undesirable metabolites when they grow above a certain level (Gram *et al.*, 2002). The growth of spoilage microorganisms in foods may result in changes in sensory properties, such as color, odor, texture, and appearance. Additionally, some microorganisms pose a health risk, and they are considered pathogenic. The characteristic of the disease will depend on a number of factors inherent to the food, pathogenic microorganism, and affected individual.

There are many microorganisms that are naturally present in foods, sometimes as contaminants. Bacteria stand out the most, both as pathogens and as spoilage microorganisms. For example, *Pseudomonas* spp. and other Gram-negative psychrotrophic microorganisms can be responsible for reduction of shelf life of high-protein, chilled foods stored under aerobic conditions, such as meat and dairy products. *Pseudomonas* is associated with postprocessing contamination of pasteurized milk Eneroth *et al.* (2000). *Shewanella putrefaciens* is an example of psychrotrophic microorganism responsible for the deterioration of chilled seafood (Chai *et al.*, 1968). Although vegetative forms of microorganisms play an important role in food spoilage, spore-forming bacteria are of great relevance for processed food, such as canned foods, vacuum-packaged meat, and thermally processed foods, because their spores survive lethal treatments (Ternstrom *et al.*, 1993).

In addition to bacteria, fungi are a very important group to be concerned with. Fungi are involved not only in food production but also in their spoilage and mycotoxin production, which may have several adverse effects to human and animal health. Fungi may gain access to foods through different routes, such as in the field (pre- and postharvest and during processing and storage). Fungi are the most diverse microorganisms of importance for food industry as they can grow on many different substrates with varied water activity (a_w), pH, and temperature (Dao and Dantigny, 2011). These microorganisms have developed mechanisms that allow them to adapt to high acidic conditions (Prusky and Yakoby, 2003), very low a_w, such as 0.75, and to survive thermal processing (Dao and Dantigny, 2011).

Viruses, protozoan, and parasites play a major role as causes of foodborne diseases in several countries. These microorganisms have variable resistance to lethal treatments used in food processing. Although they all share a common characteristic of not being able to grow in foods, they may be able to survive in these substrates.

Factors Affecting Microbial Behavior in Foods

The fate of microorganisms in foods depends not only on the physical and nutritional characteristics of the food but also on a set of extrinsic and intrinsic factors of the food and their interactions. Factors, such as temperature, pH, water activity, and redox potential, can be considered the most important factors driving microbial fate in foods. Food industry takes advantage of the fact that these factors can be conveniently manipulated to prevent microbial contamination and growth in foods.

Temperature

Among factors affecting microbial behavior in foods, temperature is for sure the most important one. According to Jay (2000), microorganisms can be classified into three groups according to their growth temperature domains: Psychrotrophs grow well at 7 °C or below and have an optimal growth temperature range of 20–30 °C; mesophiles grow well between 20 and 45 °C and have an optimal growth temperature range of 30–40 °C; and thermophiles grow well at 45 °C or higher and have an optimal growth temperature range of 55–65 °C. Most foodborne pathogens are mesophilic microorganisms, with exception of *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Clostridium botulinum* type E, which have markedly psychrophilic behavior. *Alicyclobacillys*, *Geobacillus stearothermophilus*, and *Bacillus sporothermodurans* are examples of thermophilic microorganisms of importance in foods and beverage industries.

Storage at low temperatures is one of the most important ways of slowing microbial metabolic activity in foods. However, cellular sensitivity to cold stress depends on many factors, including temperature, cooling/freezing rate, culture medium, strain, and storage time (Beales, 2004). It has been well

documented that microorganisms adjust the lipid composition of their membranes in response to temperature changes to insure membrane functionality (Russell, 1984; Mastronicolis *et al.*, 1998). Microorganisms growing at low temperatures change the fatty acid composition of their phospholipids and glycolipids to optimize membrane fluidity. This is necessary for nutrients to continue passing through the membrane and for cellular respiration (Berry and Foegeding, 1997).

Reduction of temperature leads to increase of lag time in foodborne microorganisms, culminating with the extension of shelf life. Therefore, it is one of the most used methods to preserve shelf life of minimally processed, pasteurized, and raw foods. Low temperatures will inhibit the development of mesophiles and thermophiles, but not of psychrotrophs. The growth of psychrotrophic microorganisms can be inhibited by adjusting other extrinsic and intrinsic parameters.

pH

It is well established that most microorganisms grow better in pH values close to 7.0, although a few can grow in pH values below 4.0. Bacteria tend to be more sensitive to pH than filamentous fungi and yeasts, and pathogenic bacteria are even more sensitive. Spoilage microorganisms of the lactic acid bacteria (LAB) group, for example, may grow in pH values as low as 2.0. Pathogenic microorganisms, such as *Cl. botulinum*, will not grow in pH values below 4.6. Because of its pathogenic potential, pH 4.6 is used as a limit for a food to be classified as of low acidity (>4.6) or high acidity (<4.6). The pH has a marked importance in the definition of intensity of thermal processing, with low and high acid foods being processed above and below 100 °C, respectively.

The minimum and maximum pH values tolerated by each microbial species depend also on other factors. For example, the minimum pH required for the growth of certain lactobacilli depends on the type of acid used: Citric, hydrochloric, phosphoric, and tartaric acids enable growth at lower pH values than acetic and lactic acids (Jay, 2000). Minimum concentrations of these acids or preservatives are used for inactivating or inhibiting microorganisms. In principle, growth could be inhibited by inactivation or disruption of the cell membrane, cell wall, metabolic enzymes, protein synthesis, or genetic material (Eklund, 1989).

Although the pH 4.6 marks the point below which pathogenic microorganisms cannot grow, the occurrence of several outbreaks associated with acidic products, such as fruits and fruit juices, have shown that inability to grow in foods with $pH<4.6$ should not be confounded with ability to survive.

All microorganisms have an optimal pH range for their growth and survival, and they are more sensitive to internal than external pH changes. When these changes are significant, they may lead to loss of viability. Acids usually inhibit essential reactions by increasing the concentration of hydrogen ions, which reduces the internal pH of the cell. The cell's ability to grow depends on its ability to change the environmental pH enough to reach its optimal range (Jay, 2000). Filamentous fungi have developed a mechanism to circumvent the effect of weak acids and keep homeostasis and internal pH. This mechanism requires adenosine triphosphatase (ATPase), which helps to remove excess protons from the cell (Beales, 2004). Hence, filamentous fungi and yeasts are capable of tolerating lower pH values than bacteria and are associated with the spoilage of acidified foods and products made with acidic fruits. Many microorganisms in more acidic environments than the ideal may require a higher minimum temperature for growth and even a higher minimum water activity (Booth and Stratford, 2003).

Water activity

Water activity is related to the amount of water available for the metabolic reactions within the cell. In fresh foods, a_w exceeds 0.99 (Jay, 2000). In general, bacteria need higher water activity than fungi, and Gram-negative bacteria need higher water activity than Gram-positive bacteria. Most bacteria associated with food spoilage grow at a_w above 0.91, whereas most filamentous fungi can grow at a_w as low as 0.80. *Staphylococcus aureus* can grow at a_w of 0.86, whereas *Cl. botulinum* needs a_w of at least 0.94. Like filamentous fungi, yeasts can withstand lower pH than bacteria, and the same goes for water activity. The lowest water activity required by a bacterium is 0.75 (halophilic bacteria), whereas xerophilic molds and osmophilic yeasts can grow at a_w of 0.65 and 0.61, respectively (Jay, 2000). The general effect of reducing water activity to a value below the optimum value is to increase the lag phase and reduce growth rate. Lowering the water activity causes what is known as osmotic stress.

Most microorganisms have evolved to function only within certain water activity ranges. Water activity outside the optimal range may reduce the essential metabolic functions of the cell and inhibit a large part of the physiological processes, such as nutrient absorption (Roth *et al.*, 1985) and deoxyribonucleic acid replication (Meury, 1988). In response to osmotic stress, microorganisms produce biocompatible solutes, such as trehalose, glycerol, sucrose, and mannitol. These biocompatible solutes help to balance the osmotic pressure of the cell and preserve protein function (Beales, 2004).

Other factors

It is evident that water activity and pH interact with temperature, because individual microorganisms or groups grow in a wide range of storage conditions. In addition to temperature, pH, and water activity, other factors are also important, such as the redox potential (Eh), packaging system, food structure, relative moisture, and atmospheric composition. Some anaerobic bacteria such as those from the genus *Clostridium* need an environment with reduced Eh to grow ($Eh=-200$ mV), whereas those from the genus *Bacillus* require positive Eh to grow. The bacteria that grow better in slightly reduced Eh conditions are called microaerophiles, which includes some LAB, such as *Lactobacillus* (Jay, 2000).

Studies have also discussed how the presence and concentration of some gases in the environment prevents or promotes microbial growth. Oxygen prevents the growth of anaerobic microorganisms in modified packaged foods, but high concentrations can increase the speed of oxidation reactions or even allow the faster growth of aerobic microorganisms. Carbon dioxide is the gas used in modified atmosphere packages that possess antimicrobial properties. Nitrogen is not absorbed by foods and is used as filler gas. The

atmosphere within the packages will be different depending on foods packaged, storage conditions, and package material. Several studies have been performed regarding the fate of foodborne microorganisms in modified packaged foods. However, the major concern in these products is related to the potential growth of psychrotrophic pathogens, such as *L. monocytogenes*. Some examples include the growth of *Listeria innocua* in fresh cut mixed leafy salads packaged in modified atmosphere (Scifò *et al.*, 2009) and survival of *L. monocytogenes* and *Salmonella* Enteritidis in sea bream packaged in modified atmosphere enriched with carbon dioxide (Provincial; Guillen *et al.*, 2013).

The combination or interaction of extrinsic and intrinsic parameters is very important for controlling microbial growth. Considering this, the concept of hurdle technology has been developed and applied for production of several foods, such as salami. The hurdle technology considers the combination of extrinsic and intrinsic factors aimed at increasing the stability and safety of foods, but at the same time insuring freshness and nutrition of foods.

Even with the application of multiple barriers to guarantee shelf-stable and safe products, microorganisms will use mechanisms to adapt to function optimally in physiologically normal environments. Any extreme change in environmental conditions causes stress on the microorganism, which may result in loss of viability or sublethal injuries leading to inactivation or inability to grow in foods.

To survive in foods, microorganisms have developed physiological and genetic mechanisms that enable them to withstand stressful conditions, such as cold shock, preservatives, heat shock, etc. For example, when exposed to cold shock, some microorganisms can produce specific protein groups, called cold shock proteins, which enable them to adapt and survive cold. The addition of preservatives, such as sorbates and benzoates, also activates specific repressor genes, which are probably necessary for optimal adaptation of microorganisms to preservatives, weak acids, and low pH (Beales, 2004). A low–medium pH lowers the inner pH of microbial cells to a point that the cell can no longer tolerate, inhibiting their development. However, some microorganisms can survive this stress by making use of passive and active mechanisms to regulate the inner pH of the cells.

The principles and mechanisms associated with the interaction of extrinsic and intrinsic factors should always be taken into account, whether it is for the formulation of a new product or the establishment of a new process.

Microbiological Spoilage of Foods

Bacterial spoilage of foods

Although many bacteria can grow in foods, only some specific groups are responsible for their spoilage (**Table 1**). The survival, growth, and occurrence of these microorganisms in foods are affected by many different factors, including storage temperature, oxygen availability, food composition, pH, thermal treatment, and competing microbiota, among others.

Bacterial spoilage occurs faster and more evidently in high-protein foods, such as meat, milk, fish, and dairy products. Most of these foods are rich in nutrients and have high water activity as well as pH values close to 7.0 Huis in't Veld, 1996. However, the occurrence of certain microorganisms in these foods is mainly promoted by temperature and atmosphere. Temperature is a limiting factor as it can favor the growth of some spoilage microbial groups and impair the growth of others. For example, low temperature favors the growth of psychrotrophic bacteria over the growth of mesophiles and thermophiles. In this context, *Pseudomonas* spp., a strictly aerobic psychrotrophic bacterium, prevails as the main spoilage microorganism in chilled fresh meat and may cause color, odor, and flavor changes as it degrades amino acids and fats (Gill and Newton, 1978; Labadie, 1999). They create a slimy layer on the surface of the food as they grow. This species, together with *S. putrefaciens*, *Photobacterium phosphoreum*, and some *Vibrio* spp., is the main microorganism involved in the spoilage of seafood (Broekaert *et al.*, 2011; Chai *et al.*, 1968; Gram and Huss, 1996; Gram and Dalgaard, 2002). Moreover, *Pseudomonas* spp., together with other psychrotrophic species, is responsible for proteolysis and hydrolytic rancidity of raw milk and dairy products and also for changes on the surface (slime), pigmentation, and odor of cheeses (Champagne *et al.*, 1994). Despite the high fat content of butter and cream, these can also be spoiled by this group of bacteria (Wang and Frank, 1981). In addition to changes promoted by psychrotrophic and LAB, cheese may also be spoiled by coliforms (early blowing) or gas-producing, anaerobic, spore-forming bacteria, such as *Clostridium tyrobutyricum* (late blowing) (Ledenbach and Marshall, 2010). Eggs are a very popular food and are also used as ingredients in many foods. They have many barriers that prevent the access and growth of microorganisms within them. The prevailing microbiota in the spoilage of this product is composed of Gram-negative bacteria, including *Pseudomonas* spp., which rot the product and change its color (Shebuski and Freier, 2010).

A small change in the way a food is packaged, such as the use of vacuum or modified atmosphere, can change the profile of the spoilage microorganisms from aerobic to facultative or strictly anaerobic. In meats and meat products stored under these conditions, spoilage is caused by LAB, Enterobacteriaceae, and sometimes *Brochothrix thermosphacta* (Dainty and Mackey, 1992). *Brochothrix thermosphacta* is an important species associated with the spoilage of meats and meat products and grows in both aerobic and anaerobic conditions (Pin *et al.*, 2002; Russo *et al.*, 2006).

Other changes in the spoilage microbiota of high-protein foods may be induced by changing water activity and adding a competing microbiota. For example, curing, salting, and fermenting meat products inhibits the growth of the natural spoilage microbiota and promotes the growth of bacteria better adapted to these conditions, such as species of the genera *Micrococcus* and *Staphylococcus* and some LAB (Cerveny *et al.*, 2010; Huis in't Veld, 1996; Samelis *et al.*, 2000).

The presence of complex nutrients in fruits and vegetables, such as cellulose, polysaccharides, hemicellulose, and pectin and in some cases the acidic pH, limits the bacterial spoilage of these foods. However, some bacteria are capable of producing extracellular lytic enzymes that degrade these polymers, releasing nutrients for their growth. This softens the flesh and produces acids, alcohols, and metabolites with unpleasant odors and flavors. The main bacterium species responsible for

Table 1 Main bacteria involved in the spoilage of specific food groups

Types of food	*Main groups responsible for spoilage*	*References*
Refrigerated, fresh meats (aerobic atmosphere)	*Pseudomonas* spp. and other psychrotrophs	Ellis and Goodacre (2001); Gill and Newton (1978)
Refrigerated, fresh meats (vacuum or modified atmosphere)	*Brochothrix thermosphacta*, *Clostridium* spp., *Shewanella putrefaciens*, *Enterobacteriaceae*, and lactic acid bacteria (LAB)	Garcia-Lopez *et al.* (1998); Gill and Newton (1978); Labadie (1999); Russo *et al.* (2006)
Meat products (cured, salted, or fermented)	*Micrococcus*, *Staphylococcus*, *Enterococcus*, and LAB	Cerveny *et al.* (2010); Huis in't Veld (1996); Samelis *et al.* (2000)
Seafood (fresh)	*S. putrefaciens*, *Aeromonas* spp., *Photobacterium phosphoreum*, and *Pseudomonas* spp.	Chai *et al.* (1968); Gram and Huss (1996); Gram *et al.* (2002)
Seafood (processed and salted)	*Brochothrix thermosphacta*, LAB, *Enterobacteriaceae*, and *Vibrio* spp.	Jørgensen *et al.* (2000); Leroi *et al.* (2001)
Refrigerated, raw milk	*Pseudomonas* spp., *Bacillus*, *Micrococcus*, *Aerococcus*, *Lactococcus*, and *Enterobacteriaceae*	Champagne *et al.* (1994); Ledenbach and Marshall (2010)
Ultra-high-temperature milk	*Bacillus* spp.	Stone and Rowlands (1952); Ternstrom *et al.* (1993)
Cheeses	Psychrotrophs, Coliforms, *Clostridium tyrobutyricum*, *Clostridium sporogenes*, *Lactobacillus*, and *Leuconostoc*	Hutkins (2001); Ledenbach and Marshall (2010)
Butter and cream	*Pseudomonas* spp. and Coliforms	Wang and Frank (1981)
Eggs and related products	*Serratia*, *Pseudomonas*, *Proteus*, and *Aerobacter*	Shebuski and Freier (2010)
Vegetables	*Erwinia carotovora*, *Pseudomonas* spp., *Corynebacterium*, *Xanthomonas campestris*, and LAB	Lund (1992); Tournas (2005)
Fruits	*Pseudomonas* spp., *Erwinia*, *Lactobacillus* spp., *Xanthomonas*, and *Acidovorax*	Kalia and Gupta (2007)
Fruit juices	*Alicyclobacillus* spp. and *Propionibacterium* sp.	Smit *et al.* (2011); Walker and Phillips (2007)
Bakery products	*Bacillus* spp.	Voysey and Hammond (1993)
Canned foods	*Thermoanaerobacterium thermosaccharolyticum*, *Moorella thermoacetica*, *Desulfotomaculum nigrificans*, *Clostridium butyricum*, and the facultative anaerobe *Geobacillus stearothermophilus*	Evancho *et al.* (2010)

this deterioration is *Erwinia carotovora*, present in nearly all vegetables (Tournas, 2005) and some fruits (Kalia and Gupta, 2007). However, the intrinsic factors of bakery products limit bacterial growth and favor the prevalence of filamentous fungi as the main spoilage microorganisms (Saranraj and Geetha, 2012). However, bacteria from the genus *Bacillus* spp. may cause a type of spoilage in bread known as ropiness, which is characterized by brown and black stains, release of a rotten fruit odor, and a sticky and moist breadcrumb (Rosenkvist and Hansen, 1995). *Bacillus subtilis* is the main species responsible for this spoilage, but other members of the genus, such as *Bacillus licheniformis* and *Bacillus megaterium*, have also been identified causing this spoilage (Voysey and Hammond, 1993).

Thermal treatment preserves and insures the safety of some types of food, but it also allows specific spoilage microorganisms to prevail in the treated food. In ultra-high-temperature milk, spoilage is caused mainly by spore-forming bacteria of the genus *Bacillus*, which resist thermal treatment and produce enzymes that promote sweet coagulation, gelification, and odor changes (Kalogridou-Vassiliadou, 1992; Ternstrom *et al.*, 1993). *Alicyclobacillus* spp. and *Propionibacterium cyclohexanicum* are the main microorganisms associated with the spoilage of fruit juices that survive thermal treatment and may cause off-flavors, off-odors, discoloration, and turbidity (Smit *et al.*, 2011; Walker and Phillips, 2007). Canned foods submitted to industrial sterilization methods are spoiled exclusively by facultative or strictly anaerobic spore-forming bacteria, such as *G. stearopthermophilus*, *Clostridium sporogenes*, and *Desulfotomaculuns nigrificans*, among others (Evancho *et al.*, 2010).

Yeast spoilage of foods

Yeasts are very important unicellular eukaryotic microorganisms involved in both food and beverage production and spoilage (Querol and Fleet, 2006). These microorganisms spoil foods with high sugar or salt contents, low pH, or other characteristics that give them a competitive advantage over bacteria (Kurtzman, 2006; Smits and Brul, 2005). Most yeasts produce extracellular enzymes, such as proteases, lipases, amylases, and pectinases, and also volatile and nonvolatile

Table 2 Main yeasts involved in the spoilage of specific food groups

Types of food	*Main groups responsible for spoilage*	*References*
Fresh meats	*Candida* spp., *Rhodotorula* spp., *Debaryomyces* spp., *Trichosporon* spp., and *Torulopsis* spp.	Fleet (1992); Osei Abunyewa *et al.* (2000)
Dry and salted meat products	*Debaryomyces hansenii*, *Yarrowia lipolytica*, *Candida*, *Trichosporon*, *Cryptococcus*, and *Rhodotorula*	Dalton *et al.* (1984); Encinas *et al.* (2000); Fleet (1992); Osei Abunyewa *et al.* (2000); Saldanha-da-Gama *et al.* (1997)
Yogurts	*Saccharomyces cerevisiae* and *Hansenula anomala*	Fleet (1990); Hansen and Jakobsen (2003); Rohm *et al.* (1992)
Cheeses	*Candida* spp., *Kluyveromyces marxianus*, *Debaryomyces hansenii*, and *Pichia* spp.	Johnson (2001)
Butter	*Candida parapsilosis*, *Candida zeylanoides*, and *Yarrowia lipolytica*	Lopandic *et al.* (2006)
Fruits and vegetables	*Saccharomyces*, *Candida*, *Zygosaccharomyces*, *Torulopsis*, *Rhodotorula*, *Hansenula*, *Debaryomyces*, and *Pichia* spp.	Jacxsens *et al.* (2001); O'Connor-Shaw *et al.* (1994)
Bakery products	*Saccharomyces*, *Debaryomyces*, *Kluyveromyces*, *Pichia*, *Candida*, and *Zygosaccharomyces*	Van der Zee and Huis in't Veld (1997)
Chocolate, honey, and candy	*Zygosaccharomyces* spp., *S. cerevisiae*, *Torulopsis apicola*, *Hansenula anomala*, and *Kloeckera apiculata*	Thompson (2010)
Carbonated soft drinks	*Saccharomyces* spp., *Zygosaccharomyces* spp., *Torulaspora delbrueckii*, *Pichia anomola*, *Dekkera* spp., and *Candida* spp.	Lawlor *et al.* (2010)

metabolites that affect the sensory characteristics of food, especially flavor and texture. The main genera associated with food spoilage are *Saccharomyces*, *Candida*, *Zygosaccharomyces*, *Debaryomyces*, *Rhodotorula*, and *Pichia* (**Table 2**).

Yeasts play a small part in the spoilage of meats. Despite the competition with bacteria, some yeasts manage to grow on the surface of fresh meats (Nielsen *et al.*, 2008), whereas others may have their growth promoted by changes in intrinsic and extrinsic factors that inhibit bacterial growth (Fleet, 1992; Osei Abunyewa *et al.*, 2000).

Yeasts are also known for their important role in the dairy industry, especially for the production of some fermented products, maturation of some cheeses, and whey fermentation for bioactive compound production (Marth, 1987). However, yeasts are the main cause of yogurt and fermented milk spoilage because the low pH provides a selective environment for their growth. Their presence is associated with off-flavors and gas production (Fleet, 1990; Rohm *et al.*, 1992). These microorganisms also spoil cheeses, and most contamination stems from the brine used in the production process (Kaminarides and Laskos, 1992). Yeasts are normally not involved in butter spoilage, but they have already been detected on spoiled butter (Lopandic *et al.*, 2006).

Yeasts play an important role in the spoilage of fruits and vegetables, especially because of the exposure of these foods to the environment and their minimal processing (Barth *et al.*, 2010). Some of their intrinsic factors, such as pH, also limit bacterial growth. Yeasts are also involved in the deterioration of bakery products, causing white and pink stains or fermenting the carbohydrates, with subsequent production of volatile compounds and alcoholic odor (Legan and Voysey, 1991). In carbonated beverages, the growth of spoilage yeasts is usually characterized by a great production of gas, packaging distension and rupture, abnormal effervescence, excessive turbidity, sedimentation, off-flavors, and off-odors (Lawlor *et al.*, 2010). Osmophilic yeasts, together with xerophilic fungi, are the only microorganisms involved in the spoilage of high-sugar foods with low water activity, such as chocolates, confectionary products, and honey (Thompson, 2010). *Zygossacharomyces bailli* is involved in spoilage of low-pH beverages containing preservatives and high-sugar foods because it has developed mechanisms to adapt these stress factors. These yeasts play a major role in spoilage of beverages, such as fruit juices, soft drinks, chocolate fillings, confectionary creams, etc.

Filamentous fungi spoilage of foods

Filamentous fungi are capable of growing on many different foods, such as grains, meats, milk, fruits, vegetables, seeds, and high-fat products. They are an important group of food spoilage organisms and cause significant economic losses in agriculture and the food industry (Gerez *et al.*, 2013). Spoilage caused by filamentous fungi may manifest as discoloration, off-flavors, loss of structure, loss of texture, formation of visible mycelium, and production of volatile compounds, all of which affect the quality of foods and beverages (Alvo and Raghavan, 1993). These obligate aerobic microorganisms are capable of growing in wide ranges of pH, temperature, and water activity and of using a great variety of substrates as food (Dao and Dantigny, 2011; Sperber, 2010). Filamentous fungi grow more commonly in products with low pH and water activity. They are located mainly on the surface of the food because of their dependence on oxygen. The main food spoilage fungi are *Penicillium*, *Aspergillus*, *Rhizopus*, *Mucor*, *Geotrichum*, *Fusarium*, *Alternaria*, *Cladosporium*, *Eurotium*, and *Byssochlamys* (**Table 3**). Some of these species are also known for their ability to synthesize secondary toxic metabolites called mycotoxins, constituting a problem for agribusiness and the food industry.

Controlling Microbiological Spoilage of Foods

Avoiding or retarding spoilage of raw and processed foods but at the same time preserving the sensory and nutritional

Table 3 Main filamentous fungi involved in the spoilage of specific food groups

Types of food	*Main groups responsible for spoilage*	*References*
Salted fish and similar products	*Wallemia*, *Hortaea*, *Aspergillus*, *Penicillium*, *Polypaecilum*, *Eurotium*, *Basipetospora*, *Cladosporium*, and *Scopulariopsis*	Pitt and Hocking (1999)
Cheeses	*Penicillium*, *Cladosporium*, *Byssochlamys nivea*, *Talaromyces avellaneus*, *Neosartorya fischeri* var. *spinosa*, and *Eupenicillium brefeldianum*	Hocking and Faedo (1992); Pitt and Hocking (1999)
Yogurts	*Penicillium* and *Aspergillus*	Cousin (2001); Ndagijimana *et al.* (2008)
Meat and meat products	*Aspergillus* spp., *Penicillium* spp., *Mucor* spp., *Cladosporium* spp., and *Eurotium* spp.	Deak and Beuchat (1996); Samson *et al.* (2000)
Fruits and vegetables	*Penicillium*, *Phytophthora*, *Alternaria*, *Botrytis*, *Fusarium*, *Cladosporium*, *Phoma*, *Trichoderma*, *Aspergillus*, *Alternaria*, *Rhizopus*, *Aureobasidium*, and *Colletotrichum*	Hagenmaier and Baker (1998); Nguyen-the and Carlin (1994); Tournas (2005)

Table 4 Methods for controlling the microorganisms involved in food spoilage

Principle of preservation	*Methods*
Prevent access of the microorganism to the food	Good agricultural practices Good manufacturing practices Appropriate hygienic and sanitary conditions
Microorganism removal	Washing of surfaces Centrifugation Filtration by membranes
Growth inhibition	Cooling or freezing Reduce water activity (drying and addition of solutes) Food acidification Addition of inhibitors (weak organic acids, nitrites, nitrates, sulfites, and bacteriocins) Microbial competition Atmosphere modification (vacuum or modified atmosphere packaging)
Microbial inactivation	Thermal treatments (pasteurization, sterilization, blanching, and thermization) Radiation High hydrostatic pressures Pulsed electric field processing Ultrasound

characteristics of foods is a great challenge for the industry. The methods used for controlling microbial spoilage include preventing access of the organisms to the foods, removing their cells or spores, inhibiting their growth, and using thermal and nonthermal methods to inactivate them (**Table 4**).

The good manufacturing and handling practices are very important means of reducing the baseline microbial load and increasing shelf life. They are considered the minimum hygienic requirements for the production of any type of food. These practices should be used throughout the entire food production chain, from production of raw materials to consumer consumption.

Other methods of preventing spoilage include: (1) washing the raw materials before processing, which removes microbial cells from its surface, (2) centrifuging, (3) or using membrane filtration processes, which can only be used on certain food groups. In addition to the above-mentioned methods, foods can also be preserved by manipulating factors that not only influence microorganism growth and survival but are also safe for consumers (Gould, 1995). Control of microbial growth by changing intrinsic and extrinsic factors, also called barrier theory, is the main way of reducing food spoilage. The most important barriers used for food preservation are temperature (high and low), low a_w, acidity (pH), redox potential (Eh), preservatives, and other organisms (microbial competition) (Leistner, 2000). These microbial control methods use changes in food characteristics and storage conditions to prevent or reduce microbial growth. Combining different sublethal conditions to prevent microbial growth is a successful approach mainly because it does not change the sensory characteristics of the foods significantly. The great disadvantage of these methods is that microorganisms can resume growth if favorable growth conditions occur.

Some food preservation methods focus on microbial inactivation. They can be classified as thermal or nonthermal methods of food preservation. The thermal methods, such as pasteurization and commercial sterilization, are widely used methods to efficiently inactivate pathogens and reduce the load of spoilage microorganisms. Despite this, they can also change the nutritional and sensory properties of the food. To

replace these methods and meet the increasing consumer demand for healthier products, many nonthermal methods are being developed and used. The main nonthermal methods are radiation, high hydrostatic pressure, pulsed electric field, and ultrasound. Many of these methods are still difficult to implement because of equipment cost, need of trained personnel, and consumer distrust.

Pathogenic Microorganisms in Foods

Gram-positive foodborne pathogens

Most foodborne illnesses are caused by the ingestion of food or water contaminated with microorganisms or their toxic metabolic products. Some Gram-positive bacteria, especially *St. aureus*, *Cl. botulinum*, *Clostridium perfringens*, *Bacillus cereus*, and *L. monocytogenes*, are considered important foodborne pathogens responsible for foodborne illness outbreaks everywhere in the world (**Table 5**). Most of these microorganisms, except for *L. monocytogenes* and *Cl. perfringens*, can grow on food and produce toxins that will cause food poisoning when ingested. *Clostridium botulinum*, *Cl. perfringens*, and *B. cereus* are capable of forming spores, structures that make them resistant to high temperatures and other adverse conditions.

Among Gram-positive bacteria, *St. aureus* stands out because it can grow in foods with high sodium chloride concentrations (10–20%) and low a_w (0.83–0.86). *Staphylococcus aureus* is heat labile and produces heat-resistant enterotoxins (Adams and Moss, 2008). The disease caused by *St. aureus* is due to consumption of animal-origin and excessively handled foods. *Staphylococcus aureus* have a short incubation period and the intoxication caused is self-limiting. The clinical symptoms associated with *B. cereus* poisoning are very similar to those associated with staphylococcal intoxication. However, *B. cereus* can cause two distinct types of foodborne illnesses, namely emetic and diarrheal syndromes. Emetic syndrome is caused by the ingestion of a preformed toxin (cereulide) in foods, which stimulates the vague nerve and causes nausea and vomiting (Agata *et al.*, 2002; Ehling-Schulz *et al.*, 2004). The diarrheic syndrome is an infection caused by ingesting bacterial cells, which then colonize the small intestine and produce enterotoxins *in loco* (Andersson *et al.*, 1998; Clavel *et al.*, 2004). These two syndromes are also characterized by their rapid onset and self-limiting nature, not requiring therapeutic interventions and hospitalization. Despite this, severe and even fatal cases have been reported (Dierick *et al.*, 2005; Granum, 1994; Lund *et al.*, 2000).

Clostridium botulinum, the causative agent of botulism, is a globally distributed bacterium. It causes a severe disease with high mortality rate (Lund and Peck, 2000; Smith and Sugiyama, 1988) due to ingestion of botulinum toxin. Botulinum toxin is preformed in foods and as a neurotoxin, after absorption in the intestines, reaches the nervous system and blocks the release of acetylcholine by nerve terminals (Montecucco *et al.*, 1996). Despite the severity of the illness, the associated neurotoxins are heat labile and can easily be destroyed by heating the food to 80° C for 20 min or 85° C for 5 min (Siegel, 1993).

Clostridium perfringens is another important spore-forming bacterium widely distributed in nature and capable of producing more than 15 toxins that cause different diseases in humans and animals (Lindström *et al.*, 2011). Food poisoning caused by this bacterium is among the most common foodborne illnesses in the world. Food poisoning by *Cl. perfringens* is caused by the ingestion of at least 10^7 cells of the microorganism, which sporulate in the intestines, releasing the *Cl. perfringens* enterotoxin (McClane, 2001).

Differently from the above-mentioned Gram-positive bacteria, *L. monocytogenes* is characterized by its ability to invade intestinal cells and diffuse to other organs and tissues (Orsi *et al.*, 2011). It is a ubiquitous bacterium resistant to desiccation, low water activity, and low pH and may cause anything from a mild gastroenteritis to severe infections of the central nervous system and abortion, depending on the host's susceptibility (Orsi *et al.*, 2011; Rocourt *et al.*, 2003). Listeriosis, the disease caused by *L. monocytogenes*, is a major concern for those involved in food safety because of its high mortality rates (approximately 50%). *Listeria monocytogenes* is a psychrotrophic pathogenic bacterium of very high importance for processed foods or minimally processed foods that are stored for medium to short periods.

Gram-negative foodborne pathogens

Many Gram-negative pathogenic bacteria can cause foodborne illnesses, including *Salmonella* spp., *Campylobacter* spp., pathogenic *Escherichia coli*, *Shigella* spp., *Y. enterocolitica*, *Vibrio* spp., *Aeromonas* spp., and *Cronobacter sakazakii*, among others (**Table 6**).

Among these, *Campylobacter* spp. has been identified as the main cause of foodborne illnesses and outbreaks in the USA and Europe in the past 5 years. The thermophilic species *Campylobacter jejuni* and *Campylobacter coli* are the main causes of campylobacteriosis in humans, a usually self-limiting gastrointestinal disease that can, nevertheless, cause severe complications, such as Guillain–Barré syndrome and reactive arthritis (Allos, 1997; Zilbauer *et al.*, 2008). *Salmonella* spp. also plays an important role in foodborne illness outbreaks worldwide, being an important public health problem (Payment and Riley, 2002). Most serotypes cause gastroenteritis limited to intestinal infections, but the Typhi and Paratyphi serotypes can cause enteric fevers, which are more severe illnesses and affect other organs and tissues (Crump and Mintz, 2010). Although *E. coli* are considered part of the normal intestinal microbiota of warm-blooded humans and animals, some strains can cause foodborne illnesses. These pathogenic strains can be grouped into at least six different groups: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAggEC), diffuse aggregative *E. coli* (DAEC), enterohemorrhagic *E. coli* (also known as verocytotoxin-producing *E. coli*, VTEC, or Shiga toxin-producing *E. coli*, STEC), and enteroaggregative hemorrhagic *E. coli* (EAHEC). Foodborne illness outbreaks have been particularly associated with VTEC and, to a smaller extent, EPEC, ETEC, and EAggEC. A great outbreak with EAHEC strain *E. coli* 0104:H4 occurred in Europe in 2011, with 320 bloody diarrhea cases, 850 cases of hemolytic-uremic syndrome (HUS), and 82 deaths.

Yersinia enterocolitica is one of the most interesting species within the *Yersinia* genus, and related outbreaks are mainly associated with the consumption of contaminated pork.

Table 5 Main Gram-positive bacteria associated with foodborne illnesses

Pathogen	*Characteristics*	*Symptoms*	*Incubation*	*Foods*	*References*
Staphylococcus aureus	Ingestion of one or more types of staphylococcal toxins	Intense vomiting, diarrhea, abdominal pain, and nausea	0.5–6 h	Milk and dairy products, meat products, confectionary products, and ready-to-eat foods	Adams and Moss (2008); Argudín *et al.* (2010); Gilmour and Harvey (1990); Kim *et al.* (2011); Schelin *et al.* (2011); Tranter (1990)
Clostridium botulinum	Ingestion of botulinum neurotoxin	Initial gastrointestinal symptoms, double vision, dry mouth, difficulty in swallowing and controlling tongue, and flaccid paralysis	12–36 h	Canned foods (vegetables and meats), honey, milk products, fish, and fermented seafood	Lindstrom *et al.* (2006); Lund and Peck (2000); Peck *et al.* (2011)
Clostridium perfringens	Release of *Clostridium perfringens* enterotoxin after intestinal sporulation	Acute abdominal pain, nausea, and diarrhea	8–12 h	Meat products and meat-based ready-to-eat foods	Lindström *et al.* (2011); Liu (2009); McClane (2001)
Bacillus cereus	Ingestion of cereulide toxin (emetic syndrome)	Nausea and vomiting	0.5–6h	Rice and grain-based foods	Agata *et al.* (2002); Ehling-Schulz *et al.* (2004); Shaheen *et al.* (2006)
	Toxin production in the small intestine (diarrheic syndrome)	Abdominal pain and aqueous diarrhea	8–16 h	Meats, pasta, desserts, cakes, sauces, and milk	Andersson *et al.* (1998); Clavel *et al.* (2004); Granum (1994)
Listeria monocytogenes	Invasion of intestinal epithelial cells and diffusion to other organs and tissues	Fever, headache, abdominal pain, diarrhea, chills, and complications (abortion, meningitis, and septicemia)	2 days to 3 weeks	Vegetables and salads, cheeses, milk, beef, chicken, and fish	Abadias *et al.* (2008); Cabedo (2008); Caramello and Vaudetti (1990); Kvenberg (1988)

Table 6 Main Gram-negative bacteria associated with foodborne illnesses

Pathogen	*Characteristics*	*Symptoms*	*Incubation*	*Foods*	*References*
Salmonella spp.	Invasion of intestinal cells (gastroenteritis) Typhoid fever (Typhi and Paratyphi serotypes)	Fever, headache, abdominal pain, diarrhea, and chills	12–36 h	Eggs, meats, milk, and dairy products	Crump and Mintz(2010); D'AOUST (2001); Payment and Riley (2002).
Campylobacter spp.	Invasion of intestinal cells	Fever, headache, muscle pain, diarrhea, abdominal pain, and nausea. Complications: Guillain–Barré syndrome and reactive arthritis	2–10 days	Meat and poultry products, raw milk, and contaminated water	Rao *et al.* (2001); Rautelin and Hanninen (2000); Solomon and Hoover (1999); Zilbauer *et al.* (2008)
E. coli (pathogenic) (EPEC, EIEC, EAggEC, ETEC, EHEC, and DAEC)	Adherence to intestinal cells, electrolyte imbalance, toxin production, and rare invasion of intestinal cells	Fever, abdominal pain, chills, diarrhea, and nausea Complications: hemolytic-uremic syndrome (Shiga toxins)	8 h to 4 days	Meats and meat products, milk and milk products, leafy vegetables, and fish	Abadias *et al.* (2008); Atanassova *et al.* (2008); Brandl and Amundson (2008); Eglezos *et al.* (2008)
Shigella spp.	Invasion of intestinal cells and toxin production	Fever, bloody diarrhea, chills, abdominal pain, and vomiting Complications: Hemolytic-uremic syndrome	12–50 h	Shellfish, crustaceans, fruits, vegetables, and salads	Agle *et al.* (2005); Chanachai *et al.* (2008); Kimura *et al.* (2006); Pinu *et al.* (2007); Warren *et al.* (2006)
Yersinia enterocolitica	Invasion of intestinal cells, penetration in mesenteric lymph nodes, and inflammation	Abdominal pain, fever, diarrhea, sore throat, and joint pain	1–3 days	Beef, pork, poultry, oyster, fish, milk, and milk products	Arnold *et al.* (2006); Fredriksson-Ahomaa *et al.* (2007); Yucel and Ulusoy (2006)
Vibrio cholerae	Cholera toxin production in the small intestine	Abdominal pain, aqueous diarrhea, and dehydration	6 h to 5 days	Contaminated water, vegetables, and seafood	Austin (2010)
Vibrio parahaemolyticus	Colonization of the small intestine and production of adhesins and cytotoxins	Abdominal pain, diarrhea, colic, fever, headache, nausea, vomiting, and chills	4 h to 4 days	Shellfish, raw fish, shrimp, and oyster	Chan and Chan (2008); Davis *et al.* (2007); DePaola *et al.* (2003)
Vibrio vulnificus	Colonization of the small intestine and production of adhesins and cytotoxins	Diarrhea, abdominal pain, vomiting, fever, and may cause infections in wounds	7 h to some days	Shrimp, fish, oysters, and mussels	Colakoglu *et al.* (2006); Gopal *et al.* (2005); Jung *et al.* (2007)
Cronobacter sakazakii	Opportunistic infection	Abdominal pain and bloody diarrhea Complications: septicemia, meningitis, and brain abscess		Infant foods and formulas	Strydom *et al.* (2012); Zhou *et al.* (2008)
Aeromonas spp.	Opportunistic infection	Symptoms similar to those of cholera with aqueous diarrhea and mild fever; in some cases, symptoms similar to dysentery with bloody diarrhea, fever, and abdominal pain		Fish, shrimp, milk, and bottled water	Igbinosa *et al.* (2012)

Shigella spp. are transmitted by the fecal/oral route and ingestion of contaminated food or water. These bacteria are highly infectious and can produce the Shiga toxin, which causes the HUS (DuPont *et al.*, 1989). *Vibrio cholerae*, *Vibrio vulnificus*, and *Vibrio parahaemolyticus* have been associated with foodborne illnesses due to consumption of contaminated water, vegetables, and seafood (DePaola *et al.*, 2003; Drake *et al.*, 2007; Morris, 2003).

Other Gram-negative bacteria, such as *Aeromonas* spp. and *Cr. sakazakii*, are considered opportunistic foodborne pathogens and infect specific population groups, namely newborns, the elderly, and immunocompromised individuals (Igbinosa *et al.*, 2012; Nazarowec-White and Farber, 1997).

Protozoan foodborne pathogens

Protozoans are important etiological agents of human diseases that can be transmitted by contaminated water and foods. Intestinal infections caused by protozoans include toxoplasmosis (*Toxoplasma gondii*), cryptosporidiosis (*Cryptosporidium* sp.), cyclosporiasis (*Cyclospora cayetanensis*), cystoisosporiasis (*Cystoisospora belli*), sarcocystosis (*Sarcocystis* sp.), giardiasis (Giardia sp.), and amebiasis (*Entamoeba histolytica*). More recently, *Trypanosoma cruzi*, the causative agent of Chagas disease, was included in this list because it can be transmitted by foods (Yoshida *et al.*, 2011). Among the abovementioned protozoans, *Giardia*, *Cryptosporidium*, and *Cyclospora* are the main microorganisms responsible for diarrhea in humans (Dawson, 2005). The main transmission route of these protozoans is the fecal/oral route. However, indirect transmission from pets, such as dogs and cats, and ingestion of foods contaminated with oocysts represent a threat (Karanis *et al.*, 2007; Smith *et al.*, 2007). In general, water and certain foods, such as vegetables, fruits, and seafood, which are more likely to have been in contact with diseased individuals, are the main vehicles of intestinal protozoans.

Foodborne viruses

Viruses are small intracellular parasites capable of causing diseases in plants, animals, and human beings. Many viruses can be found in the human intestines, but only some are generally recognized as important foodborne pathogens (Koopmans and Duizer, 2004). Foodborne viruses attack intestinal cells and propagate within them. Later, some types of viruses may attack other cells or invade other organs, such as the liver and the central nervous system (Bajolet and Chippaux-Hyppolite, 1998; Koopmans and Duizer, 2004). The main genera associated with foodborne illnesses are Norovirus, Sapovirus, Enterovirus, Hepatovirus, Astrovirus, Rotavirus, and members of the Adenoviridae family, among others (Vasickova *et al.*, 2005). Norovirus, Rotavirus, and the hepatitis A virus are the ones most related with foodborne illness outbreaks around the world. All these viruses can be transmitted through the fecal/oral route, either by direct contact with infected individuals or by ingestion of contaminated water and foods, such as vegetables, fruits, shellfish, bivalve molluscs, and sometimes beef (Dubois *et al.*, 2002; Keeffe, 2004; Koopmans and Duizer, 2004; Lees, 2000; Richards, 2001).

Although viruses are the most common pathogenic foodborne agents, their identification and association with foods is still difficult in the absence of systematic surveillance and appropriate legislation to establish food safety criteria (Koopmans and Duizer, 2004).

Mycotoxigenic molds

Filamentous fungi can grow in foods and cause chronic disease in humans and animals because of their mycotoxins. Mycotoxins are secondary metabolites that may cause a variety of adverse effects in human beings, such as intestinal symptoms, allergic responses, immunosuppression, mutagenesis, inhibition of protein synthesis and essential metabolic pathways, and cancer (Bennett and Klich, 2003). Mycotoxins may be produced before harvest or during storage, and filamentous fungi need favorable water activity, atmosphere (oxygen), substrates, and temperatures to grow. The main filamentous fungal genera associated with the production of mycotoxins in foods are *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp.

Aspergillus flavus and *Aspergillus parasiticus* are the main fungi responsible for the production of aflatoxin in peanuts, corn, wheat, rice, and other grains. The main types of toxins isolated from these foods are aflatoxins B_1, B_2, G_1, and G_2. They are characterized by their fluorescence under ultraviolet light. Additionally, toxic metabolites, such as aflatoxins M_1 and M_2, may be present in the flesh and milk of animals fed grains contaminated with type B aflatoxin.

Aspergillus carbonarius, *Aspergillus niger*, *Aspergillus ochraceus*, and *Penicillium verrucosum* are associated with the production of ochratoxins. An important characteristic of this mycotoxin is that it is found in many different products, such as raisins, barley, soybean and coffee products, grapes, and wines, but usually in low levels. These mycotoxins can accumulate in human or animal body tissues and fluids when contaminated foods are consumed regularly.

Citrinin is another mycotoxin produced by *Penicillium citrinum* and by some *Aspergillus* species, such as *Aspergillus terreus* and *Aspergillus niveus*, and industrially important species, such as *Monascus ruber*, *Monascus purpureus*, *Penicillium Camemberti*, and *Aspergillus oryzae*. This nephrotoxic mycotoxin is found mainly in wheat, oat, rye, corn, barley, and rice. Patulin, produced by some species of *Penicillium*, *Aspergillus*, and *Byssochlamys*, is the main mycotoxin found in fruits, such as apple, pear, and cherry, and their derivatives. This toxin may irritate the stomach and cause vomiting and nausea.

Fusarium spp. species are responsible for the production of many mycotoxins, such as fumonisins, zearalenone, and trichothecenes (deoxynivalenol, also known as vomitoxin or DON, and toxin T2); corn is the source of these mycotoxins.

The toxic effects and economic losses caused by mycotoxins are of global concern for public health and agricultural and livestock production. Mycotoxin control should be done in the entire food production chain, taking into account the interactions between toxicogenic fungi and plants, storage manner and conditions, animal contamination and metabolism, detection methods, and mycotoxin elimination from foods.

Controlling and Inactivating Foodborne Pathogens in Foods

Diverse strategies have been used to control and inactivate foodborne pathogens. These methods are used for insuring

that foods are pathogen free or for keeping them within levels that do not jeopardize consumer's health.

Most animal-origin pathogens are transmitted by the oral/fecal route, and good manufacturing practices and hygienic conditions throughout the entire food production chain can reduce the risks of contamination effectively. Use of potable water, prohibition of animals in processing units, pest control, correct hygiene practices by food handlers, and proper hygienic and sanitary conditions are important for reducing the contamination of foods by pathogenic protozoans and intestinal viruses.

Some pathogenic bacteria depend on favorable conditions to grow on foods and produce enough toxins to cause foodborne illness. The inhibition of microbial growth by changing intrinsic and extrinsic factors, especially temperature, water activity, and pH, are ways of reducing foodborne illnesses caused by these bacteria.

Foodborne pathogens have different virulence levels. Sometimes a few pathogenic cells are capable of causing disease, so their elimination is essential for food safety. Among all inactivation methods, heat can be considered the most commonly used. However, consumers' demand for minimally processed foods and essentially unchanged nutritional and sensory characteristics requires nonthermal methods of microbial inactivation. Some of these methods are irradiation, high hydrostatic pressure, pulsed electric field, and ultrasound. However, more studies are required to determine how efficiently these methods can be used in industrial scale, not only to insure inactivation of specific microbial groups but also to minimize losses on the food components and be accepted by consumers.

Technological microorganisms in foods

Microorganisms of industrial importance

Biotechnology is defined as the use of live organisms or biological systems in industrial processes and waste treatment plants (Borem *et al.*, 2003). Today, biotechnology is equally important in food science and technology. Fermentation processes are a link between the old food preparation arts (cheese and wine, among others) that use natural microbiota and the modern food fermentation industry.

There are two types of cells in nature and both types are used in industrial fermentation processes: prokaryotes (bacterial cells) and eukaryotes (fungal, animal, and plant cells). Industrial microorganisms also differ as a function of their oxygen requirement: they may be strictly aerobic, such as *Streptomyces* and most filamentous fungi; strictly anaerobic, such as clostridiums; and facultative, such as industrial yeasts.

The bacteria used in fermentation processes are mainly chemoorganotrophic, that is, they obtain their energy by oxidizing organic compounds. Lactobacilli are bacteria that ferment carbohydrates, producing lactic acid. They can be homo- or heterofermentative. Homofermentative species produce only lactic acid from glucose, whereas heterofermentative species produce ethanol, carbon dioxide, and lactic acid from glucose.

The ability to ferment carbohydrates makes lactobacilli very useful for food production, but they may also cause food spoilage. However, Streptococci, Pediococci, and Lactococci are Gram positive, homofermentative cocci that produce mainly lactic acid. Then, these genera have a major importance for industrial processes.

Molds are also chemoorganotrophic and the most important molds used in industrial fermentation are classified into two groups: (1) Zygomycota, which have nonseptate hyphae and include the genera *Mucor* and *Rhizopus* and (2) Deuteromycota, which have septate hyphae and include the genera *Trichoderma*, *Aspergillus*, *Penicillium*, *Fusarium*, and *Aureobasidium*. Some *Mucor* and *Rhizopus* species are used for the production of cheeses and fermented Eastern foods. Among the Penicillia, *P. camemberti* and *Penicillium roqueforti* stand out because they are used for producing the Camembert and Roquefort cheeses, respectively.

Yeasts are unicellular fungi that reproduce asexually or sexually. The most commonly used yeasts in industrial fermentation processes are the *Saccharomyces*, with the most important species being *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* is used for many different purposes, such as the production of breads, beverages (beers and wines, among others), alcohol, and glycerol, among other uses in technological processes. *Saccharomyces cerevisiae* does not break down lactose, so the production of alcohol and biomass from whey requires another species, such as *Kluyveromyces lactis*. *Kluyveromyces lactis* has the necessary enzymes to break down lactose. Other important yeasts are: *Candida utilis*, used for processing apple residues (Fellows and Worgan, 1987), and *Endomycopsis fibuligera*, used for ethanol production (Reddy and Basappa, 1996; Chi *et al.*, 2009).

Biotechnological use of lactic acid bacteria in food production

LAB are found in very diverse environments, such as fermented foods and beverages, plants, fruits, soil, and residual water. They are also part of the respiratory and intestinal tracts of humans and other animals (Holzapfel and Wood, 1995).

The manufacturing of fermented meat products intends to continue using a set of microorganisms because they give the product agreeable sensory, hygienic, and sanitary characteristics. This set of LAB are introduced into the product by raw materials or by starter cultures sold frozen or freeze-dried (Patarata, 2002).

From the technological perspective, LAB have many potential uses ranging from control of the fermentation process in the production of fermented foods to their use as probiotics for human and animal health Inês *et al.*, 2008. According to Klaenhammer *et al.* (2002), the main beneficial and nonpathogenic genera used by the food industry are: *Lactococcus* (milk), *Lactobacillus* (milk, meat, vegetables, and grains), *Leuconostoc* (vegetables and milk), *Pediococcus* (vegetables and meat), *Oenococcus oeni* (wine), and *Streptococcus thermophilus* (milk). These microorganisms are generally regarded as safe (GRAS), although some studies in the literature have shown that some strains become opportunistic pathogens in individuals with a weakened immune system Zé-Zé *et al.* (2004).

LAB cultures are an important group of starter cultures used by the food industry for the production of cheeses, yogurts, sausages, sauerkraut, and sourdough (Messens and De Vuyst, 2002). Starter cultures quickly acidify the raw materials and give the final products pleasant sensory characteristics (Leroy *et al.*, 2006).

The quick acidification promoted by LAB prevents the development of other microorganisms that could give the final product undesirable characteristics (Hugas and Monfort, 1997). This inhibitory activity stems from their ability to produce lactic and acetic acids, hydrogen peroxide, bacteriocins, and surfactants (Fernández *et al.*, 2000).

Other microorganisms in this group include the probiotics. The probiotics are defined as "live organisms that, when administered in adequate amounts, confer a health benefit on the host" (Guarner and Schaafsma, 1998). LAB are the most common microorganisms used as probiotics (Cinque *et al.*, 2010).

Within the *Lactobacillus* genus, the most studied strains used as probiotics are *Lactobacillus acidophilus* LA1, *L. acidophilus* NCFB 1748, *Lactobacillus rhamnosus* GG, *Lactobacillus casei shirota*, *Lactobacillus gasseri* ADH, and *Lactobacillus reuteri*. They boost the immune system, help to control pathogenic microorganisms in the intestines by competitive exclusion, and have anticarcinogenic activity Leroy and De Vuyst, 2004. The genus *Bifidobacterium*, including *Bifidobacterium breve*, *Bifidobacterium longum* BB536, and *Bifidobacterium lactis* Bb12, also promotes good intestinal function. These microorganisms reduce intestinal irritation and can be used in the treatment of allergies, improvement of diarrhea caused by rotavirus, and reduction of the incidence of traveler's diarrhea (Ouwehand *et al.*, 2002).

The use of probiotics in animal feed has also been studied (Corcionivoschi *et al.*, 2010). The advantages include the improvement of intestinal disorders, inhibition of pathogenic bacteria, and proliferative stimulation of peripheral blood mononuclear cells (Collado *et al.*, 2007; Schierack *et al.*, 2009; Strompfova *et al.*, 2006). Hence, their use in livestock feed is increasingly desirable because they may dispense with the need of some antibiotics or other pharmaceutical products.

Biotechnological use of acetic acid bacteria in food production

The production of vinegar from alcohol–water solutions has been known for at least 10 000 years since Romans and Greeks obtained vinegar by the spontaneous fermentation of wine exposed to air (Crueger and Crueger, 1989). The process consists of the production of acetic acid.

The oxidation of ethanol into acetic acid is done by a mixed culture of acetic bacteria, preferably, a mixed microbiota of *Acetobacter* with different species or strains of the same species Zancanaro, 2001. However, different *Gluconobacter* and *Frateuria* strains are normally found in the microbiota used by vinegar manufacturers (Ebner *et al.*, 1996).

Although the classification of acetic acid bacteria is very problematic (Ebner *et al.*, 1996), it is estimated that mainly 20 strains of the species *Acetobacter aceti*, *Acetobacter pasteurianus*, *Acetobacter acidophilum*, *Acetobacter polyoxogenes*, *Acetobacter hansenii*, and *Acetobacter liquefaciens* are responsible for submerged acetification in the food industry Zancanaro, 2001.

Vinegars are classified according to the raw materials used in their preparation. For example, distilled vinegar is obtained by the acetification of diluted and distilled alcohol. Wine vinegar is obtained by the acetification of grape wine (Ebner *et al.*, 1996). Nondistilled alcohol–water solutions (fruit wines, grain wines, and others) do not require the addition of nutrients for acetification. These solutions already contain the nutrients required by acetic acid bacteria, as the preceding alcoholic fermentation helped to enrich them. However, the acetification of alcohol–water solutions prepared with distilled alcohol (ethanol or distilled beverages) requires the addition of many nutrients, namely glucose and dietary minerals, including ammonium phosphate (Ebner *et al.*, 1996; Zancanaro, 2001). Finally, if the solution requires dilution, the added water should be potable, of low hardness, and free from sediments and chlorine (Zancanaro, 2001).

Acetic acid bacteria are obligate aerobes. Interruption of aeration during any phase of the submerged fermentation process may impair vinegar production. The degree of impairment increases as the duration of the interruption and the total concentration of ethanol and acetic acid increase. For example, a 2 min aeration interruption in a solution with a total concentration of 5% causes a loss of 34% of the viable cells; the same loss occurs after a 10–20 s aeration interruption in a solution with a total concentration of 12% (Crueger and Crueger, 1989; Ebner *et al.*, 1996).

Additionally, once there is no more ethanol in the solution, acetic acid may be oxidized to CO_2 and H_2O depending on the organisms present in the solution (Ebner *et al.*, 1996). When ethanol reaches a critical concentration of 0.2%, the semicontinuous process must be stopped (Crueger and Crueger, 1989).

Biotechnological use of yeasts and molds in food production

Yeasts are used for processing numerous basic food items. Bread texture is given by the activity of the yeast *Sa. cerevisiae*, which ferments small amounts of sugar and releases carbon dioxide, forming bubbles. The production of beer also depends on yeasts. Beer is a malt beverage resulting from the alcoholic fermentation of an aqueous extract of malted barley and hops. Consequently, beer production is a multiple-stage process involving the biological conversion of fresh raw materials into a final product (Walker, 2000). All traditional brewing yeasts used in beer production consist of *Sa. cerevisiae* strains, which are a diverse group of microorganisms.

Still on beverages, sparkling wines are other gastronomic inventions produced by fungi. Nowadays, most wine is produced industrially by selected *Saccharomyces* sp. strains, and small wineries even select yeasts from their own environment as starter cultures (Ortiz *et al.*, 2013). The selected strains must have the following characteristics: ethanol tolerance, high fermentation activity, ability to grow in the presence of high sugar concentrations, and resistance to certain fermentation by-products (Nikolaou *et al.*, 2006). Even when strains meet these criteria, the starter culture may fail because of the presence of wild yeasts (Capece *et al.*, 2010).

Cheeses also deserve to be highlighted in addition to the above-mentioned products. They have probably been consumed by humans since the domestication of animals because they are an efficient means of storing milk proteins for long periods of time. There are more than 500 different types of cheeses, each one bearing specific characteristics of the location they were originally produced. However, only some cheeses are produced by fungi and these happen to be those with the most appreciated flavor and texture. These cheeses are divided into two categories: Camembert-type cheeses and blue Roquefort-type cheeses. The former includes the world-famous

Camembert and Brie and the less known cheeses Troyes, Thenay, and Vendome. These cheeses are produced by two *Penicillium* species: *camemberti* and *caseiolum* (hyphomycetes).

However, the blue cheeses, namely Roquefort, Stilton, Gorgonzola, Danish Blue, and Wensleydale, are produced with *P. roqueforti*. This fungus oxidizes fatty acids into methyl ketones, producing unique flavors and odors.

Many Asian countries developed a wide range of foods and condiments fermented by fungi. The most widely known fermented Asian food item is undoubtedly shoyu, a Japanese sauce made by fermenting soybeans with *A. oryzae*. Another important Japanese product is miso, a fine paste produced by fermenting soybeans and rice or barley. The fungi used for producing miso are *Saccharomyces rouxii* and *A. oryzae* (Abe *et al.*, 2006).

Assessing the Microbiological Quality and Safety of Foods

Microbiological Sampling Plans

The International Commission on Microbiological Specifications for Foods (ICMSF) has provided guidance on the use of sampling plans and microbiological criteria for international food trade for many decades. Many microbiological criteria have been established by the ICMSF and national surveillance and public health authorities. These criteria may be important in food analysis, including the analysis of raw materials and ingredients of unknown origin. Microbiological criteria are an important part of the food protection and public health systems, and they assist in determining whether foods meet required safety needs.

Two types of sampling plans, by attribute and by variable, are used for performing microbiological analysis and making decisions on food safety and quality (ICMSF, 1986). Attribute sampling plans assess data quality and detect the occurrence of changes, without determining their degree, i.e., they provide a qualitative assessment. However, variable sampling plans assess quality characteristics quantitatively by making measurements.

Variable sampling can be used when the numbers of microorganisms in the food follow a log-normal distribution. The implementation of such a plan requires making many decisions. The first decision regards the establishment of an acceptable limit for the microbiological quality of the study lot and a maximum proportion of the lot that may exceed said limit. Furthermore, an α (standard deviation of the log-normal distribution) should be chosen to represent the maximum probability of accepting the nonconformity of the lot, so 1α is the desired probability for rejecting the nonconformity of the lot (Schothorst *et al.*, 2009). The disadvantages of this plan include the calculations required for lot assessment and for each variable and the fact that the probability distribution of each measurement must be known or assumed. The transformation that insures a normal distribution may not be known, and the estimates of variance are often different for each measurement. For these reasons, variable sampling plans are not widely used in the food industry for microbiological measurement (Midura and Bryant, 2001).

Attribute sampling plans are used when the microbiological distribution in the food is unknown or when the counts of microorganisms do not follow a log-normal distribution (Forsythe, 2000). The ICMSF describes two attribute sampling plans (ICMSF, 1986): a two-class plan and a three-class plan (**Figure 1**). The two-class plan consists of specifying *n*, *m* and *c*, where *n* is the number of units sampled from a lot, *m* is the maximum acceptable number of microorganisms per gram, and *c* is the maximum number of units that may exceed *m* (the lot is rejected if *c* is exceeded).

A sampling plan may approve a relatively bad lot of food and reject a good lot. This can be illustrated by the operating characteristic curve (OC) (**Figure 2**), where the probability of acceptance on the *y*-axis is the expected proportion of times that a lot with a certain quality is accepted, and the proportion of defective sampled units of the lot is on the *x*-axis.

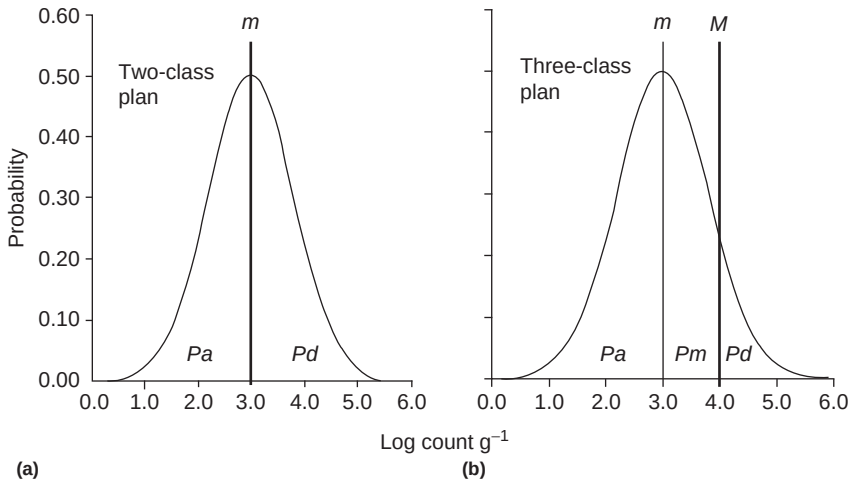

Figure 1 The relationships between acceptable and defective log concentrations for a two-class plan and acceptable and defective concentrations for a three-class plan when $m=3.0$, $M=4.0$, and distribution of organisms has mean$=3.0$ and $\sigma=0.8$. *Pa* is the proportion of acceptable material, *Pm* is the proportion marginally acceptable, *Pd* is the proportion defective, *m* is the maximum acceptable number of microorganisms per gram, and *M* is the upper bound on the marginally acceptable concentration. Adapted from Legan, J.D., Vandeven, M.H., Dahms, S., Cole, M.B., 2001. Determinig the concentration of microrganisms controlled by attributes sampling plans. Food Control 12, 137–147.

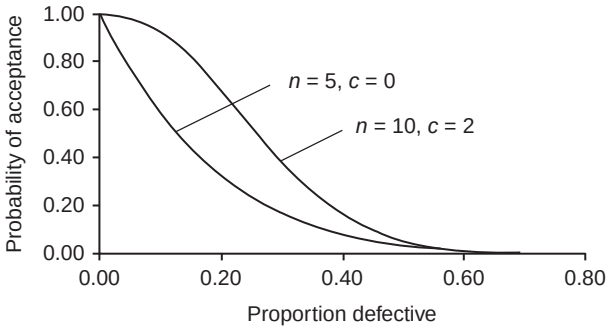

Figure 2 Operating characteristic curves showing the probability of acceptance at different defect rates for two-class plans with $n=10$, $c=2$ and $n=5$, $c=0$. n is the number of units sampled from a lot and c is the maximum number of units that may exceed m. Adapted from Legan, J.D., Vandeven, M.H., Dahms, S., Cole, M.B., 2001. Determinig the concentration of microrganisms controlled by attributes sampling plans. Food Control 12, 137–147.

The ability of a sampling plan to reject or approve the quality of a lot with high probability and the slope of the OC curve depend on n and c. **Figure 2** shows how much these characteristics change when the number of samples increases from 5 to 10 units, resulting in steeper curves and lower probabilities of acceptance, which provides better assurance that lots with high proportion defectives will be rejected (Dahms, 2004).

Three-class plans have an additional parameter called M, which is an amount chosen to distinguish marginally acceptable from unacceptable counts. Any sample with a count greater than M is rejected (**Figure 1**). Hence, a three-class plan is that in which the food may be divided into three classes according to its microbial load (Forsythe, 2010). The sample is acceptable if the counts are below m; marginally acceptable if the counts are greater than m but below M; and rejected if the counts exceed M. The strictness of a sampling plan should be given by the potential hazard of the food or its desired condition by the time it is consumed (ICMSF, 1986).

Overview of Microbiological Methods for Food Analysis

The microbiological analysis of food is part of food safety management and conformity tests that define microbiological criteria or assess the performance of control strategies based on the Hazard Analysis and Critical Control Point.

For microbiological testing of foods, rapid and conventional methods can be used. The conventional methods are called so because they were developed many years ago and have been in use ever since as the official methods of most food microbiology laboratories. These methods are described in the so-called reference publications; they are accepted internationally and recommended, for example, by the American Public Health Association, ICMSF, and the Food and Drug Administration.

The traditional methods have disadvantages associated with excessive laboratory work, time consumption, culture media, and laboratory glassware requirement. Other limitations should also be taken into account, such as technique failures related to high agar temperature and high risk of contamination because of all the stages involved in culture medium preparation and inoculation (Chain and Fung, 1991). The limitations of the traditional methods have encouraged the development of alternative methods for microbiological analysis of foods.

Many techniques for enumerating and identifying bacteria have been studied; they are currently called rapid methods. A rapid method can be defined as any method or system that reduces the time required for obtaining a microbiological test result (Feng, 1996). These include enumeration methods (quantitative methods) and detection methods (qualitative methods), which can be used depending on the objective of the study.

The direct epifluorescent filter technique consists of a rapid way of enumerating viable and unviable microorganisms using microscopy. The sample is pretreated with enzymes and surfactants and filtered with a polycarbonate membrane that traps microorganisms. Detection can be automated by connecting the microscope to an image analysis system (Jasson *et al.*, 2010). Enumeration takes 0.5–1.0 h, but the pretreatment stage may increase the total time required for analysis. The detection limit is 10^4–10^5 cells per ml. This technique has been used to enumerate bacteria in raw milk (Moran *et al.*, 1991; Rosmini *et al.*, 2004) and minimally processed vegetables (Araújo *et al.*, 2009).

Another microscope-related technique is flow cytometry, a powerful technique that uses light scattering for rapidly analyzing cells suspended in a fluid. Flow cytometry measures the optical characteristics of cells. Because most microorganisms are optically very similar, fluorescent stains may be used for verifying the microorganism's viability and their metabolic status (Flint *et al.*, 2006). This technique has been used for monitoring the load of somatic cells in milk (Gunasekera *et al.*, 2003), in wine (Malacrino *et al.*, 2001), and for assessing the microbiological quality of water (Delgado-Viscogliosi *et al.*, 2005).

Another rapid technique developed is the adenosine triphosphate (ATP) bioluminescence assay. This technique measures the light emitted by ATP when ATP reacts with luciferin and oxygen in the presence of the enzyme luciferase and magnesium cations. The amount of light produced (measured as relative light units) is proportional to ATP concentration, and consequently, to the number of microorganisms present in the sample. This technique can be used only if the microbial load is high ($>10\,000$ colony-forming units per gram, CFU g^{-1}). ATP is found not only in bacterial cells but also in any biological material, so ATP bioluminescence is generally used as a rapid indicator of organic load (Jasson *et al.*, 2010). The ATP bioluminescence assay has been used to determine the microbiological quality of food products, such as milk and dairy products, beverages, vegetables, meats, and meat products; to assess the water quality of the public water supply system; and to assess the efficiency of the cleaning and disinfection processes used by the food and pharmaceutical industries and by hospitals (Corbitt *et al.*, 2000; Griffiths *et al.*, 2000; Kennedy and Oblinger, 1985; Velazquez and Feirtag, 1997; Tydrich, 1996).

Classical or modified culture methods and molecular methods, such as enzyme-linked immunosorbent assay

(ELISA), enzyme-linked fluorescent assay (ELFA), fluorescence *in situ* hybridization (FISH), and conventional, real-time, and multiplex polymerase chain reaction, have been developed for detection of foodborne microorganisms. Classical methods are usually used for detecting pathogens in 25 g food samples. These methods require preenrichment stages because pathogens may have been injured in the study food (Wu, 2008; Jasson *et al.*, 2010). Later, other tests are used for confirming the findings.

ELISA is a biochemical technique based on antibody–antigen interaction. Detection may take from 2 to 3 h (Kumar *et al.*, 2008). ELFA is an automated test of the Bio-Mérieux's VIDAS® system that combines immunoenzymatic assay with fluorescence detection. A VIDAS® run may take from 25 to 120 min, depending on the target microorganism (Sewell *et al.*, 2003).

Molecular methods for the detection of bacteria in food, such as FISH, and multiplex and real-time PCR have also been developed and applied in microbiological analysis. FISH uses fluorescent probes to detect small deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) sequences. These probes consist of nucleotide sequences that complement the DNA or RNA sequences; they are designed to identify (Shimizu *et al.*, 2009).

PCR is a rapid, inexpensive, and safe technique based on the exponential amplification of a specific DNA region. This technique has revolutionized the scientific world and its uses are innumerous. PCR success is explained by its ability to amplify a specific DNA sequence and its simplicity, accuracy, high sensitivity, and high specificity. Owing to PCR specificity given by primers, DNA does not need to be isolated even if the DNAs from other species are present. PCR limitations include the synthesis of specific primers requires knowing the DNA sequence of their target region; PCR sensitivity allows it to detect the presence of DNA contamination, inducing incorrect results; the length of the target sequence cannot exceed 5 kb, because it is difficult to use PCR to amplify longer sequences; and incorrect bases may be incorporated into a strand.

Today there are techniques faster than PCR, such as the Bax System, which consists of automated, real-time PCR for the detection of *Salmonella*, *E. coli* 0157:H7, *L. monocytogenes*, *Listeria* spp., *Cr. sakazakii*, *Ca. jejuni*, and *Ca. coli* and some molds and yeasts. The Bax System can perform as many as 96 simultaneous analyses and an analysis takes approximately 3.5 h. This technique assesses the unique genetic structure of the microorganism, greatly increasing the sensitivity and specificity of the method (Yuste and Fung, 2007).

Other innovative techniques for the microbiological analysis of food are being tested, such as microarrays (DNA microchip). This technique consists of the hybridization of messenger RNAs (mRNA) on a surface containing millions of attached sequences of DNA. It is used for specific gene identification, genome comparison, and monitoring of gene expression (Jasson *et al.*, 2010; Ikeda *et al.*, 2006).

Biosensors are another innovative technique that may be used for detecting foodborne pathogens. They consist of biologically active material, such as enzymes, antibodies, antigens, or nucleic acids, attached to a surface. Biosensors reduce the time required for analysis and can be used for analyzing very small samples (Zhang *et al.*, 2009).

See also: Biotechnology Crop Adoption: Potential and Challenges of Genetically Improved Crops. Fermentation: Food Products. Fermented Beverages. Food Engineering. Food Labeling. Food Law. Food Packaging. Food Safety: Emerging Pathogens. Food Safety: Food Analysis Technologies/Techniques. Food Safety: Shelf Life Extension Technologies. Safety of Street Food: Indonesia's Experience

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