EDITED BY

RICHARD PODOLAK • DARRYL G. BLACK

CONTROL OF *SALMONELLA* AND OTHER BACTERIAL PATHOGENS IN LOW-MOISTURE FOODS

GMA
The Association of Food, Beverage and Consumer Products Companies

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Introduction and Overview

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1.1 Introduction

Low water activity (a_w) is a barrier to growth for many vegetative pathogens, including *Salmonella* spp. A product's water activity is used as a quantitative measure of the free moisture in foods available to microorganisms. Water in food that is not bound to food molecules can support the growth of bacteria, yeasts, and molds. The terms water activity and low moisture have been used interchangeably by food safety professionals even though they are quite different by definition. A variety of foods may have similar moisture content values but significantly different water activities. Of particular interest are the low-moisture foods, with water activity 0.85 and below. Processed products such as powdered milk, chocolate, peanut butter, infant foods, cereal, and bakery products are characteristically low water activity foods. While these products do not support the growth of *Salmonella*, all have been implicated in outbreaks of salmonellosis. Although some die-off occurs in low-moisture foods during storage, the degree of reduction depends on factors such as storage temperature and product formulation. Many other bacterial pathogens, such as toxin-producing *Staphylococci*, verotoxigenic *E. coli* (VTEC), *Cronobacter sakazakii* and aflatoxin-producing molds should also be considered in low-moisture foods. Due to its enhanced thermal residence in dry environments, *Salmonella* can survive for long periods in low-moisture food products. The heat resistance of *Salmonella* and other microorganisms of concern is affected by many factors, mostly by strain and serotypes tested, previous growth and storage conditions, the physical and chemical food composition, test media and the media used to recover heat damaged cells. The heat resistance of *Salmonella* generally increases with reducing moisture and this fact must be taken into account as a significant risk. Finally, from a quality standpoint, many spoilage organisms have been associated with low-moisture products; references will be provided in this book to aid the processor in finding the appropriate information concerning target organisms for specific low-moisture foods.

Control of *Salmonella* and Other Bacterial Pathogens in Low-Moisture Foods,
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1.2 Definition of Low-Moisture Foods (LMF) and Water Activity Controlled Foods

Manipulation of the water content of foods is a classical method for food preservation and has been used by people for centuries. Salting, curing, drying, and the addition of sugars are examples of several traditional preservation methods that have been practiced over the ages. Of particular interest are the low-moisture foods and food ingredients that are naturally low in moisture or that have been subjected to a drying process and resulted in reducing the water content, for example, as in traditional sun-dried foods. Processed products such as milk-based powders, chocolate, peanut butter, powdered infant foods, seeds, herbs and spices, cereal and bakery products, and animal feeds are the examples of this type of food. Low-moisture foods have a reduced water activity (a_w), which is a growth barrier for many vegetative pathogens, including *Salmonella* spp.

Both moisture content and water activity are key parameters in predicting the stability of low-moisture food products. The terms water activity and low moisture have been used interchangeably in the processing industry and by food safety professionals even though they are quite different by definition. Moisture content represents a measure of the quantity of water in a product, providing information about yield and texture, but it does not provide reliable information about microbial safety. However, water activity, which was originally applied by the pharmaceutical and food industries, can be used as a quantitative measure to determine the shelf-life of product. Water activity can be defined as the ratio of the vapor pressure of water in a food matrix compared to that of pure water at the same temperature (Labuza, 1980). Therefore, a water activity of 0.80 means the vapor pressure is 80% of that of pure water. Water activity can be also defined as the equilibrium relative humidity (expressed as a percentage) above the food in a closed container dived by 100. For example, an equilibrium relative humidity of 70% would be an equivalent to a water activity of 0.7. The water activity scale extends from 0 (bone dry) to 1.0 (pure water) but most foods have a water activity level in the range of 0.2 for very dry foods to 0.99 for very moist fresh foods.

In the food system, total water is present in "free" and "bound" forms. Growth of microorganisms can be limited or entirely prevented by binding water to make it inaccessible to microorganisms, for example, when salt and/or sugar are mixed with the food. The extent to which water is 'bound" in foods is expressed in terms of water activity. Bound water is necessary to hydrate the hydrophilic molecules and to dissolve the solutes but it is not available for biological functions, so it does not contribute to water activity. It is necessary for the transport of nutrients and the removal of waste materials, to carry out enzymatic reactions, to synthesize cellular materials, and to take part in other biochemical reactions. Thus, this type of water in foods cannot be used by microorganisms for growth. The remaining water in foods exists in "free" form. Water activity is a measure of the "free" water that is available in food to react with other molecules and participate in spoilage reactions, such as enzymatic browning or microbial growth.

A product's water activity is used as a quantitative measure of the free moisture in foods available for growth of microorganisms. Water that is not bound to food molecules can support the growth of bacteria, yeasts, and molds. Thus, water activity is an indicator of stability with respect to microbial growth, biochemical reaction rates, and physical properties.

When substances are dissolved in water, there is a reaction between the substance and water. If some food ingredients such as sugar, salt, dried fruits, and so on are added to food products, they will be substantially bound to the molecules of water and reduce the number of unattached water molecules, consequently reducing the amount of water available for growth of microorganisms. The amount of water available for microorganisms will depend on the water-binding capacity of the particular ingredient; thus, the water activity of a product is dependent on food composition. At the same molecular concentration, salt lowers water activity more than sugar. For example, sodium chloride has a water-binding ability almost six times higher than sucrose. The final ingredients in a product formulation and their effect on water-binding capacity are the critical factors in controlling water activity in foods. Thus, an essential component in assuring the required water activity will be the predetermination and accurate control of product formulation at the time of preparation and packing.

The water activity of low-moisture foods is also dependent on relative humidity and temperature during storage. Although microbial spoilage is prevented at a_w below 0.60, low-moisture foods are prone to gain moisture, which can be followed by undesirable changes, such as structural transformations, enzymatic changes, browning, and oxidation, depending on water activity and temperature. For example, in wafers, texture and mechanical characteristics critically depend on moisture content, due to the plasticization effect in the starch matrix (Parasoglou *et al.*, 2009). If a wafer is too dry after baking, then it is brittle and breaks easily, making further processing difficult; but if the moisture content is too high, the texture is affected and bacterial growth may result in a significant decrease in the product shelf-life (Parasoglou *et al.*, 2009). In instant coffee powder, high moisture content interferes with the flow characteristics and agglomeration of the product, while overdrying can result in a loss of volatile compounds affecting flavor. The effect of temperature on the water activity of a food is product specific. Some products increase in water activity with increasing temperature, others decrease with water activity, while in most high-moisture foods there is negligible change with temperature. Therefore, it is difficult to predict the direction of the change in water activity with temperature, since it depends on how temperature affects the factors that control the water activity in the food.

The relationship between moisture content and water activity is complex. A variety of foods may have similar moisture content values but significantly different water activities due to the different water-binding capacities of the food ingredients. An increase in water activity is almost always accompanied by an increase in the moisture content, but in a nonlinear trend, called the moisture sorption isotherm, at a given temperature. Moisture sorption isotherms are useful thermodynamic tools for determining interactions between water and food materials and provide information that can be used for selecting appropriate storage conditions and packaging systems that optimize retention of aroma, texture, nutrient and biological stability (Ariahu, Kaze and Achem, 2006). Sorption isotherms provide information on the moisture-binding capacity of products at a determined relative humidity and are useful means of analyzing the moisture plasticizing effect and the effect on mechanical properties (Bell and Labuza, 2000; Al-Muhtaseb, McMinn, and Magee, 2002).

A variety of microorganisms can grow in food products, and each microorganism can survive in different ranges of water activity. The optimum water activity for growth of most microorganisms is in the range 0.995–0.98. (Lund, Baird-Parker, and Gould, 2000). Fresh foods with a_w values above 0.95 rapidly spoil if they are not rapidly refrigerated. Bacteria require the highest amount of free water to grow and can be found in products with a_w as low as 0.75, but most are inhibited at a_w below 0.91. At lower water activity, yeasts and molds become the main spoilage organisms, with a minimum growth at a_w of approximately 0.88 and 0.75, respectively. Of the food poisoning bacteria, *Staphylococcus aureus* is one of the organisms of most concern, as it has been reported to tolerate a_w as low as 0.85 under aerobic conditions and pH = 7 (Brown, 1976; Notermans and Heuvelman, 1983).

Some species of bacteria, yeast, and molds can grow well below the minimum water activity stated and these exceptions are responsible for the food microbiologist's problems. Among these exceptional species are halophilic bacteria and osmophilic yeasts and molds that either tolerate or require high concentrations of solutes in growth medium and are able to grow on food with an a_w as low as 0.60. Several species of xerophilic spoilage molds, such as *Aspergillus chevalieri, Chrysosporium fastidium,* and several *Eurotium* species, and osmophilic yeasts, including *Zygosaccharomyces rouxii*, can grow at a_w 0.60–0.70, whereas the halophilic bacteria and halophilic molds are able to grow at a_w as low as 0.75 and 0.70, respectively. Below 0.60, yeasts, molds, and bacteria will not proliferate.

Definitions of low-moisture foods in terms of water activity values vary within wide limits. The US Food and Drug Administration (FDA) defines low-moisture foods as foods with an a_w of 0.85 and below (FDA, 2010). Also, the Codex Committee on Food Hygiene considers a low-moisture product to be a food with a water activity of 0.85 or below (CCF, 2013). This definition implies that some dried fish or meat products fall under the scope; however, the committee suggests excluding dried fish and meat products from the scope of this code. There is also a subcategory of low-moisture foods that considers foods with a_w ranging from 0.75 to 0.83 to be classified as intermediate-moisture foods (IMF) (Corry, 1976). Foods with a_w levels below 0.7 have also been classified as low-moisture foods (Blessington, Mitcham, and Harris, 2012).

1.3 *Salmonella* as a Continuing Challenge and Ongoing Problem in Low-Moisture Foods

Foodborne disease caused by contaminated low-moisture foods continues to be a challenge and problem in the United States. In 2013, the Foodborne

Diseases Active Surveillance Network (FoodNet), which monitors the incidence of laboratory-confirmed infections caused by food pathogens transmitted commonly through food in United States, identified 19 056 cases of infection, 4200 hospitalizations, and 80 deaths. The total number of *Salmonella* infections was 7277, resulting in 27 deaths; the number of incidence per 100 000 populations was 15.19. *Salmonella* serotypes implicated with outbreaks in low-moisture foods are presented in Table 1.1. Among 6520 (90%) *Salmonella* isolates, the top serotypes were: Enteritidis, 1237 (19%); Typhimurium, 917 (14%); and Newport, 674 (10%). The rate of *Salmonella* infections (15.19 per 100 000 population) decreased by about 9% in 2013 compared with the previous three years but it remains similar to 2006–2008, which is above the current national Healthy People objective and the national goal for 2020 (which are both 11.4 cases per 100 000 population). The incidence of serotype Enteritidis infection was lower in 2013 than in 2010–2012, but was not lower than in 2006–2008. This may be partly explained by the large Enteritidis outbreak linked to eggs in 2010 (CDC, 2014a).

Table 1.1 *Salmonella* serotypes implicated with outbreaks in low-moisture foods.

Food	Serotype	Reference
Chocolate	Eastbourne, Napoli, Nima, Montevideo, Oranienburg, Infantis, Typhimurium	Greenwood and Hooper, 1983; Hockin *et al.*, 1989; Kapperud *et al.*,1990; Werber *et al.*, 2005
Hard cheese	Heidelberg, Typhimurium	Van Duynhoven *et al.*, 2009
Black pepper	Oranienburg, Weltevreden	Van Doren *et al.*, 2013
Peanut/ Peanut butter	Mbandaka, Typhimurium, Tennessee, Stanley, Newport	CDC 2007a, 2009; Ng *et al.*, 1996
Paprika	Saintpaul, Rubislaw and Javiana	Lehmacher, Bockemühl, and Aleksic, 1995
Infant dried milk	Ealing	Rowe *et al.*, 1987
Savoury snacks	Manchester (yeast-based flavor), Agona	Killalea *et al.*, 1996
Infant cereal food	Senftenberg	Rushdy *et al.*, 1998
Toasted oat cereal	Agona	CDC, 1998
Coconut	Java, Senftenberg, Typhimurium	Wilson and MacKenzie, 1955
Almonds	Enteritidis	CDPH, 2002, 2004; Chan *et al.*, 2002; Isaacs *et al.*, 2005; Danyluk *et al.*, 2007; Keady *et al.*, 2004
Milk powder	Derby, Oranienburg	D'Aoust and Maurer, 2007; RASFF, 2012

1.4 Foodborne Outbreaks of *Salmonella* spp. and Other Implicated Microbial Pathogens in Low-Moisture Foods

Salmonella spp. and other bacterial pathogens are still a continuing problem in low-moisture foods. While low-moisture food products with a water activity of 0.85 and below do not support the growth of *Salmonella* spp. and other bacterial pathogens, many of them have been linked to international and domestic outbreaks (e.g., nuts, cereal products, and spices). Selected international outbreaks of *Salmonella* spp. during the period 1970–2014 that have been linked with low-moisture food products are presented in Table 1.2, and selected outbreaks between 2007 and 2015 of various other bacterial pathogens are presented in Table 1.3. Bacterial pathogens such as *Escherichia coli* O157:H7, *Bacillus cereus, Campylobacte*r species, *Clostridium botulinum, Clostridium perfringens, Cronobacter species, Listeria monocytogenes, Staphylococcus aureus*, and aflatoxin producing molds should be considered in low-moisture foods. Table 1.4 presents information on the characteristics of bacterial and viral pathogens that have caused infections or intoxications as a result of the consumption of low water activity food products. Each of these microorganisms is reviewed from the standpoint of the following: source of the microorganism, the illness, temperature and pH for growth, heat resistance, minimum water activity for growth, and selected control methods.

Table 1.2 Selected international outbreaks of *Salmonella* spp. during the period 1970–2014 linked with low-moisture food products.

Year	Product	*Salmonella* serotype	Country	References
1970	Chocolate	Durham	Sweden	Gastrin *et al.*, 1972
1973	Milk powder	Derby	Trinidad	D'Aoust and Maurer, 2007
1982–1983	Chocolate	Napoli	UK	Greenwood and Hooper, 1983
1985–1986	Chocolate	Nima	Canada, USA	Hockin *et al.*, 1989
1987	Chocolate	Typhimurium	Norway, Finland	Kapperud *et al.*, 1990
1993	Powdered infant formula	Tennessee	Canada, USA	CDC, 1993
1995	Infant cereals	Senftenberg	UK	Rushdy *et al.*, 1998
1996	Peanut butter	Mbandaka	Australia	Ng *et al.*, 1996
1998	Toasted oats cereals	Agona	USA	CDC, 1998

Table 1.2 (Continued)

Year	Product	*Salmonella* serotype	Country	References
2000–2001	Raw almonds	Enteritidis	USA, Canada	CDC, 2004
2001	Peanuts	Stanley, Newport	Australia, Canada, and UK	Little, 2001
2001	Chocolate	Oranienburg	Germany, Sweden, Denmark, Austria, Belgium, Finland, Netherlands	Werber *et al.*, 2005
2002	Tahini and Halva	Montevideo	Australia	Tauxe, O'Brian, and Kirk, 2008
2003–2004	Raw almonds	Enteritidis	USA, Canada	CDC, 2004
2006	Chocolate	Montevideo	UK	FSA, 2006
2006–2007	Peanut butter	Tennessee	USA	CDC, 2007a
2007	Children's snack	Wandsworth, Typhimurium	USA	CDC, 2007b
2008	Puffed cereals	Agona	USA	CDC, 2008
2008	Powdered infant formula	Give	France	Jourdan *et al.*, 2008
2008–2009	Peanut butter, peanut butter containing products	Typhimurium	USA, Canada	CDC, 2009
2009	Peanut butter flavored snack bars	Typhimurium	USA	RASFF, 2009
2009	Peanut butter	Typhimurium	USA	RASFF, 2009
2010	Dried sausage	Typhimurium	France	RASFF, 2010
2011	Ground cumin	Caracas	UK	RASFF, 2010
2012	Dried milk powder	Oranienburg	Belgium	RASFF, 2012
2012	Peanut butter and peanut-based products	Bredeney	USA	RASFF, 2012
2012	Dry dog food	Infantis	USA	CDC, 2012
2012	Nut butter	Braenderup	USA	CDC, 2012
2012	Turkish pine nuts	Enteritidis	USA	CDC, 2012
2013	Tahini sesame paste	Montevideo, Mbandaka	USA	CDC, 2013
2014	Nut butter	Braenderup	USA	CDC, 2014b

Table 1.3 Selected international outbreaks between 2007 and 2015 of food pathogens other than *Salmonella* spp. associated with low-moisture food products.

Year	Product	Pathogen	Country	References
2007	Rice, seeds, nuts, and almonds	*B. cereus* *E. coli* STEC *S. aureus* *Staphylococcus* spp	EU	EFSA, 2010
	Herbs and spices	*B. cereus* *C. perfringens*	France, Serbia, Sweden, UK	EFSA, 2009
	Fried rice	*B. cereus*	USA	CDC, 2012
2008	Rice	*B. cereus*	USA (Georgia)	CDC 2012
	Spanish rice	*C. perfringens*	USA (Colorado)	CDC, 2012
2009	Rice	*B. cereus*	USA (Alabama)	CDC, 2012
	Raw cookie dough	*E. coli* O157:H7	USA (30 states)	CDC, 2012
2010	Rice	*B. cereus*	USA (Florida)	CDC, 2012
	Dried tofu	*C. botulinum*	Taiwan	SFI, 2012
2011	Fenugreek seeds	*E. coli* O104:H4	Germany	EFSA, 2011
	Raw shelled walnuts	*E. coli* O157:H7	Canada	CDC, 2012
	In-shell hazelnuts	*E. coli* O157:H7	USA (3 states)	CDC, 2012
2013	Almond puree	*C. botulinum*	France, Norway	RASFF, 2013

Table 1.4 Characteristics of bacterial and viral pathogens of concern in low-moisture food.

Bacillus cereus	
Source	The normal habitat and/or distribution for *B. cereus* is dust, water, soil.
Disease, symptoms, and onset	It produces two types of gastroenteritis, emetic and diarrheal. The diarrheal syndrome (also called *C. perfringens*-like) is caused by an enterotoxin that is a vegetative growth metabolite formed in the intestine. The toxin is a protein (50 kDa) that is heat labile (56°C, 5 min) and trypsin sensitive. The illness onset for this syndrome is 8–16 h and it has a duration of 6–24 h. The symptoms include nausea, abdominal cramps, and diarrhea. The emetic syndrome (also called *S. aureus*-like) is also caused by a cyclic polypeptide toxin that is much smaller (5000 Da) and may be preformed in certain foods. As opposed to the diarrheal toxin, the emetic toxin is heat (>90 min at 121°C) and trypsin stable. The illness onset is very short, from 1 to 6 h and the duration is <24 h. Symptoms include nausea and vomiting (more severe than diarrheal). The illness is not generally fatal. Infectious dose: diarrheal, 5–7 log cells; emetic, 5–8 log cells.
Characteristics of microorganism	• Facultative anaerobe, Gram positive, spore-forming rod-shaped bacterium • Spore formers • Grows at 4–55°C (optimum 30–40°C) • Grows at pH 5.0–8.8 (optimum pH 6.0–7.0)

Table 1.4 (Continued)

Minimum a_w for growth/toxin formation and survival in low-moisture food	• Growth and toxin formation 0.92–0.93 • Spores can survive for a very long periods
Heat resistance of microorganism	• Spores are of moderate-to-high heat resistance • $D_{95°C}$ = 1.2–36 min, z-value 7.9–9.9°C
Control	• 7.5% NaCl inhibits growth • Application of preservatives/antimicrobials: sorbate, propionate, benzoate, nisin • Modified atmospheres • Radiation
References	Davidson, 2002; Granum, 2007; Schraft and Griffiths, 2005
***Campylobacte*r species**	
Source	Intestinal tract of wild and domestic warm-blooded animals. Most common contaminated foods are milk and poultry products. Can be also found in insects and water.
Disease, symptoms, and onset	*Campylobacter jejuni* causes a gastroenteritis called campylobacteriosis that has an onset time of 2–5 days and has primary symptoms of severe diarrhea and abdominal pain. Fever and headache may also be present. The duration is <1 week without treatment and the mortality rate is very low. Complications of campylobacteriosis include relapse (5–10%), bacteremia, acute appendicitis, meningitis, urinary tract infections, endocarditis (primarily *C. fetus*), peritonitis, Reiter's Syndrome (reactive arthritis), and Guillain-Barré Syndrome. Infectious dose as low as 500 cells.
Characteristics of microorganism	• Microaerophilic • Nonspore-forming, Gram negative, vibroid (helical, S-shaped, or gull-wing shaped) or spiral-shaped rods • Grows at 32–45°C (optimum 42–43°C) • Grows at pH 4.9–9 (optimum 6.5–7.5). Rapid death in foods at pH less than 4, especially at above refrigerated temperature
Minimum a_w for growth/toxin formation and survival in low-moisture food	• 0.98 (=2.0 NaCl), toxin formation 0.92–0.93 • Sensitive to drying but under refrigerated conditions can remain viable for several weeks. Food type influences survival at refrigerated and frozen conditions. Survival in food is better under refrigerated conditions than at room temperature, up to 15 times as long at 2°C than 20°C
Heat resistance of microorganism	• Rapidly inactivated by heating at 55°C and above • $D_{55°C}$ = 0.6–2.3 min, $D_{60°C}$ = 0.2–0.3 min, z-value 3.5–8°C
Control	• Chlorination of water • Pasteurization process • Avoid cross-contamination
References	Davidson, 2002; Solomon and Hover, 1999; Nachamkin, 2007; ICMSF, 2005; NACMCF, 1995

(Continued)

Table 1.4 (Continued)

Clostridium botulinum	
Source	Soil; the intestinal tract of animals, including fish. Almost all foods, especially vegetables, will contain *C. botulinum* spores.
Disease, symptoms, and onset	• The foodborne illness termed botulism is intoxication. The onset time is 12–36 h; the symptoms are blurred or double vision, dysphagia (difficulty swallowing), general weakness, nausea, vomiting; dysphonia (confused speech), and dizziness. The intoxication is due to a neurotoxin that first affects the neuromuscular junctions in the head and neck. The toxin causes paralysis, which progresses to the chest and extremities. Death occurs when paralysis reaches the muscles of the diaphragm or heart. Duration of the illness can be from 1 day to several months. A high proportion of patients require respiratory therapy. Death occurs without treatment in 3–6 days. The mortality rate was very high (30–65%) in the early part of the twentieth century but has been reduced significantly in recent years due to better detection and treatment. The treatment for botulism is administration of an antitoxin. Its success depends upon timing, since the toxin binds to myoneural junctions irreversibly. • *Clostridium botulinum* toxin is one of the most toxic substances known. The toxin is absorbed into the blood stream through respiratory mucous membranes or the walls of the stomach or small intestine. It then enters the peripheral nervous system and attaches at the myoneural junction, blocking release of acetylcholine and causing paralysis of the muscle. Heat resistance of the toxin is low, with 5–10 min at 80°C (Type A) or 15 min at 90°C (Type B) required to inactivate. • Infants less than 1 year old are susceptible to infant botulism. In adults, preformed *C. botulinum* toxin must be ingested. In infants, if as few as 10–100 spores of *C. botulinum* are ingested they may germinate in the intestinal tract and produce toxin. The illness occurs in infants most likely because their intestinal microflora is not established enough to prevent *C. botulinum* colonization. Types A and B are primarily involved. Symptoms of the illness are weakness, loss of head control, and diminished gag reflex. • Infectious dose: adult botulism, no infectious dose – toxin causes illness; infant botulism, 10–100 spores of *C. botulinum*
Characteristics of microorganism	• Anaerobe, Gram-positive, spore-forming bacterium with oval to cylindrical, terminal to subterminal spores • Nonproteolytic types grow at low temperature ≥3.3, optimum 28–30°C, most proteolytic types grow at ≥10°C (optimum 35–40°C) • Nonproteolytic types minimum pH 5.0, proteolytic types minimum pH 4.6
Minimum a_w for growth/toxin formation and survival in low-moisture food	• Nonproteolytic types: 0.97 • Proteolytic types: 0.93
Heat resistance of microorganism	• Nonproteolytic types: $D_{100°C}$ <0.1 min, z-values 7–10°C • Proteolytic types: $D_{121°C}$ = 0.21 min, z-value 10°C

Table 1.4 (Continued)

Control	• Retort product to destroy spores • Low pH and low water activity • Temperature control
References	Davidson, 2002; Johnson, 2007; ICMSF, 2005; NACMCF, 1995
Clostridium perfringens	
Source	Soil, water, dust, air, and certain raw foods such as meats and spices.
Disease, symptoms, and onset	The gastroenteritis syndrome is an infection and is the result of an enterotoxin formed in the intestine. Onset time is 8–24 h and primary symptoms include diarrhea and abdominal cramps. The duration is 12–24 h and the mortality is low. The microorganism produces a protein enterotoxin (35 kDa) during sporulation and concentration of the toxin is greatest immediately prior to cell lysis. Sporulation occurs at a high rate in the gut. Infectious dose: around 6–8 log.
Characteristics of microorganism	• Gram-positive spore-forming rod • Grows well anaerobically and in reduced oxygen conditions • Grows at 12– 50°C (optimum 43–47°C) • Grows at pH 5.5–9 (optimum 7.2)
Minimum a_w for growth/toxin formation and survival in low-moisture food	• 0.93 • Spores are highly resistant to desiccation but vegetative cells are not very tolerant of low water activity
Heat resistance of microorganism	• Spores: $D_{95°C}$ 17.6–63 min • Vegetative cells: $D_{60°C}$ 5.4–14.5 min
Control	• Proper heating and cooling of cooked foods
References	Davidson, 2002; McClane, 2007; ICMSF, 2005; NACMCF, 1995
Cronobacter species	
Source	Dry foods such as powdered baby formula, powdered milk, herbal teas, wheat, rice, and starches. It has also been found in sewer water.
Disease, symptoms, and onset	Infants – defined as children <1 year of age – and especially infants <28 days old are the primary victims of *Ent. sakazakii* infections. *Cronobacter* germs usually get in the blood or make the lining of the brain and spine swell (meningitis). In infants there are three main classes of illness associated with *Ent. sakazakii*: (i) meningitis, (ii) bacteremia or the more serious sepsis, and (iii) necrotizing enterocolitis. Sickness from *Cronobacter* in babies will usually start with a fever and poor feeding, crying, or very low energy. Some babies may also have seizures. Babies with meningitis may develop serious, long-lasting problems in their brains. The mortality rate of infants who develop *Ent. sakazakii*-associated neonatal meningitis is estimated to be 40–80%). People of all ages: *Cronobacter* can cause problems in cuts, scrapes, or places where people have had operations. *Cronobacter* can also get into the urinary tract. Older people and people whose bodies have trouble fighting germs because of a sickness they already have may also get *Cronobacter* in their blood. Mental retardation and quadriplegia have been reported. Approximately 1000 cells may be sufficient to cause an infection.

(Continued)

Table 1.4 (Continued)

Characteristics of microorganism	• Motile peritrichous Gram-negative rod-shaped nonspore-forming bacteria • Facultative anaerobe • Grows at 5.5–45°C (optimum 39.4°C) • Min pH 3.89, pH 5–9 (optimum), no maximum value found in the literature
Minimum a_w for growth/toxin formation and survival in low-moisture food	• Survive at 0.2; minimum for growth not known • Ability to survive in dry foods up to 2 years in powdered infant formula
Heat resistance of microorganism	• Spores: $D_{60°C}$ 1.05–2.5 min, z-value 5·6–5·8°C for 6 min
Control	• No synergistic interactions between inhibitory factors such as weak acids, pH, salt and temperature.
References	Breeuwer, Lardeau, and Joosten, 2003; Cordier,. 2008; Kandhai *et al.*, 2006; Lambert and Bidlas, 2007; Nazarowec-White and Farber, 1997; Townsend and Forsythe, 2008; Iversen and Forsythe, 2003, 2004
***Escherichia coli* O157:H7**	
Source	Intestinal tract of humans (transmitted via person-to-person) and animals (dairy cattle (healthy), deer, sheep) and water.
Disease, symptoms, and onset	The spectrum of human illness of *E. coli* O157:H7 infection includes nonbloody diarrhea, hemorrhagic colitis (bloody diarrhea), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP). Some persons are infected but asymptomatic. About one-third of the patients infected with *E. coli* require hospitalization. The illness caused by Enterohemorrhagic *Escherichia coli* (EHEC) has an onset time of 12–60 h. The duration of the illness may be 2–9 days, with an average of 4 days. In 2–7% of patients (most often younger age groups), HUS develops. HUS is characterized by hemolytic anemia, thrombocytopenia, and renal failure. Damage to renal endothelial cells is caused by blood clotting in the capillaries of kidney and accumulation of waste products in blood, which results in a need for dialysis. Approximately, half of the patients with overt symptoms of HUS require blood dialysis and three-quarters require transfusions of erythrocytes and/or platelets. The death rate associated with HUS is 3–5%. Thrombotic thrombocytopenic purpura is an involvement of the central nervous system that occurs primarily in elderly adults. This can lead to blood clots in the brain. The infectious dose of EHEC for susceptible persons is estimated to be as low as 2–2000 cells.
Characteristics of microorganism	• Nonspore-forming, Gram-negative, rod-shaped bacterium • Facultative anaerobe • Grows at 7–46°C (optimum 35–37°C) • Grows at pH 4.4–9.0 (optimum pH 6.0–7.0), can survive below pH 4.6
Minimum a_w for growth/toxin formation and survival in low-moisture food	• 0.95 for growth • Ability to survive in dry food such as dry fermented meats

Table 1.4 (Continued)

Heat resistance of microorganism	• Spores are of moderate-to-high heat resistance • $D_{63°C}$ 0.5 min, z-value 6°C
Control	• Proper cooking and reheating of foods • Proper refrigeration ≥4.4°C • Good sanitation and personal hygiene • Low pH and low water activity
References	Davidson, 2002; Meng, Doyle, and Zhao, 2007; ICMSF, 2005; NACMCF, 1995
Listeria monocytogenes	
Source	Occurs in human carriers (1–10% of the population), healthy domestic animals, normal and mastitis milk, silage (especially improperly fermented (high pH)), soil, and leafy vegetables.
Disease, symptoms, and onset	*Listeria* often may pass through the digestive systems of healthy people, causing only mild, flu-like symptoms or without causing any symptoms at all. Foodborne illness caused by *L. monocytogenes* in pregnant women can result in miscarriage, fetal death, and severe illness or death of a newborn infant. Pregnant women are most frequently infected in the third trimester. The mother's symptoms are influenza-like (chills, fever, sore throat, headache, dizziness, low back pain, and diarrhea). During the illness the microorganism localizes in the uterus in the amniotic fluid, resulting in abortion, stillbirth or delivery of an acutely ill baby. Once the fetus is aborted, the mother becomes asymptomatic. In newborns infected with the microorganism, perinatal septicemia involving the central nervous system, circulatory system, or respiratory system or meningitis may occur. For other target groups, meningitis, meningoencephalitis or bacteremia are the most common outcomes. In target populations the onset time for listeriosis can be as short as 1 day and as long as 91 days. In food-related human infections, *L. monocytogenes* likely enter the host via intestinal epithelial cells or Peyer's patches and are phagocytized and transported to the liver where they cause infection. Infectious doses dependent upon the immunological status of the host and type of food consumed; generally, 100–1000 cells are required to cause disease.
Characteristics of microorganism	• Nonspore-forming Gram-positive rods; they are motile via peritrichous flagella at 20–25°C but not at 37°C • Facultative anaerobe • Grows at −0.4–45°C (optimum 37°C) • Grows at pH 4.4–9.4 (optimum pH 7.0)
Minimum a_w for growth/toxin formation and survival in low-moisture food	• 0.90–0.93 for growth • Ability to survive in dry foods, dry fermented meats, and peanut butter (a_w 0.33) • Can remain viable in dry environment for long periods
Heat resistance of microorganism	• $D_{60°C}$ 1.6–16.7 min in food substrates, 70°C for 2 min (z = 13.5°C)
Control	• Proper heat treatment, low pH and low water activity, avoidance of re-contamination, addition of inhibitors to growth
References	Davidson, 2002; Swaminathan *et al*., 2007; ICMSF, 2005; NACMCF, 1995

(Continued)

Table 1.4 (Continued)

Salmonella spp	
Source	Intestinal tract of animals such as birds, reptiles, farm animals, humans and insects, water, soil. They may also be found in animal feeds and foods, including raw milk, poultry (up to 70%), raw meats, eggs, and raw seafood.
Disease, symptoms, and onset	• The nontyphoid foodborne illness caused by *Salmonella* is a gastroenteritis called "salmonellosis." It is classified as an infection. The onset time is 8–72 hours and duration is about 5 days. The primary symptoms include nausea, vomiting, abdominal pain, headache, chills, mild fever, and diarrhea. Salmonellosis may progress to septicemia or chronic sequelae such an ankylosing spondylitis, reactive arthritis, or rheumatoid arthritis. *Salmonella* cells attach to and invade gastrointestinal tissue in the small intestine. The mortality rate associated with the illness is low (<1%) but is age dependent. • Infectious dose: the number of cells required to produce symptoms varies with individual and strain and can be as low as 1 cell per gram of food up to 7 log cells. It was estimated that 6 cells per 65 g of ice cream caused a massive outbreak of salmonellosis in 1994.
Characteristics of microorganism	• Nonspore-forming, Gram-negative rods • Facultative anaerobe • Grows at 5.2–46.2°C (optimum 35–43°C) • Grows at pH 3.8–9.5 (optimum pH 7.0–7.5)
Minimum a_w for growth/toxin formation and survival in low-moisture food	• 0.94 for growth • Ability to survive in dry foods for weeks, months or years. Can remain viable in dry environment for long periods
Heat resistance of microorganism	• $D_{60°C}$ 0.1–10 min, z-value 4–5°C, heat resistance increased in low water activity and high fat foods
Control	• Proper heat treatment • Low pH • Avoidance of re-contamination • Proper hygiene of food handlers
References	Davidson, 2002; D'Aoust, and Maurer, 2007; ICMSF, 2005; NACMCF, 1995
Staphylococcus aureus	
Source	Usually humans. The microorganism is carried in the nasal cavity on the skin (arms, hands, face) and by wounds (boils, carbuncles). *Staphylococcus aureus* may also be found in air, dust, and on clothing. It may be associated with mastitis infection in dairy cattle.
Disease, symptoms, and onset	*Staphylococcus aureus* gastroenteritis is an intoxication. It has a very short onset time of around 4 h (range 1–6 h). Primary symptoms include nausea, vomiting, and severe abdominal cramps (secondary symptoms: diarrhea, sweating, headache, prostration, temperature drop). The duration is 24–48 h and the mortality rate is very low. Infectious dose: the number of cells necessary to produce enough toxin for symptoms (1 μg) is 100 000–100 000 000.