

# Extended Shelf Life Refrigerated Foods: Microbiological Quality and Safety

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## This Scientific Status

Summary addresses microbiological concerns and control methods for ensuring safety and quality of these foods.

**S**upermarket shelves today need to cater to the gourmet cook as well as the time-harried parent. Increasingly all types of consumers are demanding minimally-processed foods that are high in quality, nutritionally superior, and easy to prepare. Food processors have met this demand by developing refrigerated foods with extended shelf life. Ready-to-eat luncheon meats and complete heat-and-eat meals are some examples. By their very nature, however, these foods present challenges to ensure microbiological quality and safety. This Scientific Status Summary addresses the microbiological concerns associated with extended shelf life refrigerated foods and control measures for ensuring microbiological quality and safety.

Extended shelf life refrigerated foods are foods that have received minimal processing or pre-cooking and have an enhanced but limited shelf life; refrigeration is a key preservation measure. These foods include conventional products, such as luncheon meats and cured meats, as well as a new generation of partially processed refrigerated foods (NFPA, 1988) such as meat, seafood, egg, and vegetable salads, fresh pasta and pasta sauces, other sauces, soups, entrees, complete meals, and uncured meat and poultry items. *Sous-vide* foods, cooked inside a hermetically sealed plastic package under vacuum, are also included in this definition. If extended shelf life refrigerated foods are heat processed, the heat treatment is much less than that required for commercial sterility. Canned foods are, therefore, excluded from this food category. *Sous-vide* foods and others that receive a lower heat treatment than that used for

canning and that require refrigeration are described by some authors as "refrigerated processed foods of extended durability" (Peck, 1997).

## Microbiological Concerns

The chief microbiological concerns associated with these products center around two types of microorganisms—psychrotrophic and mesophilic pathogens—that could grow during extended refrigerated storage or temperature abuse. Psychrotrophs are bacteria, yeasts, and molds that grow, although slowly, at refrigeration temperatures (below 7°C) but grow optimally at temperatures above refrigeration, e.g., 25–30°C. Their maximum growth temperatures are 30–35°C (Kraft, 1992; Olson and Nottingham, 1980). Mesophilic pathogens could survive under refrigeration and grow during any temperature abuse of the food. Mesophiles grow well between 20–45°C with optimum growth between 30–40°C (Jay, 1992). The potential for psychrotrophic spoilage microorganisms to grow during the extended refrigerated storage period and decrease organoleptic quality or spoil the food product is also a concern.

**Pathogenic Microorganisms.** Conventional wisdom of decades ago held that properly refrigerated foods would remain safe because it was thought that pathogenic bacteria could not grow at refrigeration temperatures. Microbial growth was thought necessary to either produce a sufficient number of cells or enough toxin to cause foodborne illness. Since then, scientists have learned that several pathogens—such as *Aeromonas hydrophila*, nonproteolytic strains of *Clostridium botulinum*, *Listeria* spp., *Yersinia enterocolitica*, some strains of *Bacillus cereus*, enteropathogenic *Escherichia coli*, and *Vibrio parahaemolyticus*—can grow at refrigeration temperatures. Furthermore, scientists now know that some pathogens can cause illness when only a few cells are ingested. For example, as few as ten cells of the extremely virulent *E. coli* O157:H7 may cause hemorrhagic

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colitis (Buchanan and Doyle, 1997). Readers may refer to several texts (Cliver, 1990; Doyle et al., 1997; FDA, 1992; ICMSE, 1996; Jay, 1996) for detailed information on the characteristics of pathogenic microorganisms and the foodborne illnesses they cause.

*A. hydrophila* is a facultative anaerobe that is generally considered a ubiquitous waterborne microorganism, occurring widely in fresh and brackish waters. Recent surveys (Pin et al., 1994; Saad et al., 1995; Schweizer et al., 1995) detected *A. hydrophila* in samples of raw milk, poultry, lamb, cheese, shellfish, pork, beef, watercress, lettuce, and escarole. Most cases of illness attributed to *A. hydrophila* have been sporadic, rather than associated with an outbreak (FDA, 1992).

*C. botulinum* is a ubiquitous anaerobic spore-forming bacterium whose spores are widely distributed in soil, freshwater and marine environments, raw agricultural products, and the intestinal tracts of fish and animals (Sugiyama, 1990). Four groups of *C. botulinum* (I-IV) and some strains of *Clostridium baratii* and *Clostridium butyricum* can produce botulinum neurotoxin (Hatheway, 1992). *C. botulinum* type I (proteolytic strains) and *C. botulinum* type II (nonproteolytic strains) are responsible for human foodborne botulism (Peck, 1997). The botulinum neurotoxins are differentiated as types A through G on the basis of serological reaction. The nonproteolytic strains—type E and some type B and F—do not produce overt signs of food spoilage during growth and toxin production.

Some nonproteolytic strains of *C. botulinum* are a concern with extended shelf life refrigerated foods because with sufficient time they may be able to grow and produce neurotoxin at temperatures as low as 3.3°C. Proteolytic strains, which are mesophilic, may be able to grow and produce toxin in foods if temperature abuse occurs. Most outbreaks of botulism in the United States have been caused by home-processed vegetables, fish, or meat products (ICMSE, 1996). The incidence of botulism from consumption of refrigerated foods is exceedingly low. However, the few outbreaks

that have occurred and research challenge studies illustrate the potential *C. botulinum* hazards associated with extended shelf life refrigerated foods (Conner et al., 1989).

*L. monocytogenes*, a facultative anaerobe, is ubiquitous in the environment. *L. monocytogenes* has been isolated from soil, silage, food processing environments, and healthy humans and animals (ICMSE, 1996). A variety of foods, such as refrigerated ready-to-eat meat sandwiches and meat salads have been recalled from the marketplace because of contamination with *L. monocytogenes* (Ryser and Marth, 1991). Individuals with compromised immune systems, e.g., newborns, the elderly, and people suffering from the acquired immunodeficiency syndrome, are most susceptible to listeriosis. Outbreaks of listeriosis in North America have been associated with coleslaw, soft Mexican-style cheese, and milk (McLauchlin, 1996).

*Y. enterocolitica* is a facultative anaerobe whose main reservoir of bio-serotypes pathogenic to humans is believed to be the pig (ICMSE, 1996). Symptoms of yersiniosis, the disease caused by *Y. enterocolitica*, may include fever, diarrhea, headache, vomiting, and severe abdominal pain similar to that associated with appendicitis. *Y. enterocolitica* has been isolated from a variety of animals, foods (lamb, pork, oysters, shrimp, and crabs), and water (Doyle and Cliver, 1990; ICMSE, 1996); however, isolates are often avirulent. Outbreaks of yersiniosis, which are relatively uncommon in the United States, have been caused by contaminated chocolate milk, recontaminated pasteurized milk, bean sprouts, tofu, and chitterlings (raw pork intestine).

*B. cereus*, an aerobic spore-former including psychrotrophic and mesophilic strains, is widely distributed in nature and in foods. *B. cereus* is commonly found in soil, milk, cereals, starches, herbs, spices, and other dried food products and on the surfaces of meats and poultry. *B. cereus* can produce two toxins that cause two distinct types of illness—a diarrheal illness and an emetic illness characterized by nausea and vomiting. Every well-documented report of *B. cereus* intoxication has described time and temperature abuse that enabled initially relatively low (innocuous) levels of *B. cereus* in foods to increase greatly. In most incidents, the food vehicle was a cereal or cereal-

spice-containing product (ICMSE, 1996).

A few serotypes and strains of *E. coli*, a facultative anaerobe that is part of the normal microflora of the intestinal tract of humans and most warm-blooded animals, can cause illness. Although they are not considered true psychrotrophs, some of these strains can grow at 6.9°C and below (Kraft, 1992; Palumbo et al., 1994). Pathogenic *E. coli* are categorized into six groups—enteropathogenic, enteroinvasive, enterotoxigenic, enterohemorrhagic (EHEC), enteroaggregative, and diffusely adherent (Buchanan and Doyle, 1997). Foods involved in outbreaks caused by pathogenic *E. coli* include meat, poultry, fish, vegetables, apple cider, raw milk, Brie and Camembert cheese, water, and radish and alfalfa sprouts. Some strains of *E. coli*, including some EHEC strains, are acid tolerant, a complex phenomenon that is growth phase dependent and inducible; acid tolerance may persist for extended periods at refrigeration (Buchanan and Doyle, 1997).

*V. parahaemolyticus* is a facultatively anaerobic halophile (requiring sodium chloride for growth) occurring worldwide in inshore marine waters and frequently associated with molluscs, crustaceans, and fish (ICMSE, 1996). Although the microorganism is considered mesophilic, growth has been demonstrated at temperatures as low as 5°C (Twedt, 1989). The microorganism is the most common cause of foodborne illness in Japan because it frequently contaminates seafood, often eaten raw in that country. Contaminated raw, improperly cooked, and cooked recontaminated fish and shellfish have been implicated in cases of gastroenteritis.

**Spoilage Microorganisms.** With sufficient time at refrigeration temperatures, several types of psychrotrophic bacteria, yeasts, and molds may grow to levels sufficient to cause food spoilage. These microorganisms include the *Acinetobacter-Moraxella* group, *Alcaligenes* species, *Flavobacterium* spp., *Microbacterium* spp., *Xanthomonas* spp., and the microorganisms of primary concern in extended shelf life refrigerated foods: *Brochothrix thermosphacta*, lactic acid bacteria (LAB), and *Pseudomonas* spp.

*B. thermosphacta*, which is aerobic (requiring free oxygen) to facultatively anaerobic (growing well either aerobically or anaerobically), has been recovered from vacuum-packaged beef, pork, lamb, and heat-processed cured meats such as sliced cooked ham, corned beef, and others (Kraft, 1992). The extent of spoilage of

vacuum-packed meat by *B. thermosphacta* varies with product pH and with oxygen permeability of the packaging material (Egan and Grau, 1981). Spoilage may involve development of sliminess and production of off-odors and off-flavors conferred by short chain fatty acids (Jay, 1992).

The LAB, such as *Lactobacillus* spp., *Leuconostoc*, and *Pediococcus*, are facultatively anaerobic. The type of spoilage produced by the LAB is determined by the nature of the bacteria. Homofermentative LAB produce primarily lactic acid during sugar fermentation. Heterofermentative LAB produce acetic and formic acid, ethanol, and carbon dioxide, in addition to lactic acid. LAB can spoil a variety of foods, including milk and milk products, meats, vegetables, fruit juices, sugary products, alcoholic beverages, and products preserved with vinegar (Sharpe and Pettipher, 1983).

*Pseudomonas* spp., which are aerobic, are among the most common spoilage agents of refrigerated foods (Gill, 1986; Greer, 1989; Kraft, 1986; Splittstoesser, 1976). Growth of *Pseudomonas* spp., like that of other Gram-negative psychrotrophs, is affected by oxygen tension, salt and other food additives, water activity ( $a_w$ ), pH, and other factors. During growth, pseudomonads produce proteases and lipases that can catalyze reactions causing degradation of protein and fat. The consequence of these reactions is formation of peptides and fatty acids of undesirable flavor (e.g., bitterness, rancidity) and odor. Sometimes these bacteria also produce unsightly green pigments.

Many yeasts and molds given sufficient time under refrigeration temperatures can spoil fruit juices, meat products, vegetables, dairy products, and possibly other foods (Jay, 1987; Splittstoesser, 1987). Some yeasts in the genera *Candida*, *Hanseniaspora*, and *Saccharomyces* can grow in fruit juices at  $-5.5^\circ\text{C}$  to  $-2.2^\circ\text{C}$ , just above the freezing temperature for these foods (Pederson et al., 1959). Several genera of yeasts are found on fish and meat products. These include *Candida*, *Cryptococcus*, *Debaromyces*, *Hansenula*, *Pichia*, *Rhodotorula*, *Saccharomyces*, *Sporobolomyces*, *Torula*, *Torulopsis*, and *Trichospora* (Jay, 1987). Growth of yeasts on foods is commonly accompanied by production of carbon dioxide and yeasty, fruity, or alcoholic off-flavors and odors.

Psychrotrophic molds include *Botrytis cinerea*, *Geotrichium candidum*, *Pullaria pullulans* (*Aureobasidium pullulans*), and some species in the genera *Alternaria*,

*Monilia*, *Mucor*, *Penicillium*, *Sporotrichium*, and *Rhizopus*. Not only does the visible presence of mold indicate spoilage, molds also commonly produce enzymes that degrade carbohydrates, fats, and proteins, causing softening of foods and flavor and aroma deterioration. Some species of molds, especially those in the genera *Aspergillus*, *Fusarium*, and *Penicillium*, can produce mycotoxins. *Aspergillus* spp. cannot produce mycotoxins at refrigeration temperatures, whereas certain species of *Fusarium* and *Penicillium* can (Frisvad and Samson, 1991).

### Control Measures

Several types of control methods are effective in preventing or minimizing microbial contamination of product and inhibiting the growth of or destroying microbial contaminants.

**Good Manufacturing Practices (GMPs), Sanitation, and Hygiene.** Processors need to select high-quality raw materials with low levels of microorganisms, especially psychrotrophs. They need to determine potential microbiological hazards of ingredients, possibly using microbiological specifications for ingredients to minimize risk (Moberg, 1989).

Fabrication of raw materials into finished products under hygienic conditions is also important. Food processing equipment must be designed and constructed so that it: (1) is inert to the product, (2) has smooth and nonporous product-contact surfaces, (3) is readily accessible for cleaning and inspection, (4) is self-emptying or self-draining, (5) has covers to prevent external contamination, and (6) has readily cleanable surfaces that do not contact the product and do not harbor contaminants (Cliver and Marth, 1990). The equipment should be cleaned and sanitized as often as is necessary during a day's operation to prevent development of a biofilm that can contaminate subsequent lots of product. Cleaning and sanitizing adequacy can be determined using the more traditional swab procedures, the RODAC (agar contact) method, or the newer rapid ATP (adenosine triphosphate) bioluminescence assays.

Filtration of air entering the food processing area reduces the number of airborne contaminants. If processed foods will not receive a heat treatment or will have few barriers to microbial growth, use of "absolute" (high efficiency) air filters can virtually eliminate microbial contamination. If an air condi-

tioning system is present, the system must be maintained properly so that condensate drains freely and does not contaminate the product. Food processing personnel must use hygienic practices and must be barred from moving from areas containing raw materials to areas containing finished products.

GMPs, sanitation, and hygiene are necessary prerequisites for implementing an effective Hazard Analysis Critical Control Point (HACCP) system, which enables the highest level of food safety assurance possible. HACCP is a systematic approach to the identification, evaluation, and control of food safety hazards, from raw material production and procurement to distribution and consumption of the finished product (NACMCF, 1997). HACCP is based on seven principles: (1) conduct a hazard analysis, (2) determine the critical control points, (3) establish critical limits, (4) establish monitoring procedures, (5) establish corrective actions, (6) establish verification procedures, and (7) establish record-keeping and documentation procedures.

**Multiple Barriers/Hurdles.** Referred to as the hurdle concept or hurdle technology (Leistner and Gorris, 1995; Scott, 1989), this approach combines several factors at subinhibitory concentrations that can effectively control microorganisms in refrigerated foods. Common hurdles include physical elements—such as refrigeration, modified atmosphere packaging (MAP), and heat treatment—and physicochemical factors—such as  $a_w$ , pH, and preservatives. When used together, hurdles interact, sometimes synergistically, enabling use of lower intensities of each factor than would be necessary if each were used alone.

Scott (1989) recommended that challenge studies be conducted to verify the effectiveness of the combination of hurdles. For example, Simpson et al. (1995) demonstrated the antibotulinal effects of salt and pH in minimally processed *sous vide* spaghetti and meat sauce on proteolytic *C. botulinum* types A and B spores and toxin production. The challenge studies indicated that the probability of toxigenesis increased with storage time, but that it decreased as either the  $a_w$  or pH was decreased. Growth of the spores and toxin production were prevented in the product that was processed at  $75^\circ\text{C}$  for 36 min and held at  $15^\circ\text{C}$  (simulating mild temperature abuse) for 42 days by  $>1.5\%$  salt ( $a_w$  0.983) and pH 5.5.

**Ingredients.** Some products can be

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formulated with ingredients that are barriers to microbial growth. For example, organic acids, particularly acetic but also lactic and citric, can inhibit bacterial growth (Ahmad and Marth, 1989; El-Shenawy and Marth, 1989a; Park et al., 1970). Sorbate, propionate, and benzoate have both antibacterial and antifungal properties (El-Shenawy and Marth, 1988 a,b; El-Shenawy and Marth, 1989a,b; Park and Marth, 1972; Ryser and Marth, 1988). Although salt has antimicrobial properties it is not commonly used in high enough concentrations to be effective; this is particularly true of "low-sodium" foods. When appropriate, use of salt and other ingredients to reduce the  $a_w$  to 0.980 or below will lengthen the lag phase of most bacteria and will further reduce the rate of any subsequent growth (Cliver and Marth, 1990).

**Heat Treatment.** Heating foods will reduce the microbial population; the degree of reduction depends on the magnitude of the heat treatment, i.e., time and temperature. The magnitude of heat treatments commonly used is pasteurizing (destructive to vegetative pathogens) rather than sterilizing. Heat treatments lower than those producing commercial sterility are likely to inactivate vegetative cells, but not bacterial spores. Once the heating is completed, stringent hygienic measures must be implemented to pre-

vent recontamination of the food with psychrotrophic spoilage or pathogenic microbes.

**Modified Atmosphere Packaging.** MAP extends product shelf life by reducing oxygen and/or increasing gases, such as carbon dioxide, in the food product environment. MAP inhibits the growth of aerobic spoilage microorganisms, such as *Pseudomonas* species, but allows facultative anaerobes such as LAB to grow. Integrated with aseptic packaging, the technology has experienced rapid growth in the minimally processed refrigerated foods sector (Brody, 1996).

Use of MAP is not without some risk, however. Any facultatively anaerobic or anaerobic psychrotrophic pathogens, such as nonproteolytic *C. botulinum* type E or *Y. enterocolitica*, may be able to grow until the LAB have reduced the pH of the product to inhibitory levels. Further, unlike aerobic spoilage microorganisms, growth of LAB may not be accompanied by overt evidence of spoilage. Moreover, if the MAP product received a nonsterilizing heat treatment, any surviving *C. botulinum* spores may, upon temperature abuse of the product, germinate, grow, and produce toxin without organoleptic signs of spoilage.

**Storage Temperature and Shelf life.** Microbial lag phases (during which there is no growth or a decline in microbial numbers) and generation times (duration between formation of a daughter cell and its division into two new cells) increase as refrigeration temperature decreases (Table 1, 2, and 3). Thus, product temperature should be maintained just slightly above freezing. Acceptable product shelf life (e.g., days or weeks) at specified temperature limits should be established and monitored to help manage food quality and safety. Because the potential exists for temperature abuse at some point during handling or for storage past the intended shelf life, time-temperature indicators or integrators can be useful in determining when refrigeration temperatures or intended storage times have been exceeded (Labuza, 1996; Taoukis et al., 1991).

**Other Control Measures.** Table 4 lists potential non-thermal methods to extend shelf life and their mode of action on microbial cells. Except for the limited use of food irradiation, the bacteriocin nisin, and high hydrostatic pressure (HHP), these methods are not yet fully developed nor commercially applied.

**Table 2** Lag time and generation time of *Listeria monocytogenes* in fluid dairy products at various temperatures. Adapted from Rosenow and Marth (1987).

Temperature °C	Lag time (h)	Generation time (h)
4	120-144	33.3-36.3
8	24-48	10.6-13.1
13	10	5.8-6.0
21	5	1.7-1.9

Ionizing radiation, from gamma rays (produced by the radioisotopes cobalt-60 or cesium-137), machine generated x-rays (with a minimum energy of 5 million electron volts, MeV), and electrons (with a maximum energy of 10 MeV) has been studied extensively. The United States has accepted this nonthermal processing technology for insect disinfestation of wheat, wheat flour, and fresh fruits and vegetables, inhibition of maturation of fresh fruits and vegetables, sprout inhibition of potatoes, inactivation of *Trichinella spiralis* in pork, and microbial decontamination of spices, herbs, vegetable seasonings, poultry, and red meats. Commercial application of ionizing radiation to foods in the United States, however, has grown slowly (Olson, 1998). Widespread application to refrigerated foods requires consumer acceptance.

Pulsed electric fields technology (PEF), also nonthermal, uses very short pulses of high intensity electric fields to inactivate microorganisms. It has been largely limited to liquids such as juices, milk, and liquid egg. Applied to foods, PEF has the potential to equal conventional pasteurization (Yousef, 1996).

Pulsed high-intensity light is a non-thermal technology that uses a xenon flashlamp to generate extremely brief ( $\leq 2$  msec) flashes of intense broad-spectrum (200 to 1,100 nanometer wavelength) light to inactivate microorganisms (Yousef, 1996). Accepted by the Food and Drug Administration (FDA, 1996) for controlling microorganisms on the surface of food, the technology is also useful for treating surfaces of equipment and packaging materials. Successful commercial application to food requires further development because the method as currently understood suffers from limited penetration into food and may cause lipid oxidation (Yousef, 1996).

HHP is effective in controlling microorganisms. Raffalli et al. (1994) demonstrated that *L. innocua* added to 35% fat cream at  $10^7$  cells per milliliter was re-

**Table 1** Generation times of psychrotrophic *Pseudomonas* species during growth in food. Adapted from Snyder (1996).

Temperature °C	Temperature °F	Generation time (h)	Food
0	32	26.6	Dairy product
0	32	30.2	Fish
2.5	36.5	7.7	Dairy product
2.5	36.5	8.0	Chicken
2.5	36.5	13.8	Meat
4.5	40	11.7	Dairy product
4.5	40	6.7	Fish
4.5	40	5.0	Dairy product
10	50	5.4	Dairy product
10	50	2.6	Dairy product
10	50	2.7	Chicken
10	50	1.9	Fish

duced 98.7–99.99% after treatment at 25–26°C for 10–30 min. The D-value for *L. innocua* was 7.4 minutes. After treatment for 20 and 30 min, all surviving microorganisms were injured; a resuscitation step was needed before they were able to grow on a selective medium. HHP is applied commercially to refrigerated avocado products.

Bacteriocins are antimicrobial proteins produced by certain bacteria. The best known bacteriocin is nisin (also designated as a lantibiotic), produced by certain strains of *Lactococcus lactis* subsp. *lactis* (formerly *Streptococcus lactis*). In the United States, nisin is generally recognized as safe (GRAS) for limited use in pasteurized processed cheese to control growth of and toxin production by *C. botulinum* (FDA, 1988). GRAS petitions have been filed for use of nisin in reduced cholesterol liquid whole eggs, sauces, and nonstandardized salad dressings (FDA, 1994, 1995). In recent years, an array of other bacteriocins, many of which are inhibitory to foodborne pathogens, has been discovered. These include unnamed bacteriocins produced by enterococci (Martin et al., 1994; Tarelli et al., 1994), pediocin produced by *Pedococcus acidilactici* (Huang et al., 1994), bavaricin produced by *Lactobacillus bavaricus* (Larsen and Norrung, 1993), mesenterocin produced by *Leuconostoc mesenteroides* (Huang et al., 1993; Maftah et al.,

1993), carnocin produced by *Carnobacterium piscicola* (Bagi and Buchanan, 1994; Mathieu et al., 1994), sakacin produced by *Lactobacillus sake* (Holck et al., 1994), and curvaticin produced by *Lactobacillus curvatus* (Garver and Muriana, 1994).

**Labeling**

Manufacturers recognize the potential for temperature abuse during distribution or storage of foods requiring refrigeration. Hence, they voluntarily use label statements, such as “keep refrigerated” or “refrigerate after opening,” to inform consumers of the need to maintain product at refrigeration temperatures (FDA, 1997).

The FDA determined in 1997 that the labeling of potentially hazardous foods that need refrigeration by consumers should be more specific about the types of hazards present and the necessary storage conditions after the food is opened and issued labeling guidance (FDA, 1997) to food manufacturers. The agency delineated foods that need refrigeration into three groups and developed model label statements and guidance on label placement and prominence. The model label statements refer to the importance of refrigeration for foods in two of the groups

to maintain safety and the use of refrigeration for foods in the third group to maintain quality.

**Summary**  
A variety of high quality extended shelf life refrigerated foods is available. With attention to GMPs, sanitation, hygiene, product formulation, storage temperature, length of refrigerated storage, and microbial control treatments, extended shelf

**Table 4 Nonthermal methods to treat foods. Adapted from Yousef (1996).**

Method	Mode of action on microbial cells
Pulsed electric fields	Rupture of cell membrane
Pulsed light	UV (or thermal) effect
Ionizing radiation	DNA damage
High hydrostatic pressure	Denaturing of protein
Bacteriocins	Damage of cell membrane

life refrigerated foods will be of high quality and minimal risk for foodborne illness.

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**Table 3 Generation times or time until toxin formation by some psychrotrophic pathogens during growth in food. Adapted from Snyder (1996).**

Pathogen	Temperature °C	Temperature °F	Generation time (h)	Food
<i>Listeria monocytogenes</i>	0	32	110.0	Corned beef
	3	37	37.6	Roast beef
	4	39	36.0	Milk
	5	41	43.0	Raw cabbage
	5	41	44.0	Cooked meat
	5	41	33.2	Ham
	10	50	21.7	Lettuce
	10	50	8.2	Corned beef
<i>Yersinia enterocolitica</i>	0	32	67.4	Imitation crab legs
	0	32	44.0	Oysters
	3	37	18.0	Boiled shrimp
	7	45	10.3	Cooked beef
10	50	12.0	Imitation crab legs	
<i>Escherichia coli</i>	10	50	5.2	Culture medium
Pathogen	Temperature °C	Temperature °F	Time to toxin formation (h)	Food
<i>Clostridium botulinum</i> type E	3.3	38	744	Beef stew
	3.3	38	964	Fish
	4.0	39	644	Fish
	4.4	40	1320	Crabmeat
	5.0	41	426	Fish
	6.0	43	456	Beef stew
	7.0	45	243	Fish
	9.0	48	163	Fish
	10.0	50	138	Fish

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