Chapter 4

Analysis of Microbial Hazards Related to Time/Temperature Control of Foods for Safety

1. Introduction

To make decisions on whether a food requires time/temperature control for safety, the properties of the food itself must be considered. This chapter describes properties of common food commodities, including added preservatives and processing steps, and the environmental circumstances that may affect their microbial ecology. The microbiological hazards that may occur from consuming particular food commodities or their derived products are also discussed. The chapter emphasizes microbial concerns that would be associated with temperature abuse of the products, and discusses foods for which time/temperature control may be necessary for safety and those that might be safely stored at room temperature. Consideration is also given to processing technologies or other methods that may be useful in minimizing hazards. Special considerations unique to each food category are also provided. Pathogens of concern and control methods for the various product categories evaluated in this chapter was summarized by the panel and are listed in Table 4-1.

2. Meat and poultry products

2.1. Types of products

Raw meat and poultry products consist of raw products; shelf-stable, raw-salted and salted-cured products (salt pork, dry-cured bacon, country ham); perishable raw-salted and salted-cured products (fresh sausage, chorizo, bratwurst, Polish and Italian sausage); marinated products; and raw breaded products. Ready-to-eat products include perishable cooked uncured products (cooked roast beef, cooked pork, cooked turkey); perishable cooked cured products (franks, bologna, ham, and a variety of luncheon meats); canned shelf-stable cured products (Vienna sausages, corned beef, meat spreads, small canned hams, canned sausages with oil and water activity [a_W] <0.92, dried beef, and prefried bacon); perishable canned cured products (ham and other cured meats); shelf-stable, canned uncured products (roast beef with gravy, meat stew, chili, chicken and spaghetti sauce with meat); fermented and acidulated sausages (German and Italian style salamis, pepperoni, Lebanon bologna, and summer sausage); and dried meat products (jerky, beef sticks, basturma, and other dried meats). Because of the complexity of the product/processing matrices, product parameters (moisture protein ratio, a_W, and pH) and processing schedules are needed to ascertain whether ready-to-eat products require time/temperature control for safety or are shelf stable.

2.2. Microbial concerns

Red meats and poultry come from warm-blooded animals and, as such, their microbial flora is heterogeneous, consisting of mesophilic and psychrotrophic bacteria. These bacteria include pathogenic species from the animal itself and from the environment, and bacterial species introduced during slaughter and processing of raw products. Raw meat and poultry have an a_w >0.99 and a pH range of 5 –7, which is an optimal combination for microbial growth. When red meats and poultry are cooked or processed and subsequently refrigerated, the bacterial load from the raw tissue is greatly reduced, leaving only spore-formers, enterococci, micrococci, and some lactobacilli. In addition, environmental post-processing pathogen contamination can occur and the reduction in competitive bacterial flora may allow for pathogen growth. Some products are shelf stable because they received either a botulinum cook or a lesser cook in combination with other controls, such as acidity or other additives (for example, spaghetti meat sauce and Sloppy Joe mix).

2.3. Pathogens of concern

The principal pathogens of concern are *Staphylococcus aureus*, enterohemmorrhagic *Escherichia coli* (ruminants), *Salmonella* spp. (all meats), *Listeria monocytogenes* (all meats), *Campylobacter jejuni/coli* (poultry), *Yersinia entercolitica* (pork), and *Clostridium perfringens and Clostridium botulinum* (mainly processed products*)*. There is a particular concern when these species are present and/or can grow in cooked products without competition.

2.4. Effects of processing

Meat and poultry products require a wide array of control measures in their processing. Cured meats and some sausage products utilize additives such as salt, nitrate, nitrite, and sugars with processing procedures such as cooking and smoking. Salt, for example, may restrict bacterial flora to salt-tolerant species. Smoking and/or cooking will destroy many vegetative cells. However, the processing environment and product handling and packaging may introduce microorganisms, including pathogens, into the packaged product that also must be considered.

While some canned products may be processed as “commercially sterile”, others are canned "semi-preserved" and must be stored under refrigeration. Some products utilize a secondary control such as acidity and are shelf stable though not necessarily "commercially sterile." Specific labeling for refrigeration is required on the semi-preserved products that require refrigeration as a control. Pickled products depend on a low pH, absence of oxygen, and the lack of a fermentable sugar to inhibit the growth of most bacteria. Acid-tolerant species may develop, such as certain lactobacilli, and if air is available, certain yeast and molds may grow. The activity of lactic acid bacteria in fermented sausages is desirable and is an integral part of the process control for achieving the desired pH for these products.

Because of the complexities of products and processing, the USDA Food Safety and Inspection Service (FSIS) has provided guidelines for product parameters in its "*Food Standards and Labeling Policy Book*" (USDA 1996, with change 98-01). The FSIS guidelines include product specifications such as “meat sticks and cheese”, along with general topic categories such as for example "Sausage - Shelf Stable"; “Moisture Protein Ratio –MPR;" and "Moisture Protein Ratio - pH.” These policies must always be considered in conjunction with process controls under the HACCP Rule, 9 C.F.R. 417. A product processed in the retail environment is not covered by this rule; however, the variance requirements of the Food Code should require that meat and poultry products have equivalent product specifications for shelf stability and process records documenting control of hazards.

There is substantial history of safety of meat and poultry products that meet these criteria. In addition to the above criteria, certain combinations of pH, a_w, and /or other factors can be used to prevent pathogen level increase when meat products are held at ambient temperatures. Products processed in the retail environment and exempt from the HACCP Rule should also follow these guidelines and maintain records documenting control of hazards.

2.5. Time/temperature control

Unless the specific product parameters referenced in the previous section are met, meat and poultry products must be considered as requiring time/temperature control. Raw meat and poultry products currently require safe-handling instruction labeling that includes a time/temperature control provision. For ready-to-eat foods, product parameters and processing schedules are needed to ascertain whether temperature control for safety is required. Post-processing contamination is also an important consideration and should not be overlooked. Because meat offers a rich nutrient media for microbial growth, products that incorporate meat and poultry as ingredients, such as meat salads and meat pastries, also must be considered as requiring time/temperature control.

3. Fish and seafood products

3.1. Types of products

Fish and seafood products include fresh and frozen fish and crustaceans; cooked crustacean products; breaded and prepared seafood products; salted and smoked seafood products; sushi and seafood products such as minced fish flesh, surimi, pickled fish products, fermented fish, and seafood analogs; and molluscan shellfish (oysters, mussels, and clams).

3.2. Microbial concerns

Seafood is more perishable than other high-protein products due to the high level of soluble nitrogen compounds in the tissue. Microbial activity is responsible for changes in flavor, odor, texture, and color that reflect the extent of decomposition. Seafood is largely harvested from the wild and is subject to environmental contaminants, including pathogens, from the harvest site and on-board-ship handling practices. The numbers and types of indigenous microorganisms on freshly harvested fish, crustaceans, and mollusks depend on the geographical location of the harvest site, the season, and the method of harvest. While microbial concerns center mainly on foodborne illness, poor quality (spoiled or decomposed) products rarely cause illness because they usually are discarded before consumption. With the exception of scrombroid poisoning in other foods, problems generally arise from contaminated harvest sites or from mishandling during or after processing.

3.3. Pathogens of concern

Inshore water sites increase the likelihood of enteric pathogen contaminants. Indigenous pathogens including *Vibrio vulnificus*, *Vibrio parahaemolyticus*, *Vibrio cholerae,* and *C. botulinum* Type E, and enteric microorganisms such as *Salmonella* spp. and *Shigella* spp. have been isolated from freshly caught fish, crustaceans, and mollusks due to contaminated harvest waters, but they are not present in deep sea waters. Other non-indigenous pathogens such as *L. monocytogenes* and *S. aureus* can be present in cooked products as a result of processing, handling, or post-processing environmental contamination.

Sushi products that incorporate raw fish as an ingredient must meet the additional requirements of a process for destruction of parasites. Sushi is also made from acidified rice and other ingredients that are subject to the environmental/processing contamination already discussed. Rice, without proper acidification control, introduces a risk of toxin formation from *Bacillus cereus*.

Cooked seafood, especially crustaceans that are heavily handled during processing, is subject to contamination by *S. aureus*, *Salmonella* spp., *L. monocytogenes*, *Shigella* spp., and other enteric microorganisms. In addition, poor manufacturing practices may result in cross contamination by indigenous pathogens, especially *V. parahaemolyticus. Clostridium botulinum* spores may survive depending on the nature of the heating process.

3.4. Effects of processing

Since 1997, all seafood processors must comply with the HACCP rule, mandated by FDA in an attempt to minimize the microbiological hazards in the final products. Seafood can be sold raw, frozen, canned, cured, smoked, or fermented. Much seafood is frozen, a factor that does not affect the level of pathogens, except in the case of *Vibrio* spp., which are sensitive to freezing temperatures. *Vibrio parahaemolyticus*, for instance, has been shown to survive freezing at sufficient levels to cause illness.

The cooking process usually eliminates vegetative pathogens. However, to maintain quality, the duration of these cooks may be shortened and may not fully destroy all pathogens. In addition, meat from cooked crabs and lobsters is picked by hand, a practice which can cause contamination by *S. aureus* and by *Salmonella* spp., *Shigella* spp., and other enteric pathogens. *Listeria monocytogenes* is also a significant contaminant in cooked/processed seafood because the cool, wet processing environment is conducive to its presence and subsequent product contamination. Cooked seafood products should be cooled and refrigerated immediately.

Canned seafood given a full retort process is shelf stable. Of concern with canned fish are those species with high histidine levels, such as tuna, mackerel, and sardines, that have been mishandled when fresh, and that may develop significant levels of heat-stable histamine and cause food illness.

Large amounts of fish are cured and/or smoked as a preservation technique. These products are subject to contamination by environmental species, especially *L. monocytogenes.* The curing process uses salt to lower a_W and sometimes uses smoke to provide flavor. A wide variety both hot- and cold- smoked products are available. The safety of these products relies on the amount of water phase salt, preservatives, and the type and amount of heat treatment. Except for a fully salted and dried product (>20% salt), these products cannot be considered shelf stable without full validation and process control. Cold-smoked fish that are vacuum-packaged have been implicated in outbreaks from *C. botulinum* toxin. In addition, the high frequency of isolations of *L. monocytogenes,* especially in cold-smoked fish, has resulted in numerous product recalls. Several states have specific requirements based on the Association of Food and Drug Officials' Model Code: "Cured, Salted and Smoked Fish Establishment GMPs," for the amount of water-phase salt, heat treatment, and storage temperature for salt-cured, smoked fish (AFDO 1991). Curing and smoking of uninviserated fish is prohibited.

Fish is also preserved by fermentation. Fermented seafood uses salt and acids, such as vinegar, to produce acidic products with high salt contents that preclude pathogen survival.

3.5. Time/temperature control

Most seafood, including cooked seafood and sushi, requires time/temperature control. Only fully retorted or fully dried and salted products are considered shelf stable. Most smoked seafood products require time/temperature control because of the concern with *C. botulinum* growth and toxin production, in addition to their being highly perishable. Heavily smoked products with low water activities are spoiled primarily by molds.

4. Fruits and vegetables

4.1. Types of products

Fruits are the portions of plants that bear seeds, while vegetables are the edible components of a plant, including the leaves, stalks, roots, tubers, bulbs, flowers, and seeds (ICMSF 1998, p 253). A wide variety of products, including citrus fruits, apples, pears, bananas, tropical fruits, compound fruits (for example, berries), tomatoes, olives, cucumbers, and melons, as well as vegetables ranging from asparagus to zucchini, are available in the market place (ICMSF 1998, p 215-273).

Fruits and vegetables and related products include foods that are sold fresh, minimally processed (for example, cut, sliced, chopped, shredded, or peeled), canned, frozen, juiced, or dried. Some commodities are retained in storage under controlled or modified atmospheres before packaging, while others are packaged by using modified atmospheres in films that control the permeability of gases. In addition to being sold fresh, fruits are also sold dried and packaged with preservatives. Dried fruits are also used in a variety of products such as confectionary bars, cookies, chocolates, breads, and many cereal based products. Minimally processed fruit can be sold as fruit salads or incorporated into dairy products such as yogurt, cottage cheese, or ice cream (ICMSF 1998, p 253).

Fresh-cut vegetables include ready-to-eat washed, sliced, chopped, or shredded vegetables, dry coleslaw mixes (without dressing), and complex mixed salads, as well as stir-fry products. Raw or cooked vegetables (with or without fruit and meat or poultry) are used as ingredients in prepared (deli) salads with mayonnaise or other types of dressings. Due to their highly perishable nature, most fresh fruits and vegetables need temperature control to extend their shelf life. Preservation of fruits and vegetables is achieved by drying, salting, freezing, refrigeration, canning, fermentation, irradiation, and packaging under vacuum or modified atmospheres (ICMSF 1998, p 215).

Over the past several years, seeds, either fresh or cooked, have become a commonly consumed produce item. Seed sprouts may harbour very low levels of pathogens (*Salmonella* serotypes, *B. cereus*, *E. coli* O157:H7, and *Y. enterocolitica)* that can multiply to very high levels during the 3 to 10 d sprouting process and survive through the typical refrigerated shelf life of the products (IFT 2001). Whereas mung bean sprouts are often stir-fried or otherwise heated prior to consumption, which would reduce the risk of disease, other seed sprouts are often consumed raw and have been associated with foodborne illness (IFT 2001). For these products, time/temperature control would not prevent microbial hazards and, therefore, sanitation procedures that would reduce the contamination and growth of pathogens growth should be in place.

4.2. Microbial concerns

The initial bacteria of fresh produce derive from contamination from air, soil, water, insects, animals, workers, and harvesting and transportation equipment. In fruits, bacteria are usually present in low numbers, but contamination by yeasts and molds is more prevalent due to the lower pH of fruits and the lack of competition from other microorganisms (ICMSF 1998, p 253). Microorganisms also found in vegetables include *Pseudomonas* and *Erwinia* as well as coryneforms, lactic acid bacteria, spore formers, coliforms, and micrococci. Yeasts and molds are often present but in lower numbers than bacteria (ICMSF 1998, p 216). Sufficient moisture, abusive temperature, and adequate time will ensure a continuing increase in the bacterial population on fruits and vegetables, particularly in fresh-cut products.

4.3. Pathogens of concern

Since 1973, the number of reported outbreaks of foodborne illness associated with produce has more than doubled. As a result, pathogens on fresh fruits and vegetables have become a major concern. Pathogenic bacteria are not usually associated with fruit, but pathogens can be present due to fecal contamination. There have been a number of outbreaks of salmonellosis and *E. coli* O157:H7 infection associated with the consumption of a variety of fruits, including raw tomatoes, sliced watermelons, cantaloupes, and unpasteurized apple and orange juice. Human pathogens have been isolated from more than thirty kinds of vegetables and include *Salmonella* spp., *Shigella* spp, *Y. enterocolitica*, *E. coli* O157:H7, *L. monocytogenes*, *C. botulinum,* and *B. cereus* (ICMSF 1998, p 221). Fresh-cut produce presents a special concern because of the disruption of natural protective barriers that may result in increased pathogen multiplication.

4.4. Effects of processing

Fruits and vegetables are frequently consumed raw without being exposed to a process that reliably eliminates pathogens. Washing fruits and vegetables in chlorinated water can reduce bacterial levels but cannot be relied upon to eliminate pathogens. Traditional processing methods such as freezing, canning, dehydration, fermentation, and acidification are used to improve the stability of fruits and vegetables.

4.5. Time/temperature control

Outbreaks of salmonellosis and *E. coli* O157:H7 infection linked with a variety of fruits and vegetables have increased the concerns as to the safety of these foods. Strategies to reduce microbial hazards in produce include the implementation of Good Agricultural Practices on farms, and Good Manufacturing Practices in packing, handling, and storage. Due to their highly perishable nature, most fresh fruits and vegetables need time/temperature control to extend their shelf life. In any case, attention should be paid to storage times and temperatures since pathogens, if present, are able to grow—
particularly in the case of fresh-cut produce or where internalization is possible. Storage temperature and time management are important in reducing the risks of foodborne illness, and become critical parameters for any fresh-cut produce. However, as mentioned above, the time/temperature for seed sprouts will not reduce the risk of presence of high levels of pathogens. While, traditional processing methods such as freezing, canning, dehydration, fermentation, and acidification are used to improve the stability of fruits and vegetables, and time/temperature control may not be a requirement for these processed products.

5. Cereal grains and related products

5.1. Types of products

Cereal grains and related products include baked goods (breads, muffins, cakes, pastries, cookies, biscuits, bagels, and so on), frozen and refrigerated dough, breakfast cereals (cold cereal, oatmeal, grits, and so on), refrigerated or dry pasta and noodles, and cooked grains (for example, rice). Some products, such as baked goods, have a long history of safe storage at room temperature; others, such as rice, require time/temperature control after preparation.

5.2. Pathogens of concern

Grains and milled products are raw agricultural commodities; therefore, a variety of microorganisms, including mold, yeast, coliforms and other bacteria, occur naturally. Grains and milled products are dried to inhibit mold growth during storage, a process that easily controls growth of bacterial pathogens. Therefore, while organisms such as *Salmonella* spp. may be present, the prevalence and levels are low (usually $<1\%$). Raw ingredients used to prepare dough products (for example, eggs, dairy products, meats) may introduce *Salmonella* spp., and need to be considered when analyzing potential hazards. *Staphylococcus aureus* may present a potential hazard for certain raw dough, such as pasta dough processed at warm temperatures for extended periods of time (days); however, yeast leavened dough and cookie dough control the organism through competitive inhibition and low a_W, respectively. *Bacillus cereus* presents a concern in cooked rice.

5.3. Effects of processing

Baking, boiling, steaming, or frying are the methods used to cook the cereal-grain products. The temperatures required to achieve product quality easily destroy vegetative pathogens that may be present. These temperatures are needed to properly set the starch structure and/or to rehydrate dry products. Baking and frying not only destroy vegetative pathogens such as *S. aureus* and *Salmonella* spp., but they also remove moisture from the product—especially at the exterior surface. This dehydrated surface inhibits the growth of most bacteria; thus, mold is the primary microbial mode of failure for baked goods. When stored at room temperature, baked and fried products typically continue to lose moisture to the atmosphere, further reducing the potential for pathogen growth. Thus, baked and fried cereal-grain products such as cakes, breads, muffins, and biscuits have a long history of safe storage at room temperature despite having an internal a_W of approximately 0.94-0.95 (but may be as high as 0.98).

While boiled or steamed cereal products achieve temperatures lethal to vegetative pathogens during the cooking process, these products increase in a_W to levels that support the growth of many microbial pathogens. Thus, time/temperature control is required to assure the safety of these products. For example, numerous *B.cereus* outbreaks have been associated with fried rice prepared using boiled rice that was held for hours at room temperature.

5.4. Time/temperature control

Although baked and fried cereal-grain products (for example, cakes, breads, muffins, and biscuits) have a high a_W, a number of reasons may justify their shelf-stability: they have a long history of safe storage at ambient temperature; processing temperatures and moisture reduction, especially on the surface, preclude the growth of pathogens; and they are often formulated to include ingredients that enhance product safety and stability so as to permit distribution without temperature control for limited periods of time. Ingredients that are used to enhance safety and stability include humectants to reduce a_w (sugars and glycerine), preservatives (calcium propionate, potassium sorbate, sorbic acid), acids to reduce pH (vinegar, citric acid, phosphoric acid, malic acid, fumaric acid), spices with antimicrobial properties (cinnamon, nutmeg, garlic), and water-binding agents to control free water (gums, starches). The primary mode of spoilage of baked goods is mold growth, which is visible and alerts the consumer to avoid consumption, further reducing the risk of illness due to spoiled product. These characteristics plus their long history of safe storage at room temperature would allow these products to be stored at ambient temperature. Boiled or steamed cereal products, such as rice, require time/temperature control after preparation due to the increase in a_w.

Dough is frequently used to enrobe other food ingredients. Careful consideration must be given to these combination products to accurately assess the need for time/temperature control. For example, egg and dairy ingredients baked inside a pastry, such as cream-cheese croissant, will receive sufficient heat treatments to destroy vegetative pathogens and may therefore be stable at room temperature with water activities above 0.86. However, if the filling is injected after the baking process, as in the case of a cream-filled éclair, the potential for contamination must be assessed. Meat and vegetable-filled cereal products with high water activities (>0.94) and neutral pH generally require time/temperature control because the baking process can activate spore formers such as *C. botulinum* that are present in these ingredients.

6. Fats, oils, and salad dressings

6.1. Types of products

Fats and oils are primary components of many foods that are emulsions comprised of oil as the continuous phase and water as the discontinuous phase. Mayonnaise, salad dressings, and related products are examples where water is the continuous phase and oil (fat) the discontinuous phase. Product types have grown to also include pourable dressings and starch-based dressings that resemble mayonnaise. In addition, in recent years products such as garlic-in-oil, various herb/spices-in-oil, and flavored oils have proliferated.

6.2. Microbial/pathogen concerns

The form of the water-in-oil emulsion in mayonnaise and salad dressings, particularly the chemical composition of the water phase, plays a key role in their microbiological stability. The pH range is 3.2 to 4.0 due to acetic acid; the oil content, 65 to 80%; the aqueous phase salt content, 9 to 11%; and the sugar content is 7 to 10%. This composition provides an a_w of ~ 0.925. Pourable dressings have a pH in the range of 3.5 to 3.9. Microbial stability is largely related to the maximum preservative effect of acetic acid, mostly undissociated at those low pH levels. Although the a_W of mayonnaise and salad dressings is not sufficiently low to preclude growth of *S. aureus,* at pH 4.1 and below, *S. aureus* does not survive. Additionally, mayonnaise and salad dressings do not support the growth of *C. botulinum* because of the low pH and a_W. The low a_W also precludes the growth of *B. cereus*. The few documented cases of *Salmonella*-related foodborne illnesses have been related to deviations in pH and in the proportion of egg yolk and vinegar. These deviations typically occurred with non-commercially prepared products that lack the proper control of pH and the hold time to allow pathogen die-off.

Oil products that can create anaerobic sites of sufficient a_W favorable for *C. botulinum* growth and toxin production are problematic; for example, the addition of fresh garlic to oil. The moisture surrounding the garlic fragments coupled with no acidulant creates the conditions necessary for *C. botulinum* growth and toxin production. To maintain a pH that precludes growth and toxin production, an acidulant is required in these products.

6.3. Effects of processing

Following Good Manufacturing Practices can protect these products from contamination. Formulating with appropriate levels of acetic acid is essential to protect fats and oils against pathogenic bacteria; salad dressings with a pH less than 4.0 are very safe. Refrigeration after opening is recommended to prevent oxidation of the oils and product separation, but not for safety. A recent review of the microbiological safety of mayonnaise, salad dressings, and other sauces revealed that *Salmonella*, *E. coli* O157:H7, *L. monocytogenes*, *S. aureus*, and *Y. enterocolitica* die when inoculated into mayonnaise and dressings (Smittle 2000).

6.4. Time/temperature control

Products with formulations that do not meet a_W, pH, and acidity requirements as outlined above may require time/temperature control. Addition of flavoring components to traditional oils must be done in conjunction with added acidifying agents. Addition of other ingredients, such as garlic or herbs, would require an assessment or challenge testing before the product is designated shelf-stable.

7. Butter and margarine

7.1. Types of products

Butter, one of the few foods defined by law, must be at least 80% milk fat. It is a water-in-oil emulsion that can be salted or unsalted and may contain starter cultures for additional flavor. The composition and manufacturing process of butter are critical to its stability because uneven churning of butter may result in pockets of high moisture that would permit microbial growth if contamination is present. Additional stability is provided by salt, which normally results in a water-phase salt level around 16%.

Regular margarine, as defined in CFR 21.166.110, includes any plastic fat composition emulsified to at least 80% fat and with moisture in excess of 1%. A wide range of fats and oils are used to process margarine. Other ingredients in margarine include salt, emulsifiers, and preservatives, and some margarines may contain milk solids. Other margarine products may contain 40% to 60% fat with a corresponding increase in moisture content. Margarine spreads have various oil contents and usually do contain milk solids.

7.2. Microbial concerns

The bacteria found in butter products reflect the initial microflora of the cream and the sanitary condition of the processing operation, as well as the sanitary condition of the environment and handling during packaging. The high water-phase salt content of salted butter precludes all but *S. aureus* as a pathogen of concern. As with butter, the salt content of the aqueous phase in margarine and its distribution through a fine water dispersion are critical to product stability and safety. Three percent salt results in 15.8% water phase salt in the product that is inhibitory to all foodborne pathogens except *S. aureus*. On the other hand, 1% salt drops the level in the water phase to 5.9%, which would permit the growth of most foodborne pathogens. The need for time/temperature control depends on the pH and a_w of the product, and on whether other preservatives have been added to the formulation. A special concern would be the post-processing contamination by psychrotrophic pathogens such as *L. monocytogenes,* which have been demonstrated to survive processing if introduced after pasteurization.

7.3. Effects of processing

The high-temperature-short-time pasteurization of the cream used for butter destroys all but the most heat-resistant vegetative forms of microorganisms, including pathogens. Toxin formed by *S. aureus* prior to pasteurization in poorly handled cream will result in toxin carryover to the finished butter product. Contamination from lack of sanitation during processing can carry pathogens into the finely dispersed water droplets where, if nutrients are present, they could multiply. Post-processing contamination of the pasteurized cream and/or butter by *L. monocytogenes* and/or *Y. enterocolitica* is a concern since survival and growth of both microorganisms at refrigerated temperatures have been shown to be possible. This is true for both butter and margarine products containing added milk solids.

7.4. Time/temperature control

Traditional butter and margarine have had a long history of safety without time/temperature control. The few problems that have occurred are related to modified products. As these traditional products have been modified by reducing the fat levels, increasing the water content, and reducing the salt levels, the built in microbiological inhibitory factors can also be expected to change. For example, a *S. aureus* enterotoxin outbreak has been associated with a whipped butter that had been temperature abused over an extended time period. Therefore, as these traditional product compositions are changed, other microbial inhibitors such as preservatives may have to be considered to enhance the safety of the finished product during its intended use. The need for time/temperature control depends on the pH and a_w of the product, and on whether other preservatives have been added to the formulation.

8. Sugars and syrups

8.1. Types of products

A wide variety of products fall into the sugar and syrup category. Some of these products include beet and cane sugar, corn syrup, maple syrup, table syrups, and other specialty sugar syrups, such as cane syrup.

8.2. Microbial/pathogen concerns

Because of the high sugar content and resulting low a_W, pathogen survival and growth is not an issue with these products. Some may, however, require refrigeration to prevent yeast and mold growth after opening if the a_W is high enough to support growth. *Clostridium botulinum* may be a concern in light syrups, and acidulants are often used to inhibit growth and toxin production.

8.3. Effects of processing

Syrups are heated during processing to facilitate clarification and handling. Clarification steps involving precipitation and filtration serve to remove some of the microorganisms.

8.4. Time/temperature control

Traditional syrups do not need time/temperature control for safety because of high sugar content and low $a_{W.}$ Traditional syrups may be modified by reducing the caloric or reducing the sugar content which could result in a change in the microbial inhibitory characteristics of these modified products. As traditional products are modified, the changes could result in variations in the sugar to water ratios that could provide opportunity for the growth of pathogens. Therefore, the use of other microbial inhibitors may be necessary to prevent pathogen growth at ambient temperature. Using such ingredients as acidulants and preservatives as microbial inhibitors may maintain the modified syrups as shelf-stable products.

9. Eggs and egg products

9.1. Types of products

"Eggs," as a product category, refers to eggs in the shell. "Egg products" refers to eggs that have been separated from their shells to produce liquid, concentrated, dried, crystallized, frozen, coagulated, and reduced cholesterol products (ICMSF 1998, p 495). In the United States, approximately 83 % of the eggs are sold as shell eggs (ICMSF 1998, p 480). Liquid eggs are usually homogenized as whole eggs or separated into white and yolk. Sugar, salt, or acidulants may be added to yolks that will be further processed. All liquid eggs are usually pasteurized and require temperature control at refrigeration or frozen temperatures. Liquid egg products are used as ingredients in a wide variety of processed products including bakery products (meringues, custards, cream, angel food cakes, and egg washes), confectionary products, drinks, special dietary foods, infant products, sauces and dressings, mayonnaise, and noodles (ICMSF 1998, p 480).

9.2. Microbial concerns

Eggs can become contaminated through trans-ovarian or trans-shell infection (ICMSF 1998, p 481). Freshly laid eggs may be contaminated through the oviduct of an infected hen. The shell of a newly formed egg can become contaminated with a variety of microorganisms from the environment where the egg is laid. Although there are a number of antimicrobial barriers present in eggs (lysozyme, conalbumen, avidin, and alkaline pH), spoilage and pathogenicity are related to the ability of microorganisms to penetrate the shell and overcome these barriers (ICMSF 1998, p 479). The bacterial ecology of eggs is varied and consists of psychrotrophic (primarily Pseudomonads) and mesophilic bacteria and can also include some pathogens. Federal regulations stating that shell eggs must be kept refrigerated prior to use have been recently implemented ("Food Labeling, Safe Handling Statements, Labeling of Shell Eggs; Refrigeration of Shell Eggs Held for Retail Distribution," 65 FR 76092 [Dec. 5, 2000]). When properly cooked or processed (pasteurized) and stored at appropriate temperatures, the bacterial loads in these products are greatly reduced. Heat treatments used for liquid eggs do not produce shelf-stable products, so proper temperature control and safe handling after opening or thawing are necessary to prevent post-process or cross contamination and growth of pathogens.

9.3. Pathogens of concern

The principal human pathogens of concern in eggs and egg products are of the genus *Salmonella* (primarily *Salmonella* Enteritidis). These pathogens can enter the egg either by trans-ovarian transmission or by penetrating the surface of the egg in a way similar to that of spoilage organisms (ICMSF 1998, p 492). *Listeria monocytogenes* is also a concern in processed eggs, particularly in products with extended shelf life.

9.4. Effects of processing

Shell eggs are usually fried, boiled, or baked. In these cooking methods, it is important that eggs reach appropriate temperature to destroy any salmonellae that may be present. Eggs boiled or cooked long enough to solidify the yolk (~ 10 min of boiling) are heated sufficiently to inactivate salmonellae, but other cooking procedures that leave the yolk in a liquid state (for example, soft boiled and fried eggs "over easy") are not always sufficient to inactivate *Salmonella* spp (ICMSF 1998, p 493). Liquid eggs, white, and yolk that do not contain chemical additives are usually pasteurized at temperatures that vary from 55.6 °C (132 °F) to 69 °C (156 °F) at processing times that vary from 10 to 1.5 min. Lower temperatures and shorter processing times increase the risk of survival of *Salmonella* spp., whereas higher temperature and longer processing times increase damage to the functional properties of the egg (ICMSF 1998, p 496). It should be noted that reduced a_w and longer heating times are required to achieve the same level of pathogen reduction. In the United States, pasteurization requirements are 60 °C (140 °F) for 3.5 min, which achieve more than a 3 log reduction of salmonellae (ICMSF 1998, p 497). Proper pasteurization reduces the initial level of other microorganisms; however, if the product is temperature abused, some bacteria, such as micrococci, staphylococci, *Bacillus* spp., enterococci, and catalase negative bacterial rods, survive the process and can grow.

9.5. Time/temperature control

Eggs and egg products will easily support the growth of spoilage and pathogenic microorganisms and clearly require time/temperature control to assure safety. Control methods require an integrated approach that begins at the egg production facility, and carries through to processing and further processing operations as well as to retail and food service facilities. Temperature control of shell eggs, followed by thorough cooking and proper handling, are essential in assuring safety.

As mentioned above, heat treatments used for liquid eggs do not produce shelf-stable products, so they should be kept refrigerated or frozen. These products should be safely handled to reduce the likelihood of post-process and/or cross contamination.

10. Milk and milk products (except cheeses)

10.1. Types of products

Milk, the lacteal secretion from warm-blooded animals, is commercially available most commonly from cows, goats, and sheep. Milk may be available to consumers as a single- or multiple-ingredient fluid pasteurized product. It can also be obtained in a concentrated form, such as evaporated or condensed milk, or in a dry form. Bacterial cultures can be used in making other products such as cultured milk, yogurts, and cheeses. Milk and milk products are also included as major ingredients in other food forms ranging from ice cream to prepared foods.

10.2. Microbial concerns

Milk is an excellent growth medium for many kinds of microorganisms, as it provides rich nutrients for microbes, is high in moisture, and has neutral pH. Due to these factors, it is subject to microbial spoilage from the moment it is secreted from a healthy animal. Milk is exposed to the potential for microbial contamination during collection, storage, transportation, and processing. Without basic sanitary practices in place and temperature control during handling, the product will quickly spoil and become unacceptable for human consumption. Uncontrolled microbial growth affects the flavor and appearance of the product and can affect its safety. On the other hand, controlled use of microbial cultures can produce many flavorful products and can also preserve milk and milk products. Milk and milk products are normally consumed after the application of a processing step to reduce pathogenic microorganisms.

10.3. Pathogens of concern

The principal pathogens of concern associated with milk and processed milk products are *Salmonella* spp., *L. monocytogenes*, *S. aureus*, enterohemorrhagic *E. coli*, *Campylobacter jejuni*, *C. botulinum*, and *B. cereus*.

10.4. Effects of processing

Non-spore forming pathogens are reduced in fluid milk through pasteurization. Milk used as an ingredient in other products is normally pasteurized or thermally processed in some form to reduce possible pathogens. The exceptions would be some cheese-making processes that rely on microbial cultures and the effects of their growth in the milk medium over time to render the finished food safe.

While most milk and milk products are sold refrigerated to prevent spoilage, some dairy products are shelf stable due to a combination of moisture content, salts, and pH that control the growth of microbes. Canned milks are shelf stable due to thermal processing of the product within the individual containers. Some milk and milk products may be aseptically processed and packaged to enable the product to be shelf stable. Other dairy products may be thermally processed and packaged hot in conjunction with product formulations designed to inhibit the growth and survival of pathogenic organisms in products stored at room temperature. Microbial growth in dried milk is prevented by removing most of the moisture in fluid milk. Other dairy products, such as ice cream, are sold in a frozen state to limit the growth of microbes.

Protection from post-pasteurization contamination before the milk product is packaged is a critical factor in achieving a safe food. Multiple-ingredient dairy products may raise the concern of contamination depending on the characteristics of the product and the location where the ingredient may be added in the process. Ingredients added after pasteurization of the milk portion of the food can be a source of pathogens. The control of potential sources of contamination can be addressed by following production practices based upon Good Manufacturing Practices.

10.5. Time/temperature control

During handling, basic sanitary practices and temperature control are required to maintain acceptable sensory qualities of milk and milk products. Similarly, most milk and milk products are sold refrigerated to prevent spoilage. Exceptions include canned milks, dried milk, ice cream, asceptically processed and packaged products, and thermally processed products that are packaged hot in conjuntion with specific product formulations. These milk products do not require refrigeration because of the combination of moisture content, salts, and pH that control the growth of microbes.

11. Cheeses

11.1. Types of products

Cheese is the product of milk coagulation, followed by curd separation and ripening. More than 500 cheeses are manufactured worldwide, with variations deriving from modifications in the cheesemaking technique; for example, type of milk, coagulation method, starting culture, addition of salt or other additives, and ripening period. The changes, including microbiological changes occurring during cheesemaking, are complex. Cheeses types can be can be classified according to many different criteria, but a general classification divides cheeses into fresh or unripened, soft, semisoft, hard, and processed cheese.

11.2. Microbiological concerns

The survival and growth of pathogens in cheese depend on the many factors affecting the cheesemaking process, including time and temperature during the ripening process, variations in pH and $a_{w,}$ competing microflora, biochemical changes during ripening, and addition of antimicrobials. The microbiological quality of the milk will also contribute to the microbial ecology of the final product, especially in cheeses where milk is not pasteurized. *Salmonella* spp., *L. monocytogenes* (mainly in soft, high moisture, high pH cheeses), enterohemmorrhagic *E. coli* O157:H7 (due to post-process contamination), *S. aureus* (due to faulty cheesemaking process), *Shigella* spp. and *C. botulinum* (due to faulty process) have been implicated in outbreaks associated with the consumption of various types of cheeses.

11.2. Effects of processing

Cheeses made with pasteurized milk generally would not be a concern unless post-process contamination with pathogenic vegetative cells occurs. To minimize post-process contamination, strict plant sanitation and Good Manufacturing Practices need to be followed throughout the cheese making process. In the United States, cheeses made with raw milk need to be ripened for at least 60 d to control for pathogens. If ripened for more than 60 d, pH, $a_{w,}$ salt, and other parameters were thought to inhibit the growth of pathogens. However, recent studies have shown that low levels of certain pathogens such as *E. coli* O157:H7 can survive beyond 60 d curing in hard cheeses (Reitsma and Henning 1996). In general, there have been very few documented illness outbreaks linked to consumption of properly ripened hard cheese. Therefore, time/temperature control of hard cheeses is primarily needed not for safety reasons but to maintain the organoleptic quality of cheese. However, if the cheesemaking process is faulty (for example, high pH) or if post-process contamination occurs, the potential growth of pathogens is possible and time/temperature control is needed for safety. Soft cheeses (ripened or unripened), which have a higher moisture content, do require time/temperature control for safety.

In processed cheese, heat and sanitary packaging are used to prevent microbial hazards unless the cheese is contaminated with heat-resistant pathogenic spores. If the product is contaminated with spore-formers such as *C. botulinum*, however, germination and toxin formation can cause serious public heath concerns, especially if the product is intended to be used at ambient temperature. In this case, pH, $a_{w,}$ moisture content, and antimicrobials (for example, phosphate, salt) become critical parameters that may preclude pathogenic growth and toxin formation and will determine the need for time/temperature control. Post-process contamination after opening is also possible, and therefore, processed cheeses often need refrigeration after opening.

11.3. Time/temperature control

For traditionally made hard cheeses, unless pH is high or post-process contamination occurs, time/temperature control for safety reasons is not required. Time/temperature control is needed, however, for high moisture soft cheeses because of the potential growth of pathogens. With processed cheeses, there is a concern with the growth and toxin production of *C. botulinum.* If a processed cheese is intended for use at ambient temperature, pH, a_w, moisture content, and antimicrobials should be appropriately adjusted to inhibit botulin toxin formation.

12. Combination products

12.1. Type of products

The "combination products" category refers to products whose formula contains distinct food systems (for example, cheese with vegetable pieces), or products whose components are processed separately and assembled later (for example, pumpkin pie with crème topping). Examples of products that fall into this category are foccaccia breads, meat salads, meat-filled pastry and other stuffed products, and prepared foods (for example, fettuccine alfredo with chicken).

12.2. Microbial concerns

These products present special challenges to their identification as "potentially hazardous foods." Combination foods present the added complexity of the various components' microbial ecology compared to the ecology of single-component foods. The microbial concerns associated with combination products greatly depend on the food components from which they are processed. (For microbial concerns on products, see the hazard analysis of dairy, eggs, fruits and vegetables, meat and poultry, seafood, and cereal products earlier in this chapter.)

The interactions among the various foods combined, which contributes to the uniqueness of each food product, also need to be considered. Components of significantly different pH or a_W produce an altered microenvironment at the interface of the components. An example of this scenario is a donut filled with an acidified filling. The donut has higher pH and lower a_W than the filling. The pH and a_W at the interface will be affected by this difference, which may result in the growth of microorganisms if the product has a long enough shelf life. Obviously, these changes may affect the survival and growth of microorganisms in a less predictable manner than they might in single component foods. In addition to pH and a_w, other food characteristics such as redox potential and the effectiveness of antimicrobials are likely to differ at the interfaces, possibly resulting in unexpected pathogen behavior.

Another feature of combination foods that may affect their microbiological safety is the fact that products often are handled by employees, resulting in an increased risk of microbial contamination. Opportunities for post-processing contamination during handling may result in safety hazards associated with *S. aureus*, *L. monocytogenes*, *Shigella, E. coli* O157:H7*, Salmonella* spp., and other enteric pathogens. *Clostridium botulinum* is also of concern, especially for certain modified atmosphere, controlled atmosphere, and vacuum packaged products.

12.3. Effects of processing

Often, the food composed of other products is subjected to processing before consumption. For example, focaccia bread and fruit pastries are baked and the meat in meat salads is cooked. (For the effect of processing methods in microbial reduction, see the hazard analysis of dairy, eggs, fruits and vegetables, meat and poultry, seafood, and cereal products.) When considering the effect of processing in the microbial load of the product, one needs to consider if the components have been processed separately or after assembly. Processing of the food after assembly decreases the chances for contamination and growth of pathogens as compared to assembling the different components before processing.

12.4. Time/temperature control

In combination foods, the need for time/temperature control depends on the nature of the product. Both the potential for the development of microenvironments and the existence of interface areas contribute to the difficulties in accurately measuring the intrinsic factors of the food. Because of the complex interactions in multiple component foods, one cannot rely on the pH, a_w, or other parameter measurements and, therefore, challenge studies are often performed to decide if the food requires time/temperature control for safety.

Table 4-1. Pathogens of concern and control methods for various product categories (including examples of foods that may need to be evaluated for time/temperature control needs for safety).

Product Category (examples of possible foods for evaluation)	Pathogens of Concern	Types of Process Control[1] (Alone and in Combination)
Meats and poultry (fermented sausage)	*Clostridium botulinum*[5] *and Clostridium perfringens, Salmonella* spp., enterohemmorrhagic *Escherichia coli, Camplylobacter jejuni, Yersinia enterocolitica, Staphylococcus aureus, Listeria monocytogenes*	Time/temperature, pH, a_w, preservatives, moisture protein ratio, fermentation, heat processing
Fish and seafood (smoked fish)	*Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio cholerae, C. botulinum*[5], *L. monocytogenes, Salmonella* spp., *Shigella* spp., *S. aureus*	Time/temperature, harvest site control, fermentation, pH, a_w, water-phase salt, preservatives, drying, salting
Fruits and vegetables (peeled carrots)	*Salmonella* spp., *Shigella* spp., enterohemmorrhagic *E. coli*, *L. monocytogenes, Bacillus cereus, C. botulinum*[5], *Y. enterocolitica*	Production control (Good Agriculture Practices), time/temperature, cooking, preservation techniques
Cereal grains and related products (fresh pasta, foccacia bread)	*Salmonella* spp., *S. aureus, B. cereus, C. botulinum*[5]	Cooking, a_w, pH, preservatives, time/temperature
Fats, oils & salad dressings (garlic-in-oil)	*S. aureus*[2], *Salmonella* spp. [2], *B. cereus*[2], *C. botulinum*[2,]	pH, a_w, salt
Butter and margarine (light salted butter)	*S. aureus, L. monocytogenes, Y. enterocolitica*	Production/raw ingredient quality control, moisture droplet size in the water-in-oil emulsion, water phase salt, a_w
Sugars and syrups (light maple syrup)	*C. botulinum*[3,]	a_w, acidification (light syrups)
Eggs and egg products (merengue)	*Salmonella* spp.[4], *L. monocytogenes*[4]	Production control, cooking/pasteurization, time/temperature
Milk and milk products (yoghurt)	*Salmonella* spp.[4], *L. monocytogenes*[4], enterohemmorrhagic *E. coli*[4], *S. aureus*[4], *B. cereus* (cells[4] and spores[5]), *C. botulinum* (cells[4] and spores[5]), *Campylobacter jejuni*[4]	Production control, time/temperature, cooking/pasteurization, a_w, preservatives
Cheese and cheese products (Natural Swiss cheese)	*Salmonella* spp. [4], *L. monocytogenes*[4], enterohemmorrhagic *E. coli*[4], *S. aureus*[4], *Shigella* spp. [4], *C. botulinum* (cells[4] and spores[5])	Production control, moisture content, a_W, pasteurization, preservatives, pH
Combination products (cheese with veg. pieces, pumpkin pie, stuffed pastry)	Variable, based on raw materials and processing	Variable, based on raw materials and product

[1]Good Manufacturing Practices would help in reducing the hazards. For meats, poultry, and fish and seafood products the Hazard Analysis Critical Control Point principles should be implemented as a control system.
[2]A pH > 4.0 and $a_w \sim 0.92$ in salad dressings and mayonnaise would preclude the growth of pathogens of concern.
[3]Only a concern in light syrups and can be controlled by acidification.
[4]In pasteurized products, all pre-processing vegetative pathogens would be controlled.

[5]Only a concern in anoxic environments.

References

[AFDO] Association of Food and Drug Officials. 1991 June. Cured, salted, and smoked fish establishments good manufacturing practices [model code]. [York (PA)]: Association of Food and Drug Officials. 7 p.

[ICMSF] International Commission on Microbiological Specification for Foods. 1998. Microorganisms in foods. Volume 6, Microbial ecology of food commodities. New York: Blackie Academic & Professional. 615 p.

[IFT] Institute of Food Technologists, Dept. of Science and Technology Projects. 2001. Analysis and evaluation of preventive control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce. Beuchat LR, Busta FF, Farber JM, Garrett EH, Harris LJ, Parish ME, Suslow TV, editors. Chicago: IFT. (Journal of Food Science Supplement). Forthcoming.

Reitsma CJ, Henning DR. 1996. Survival of enterohemmorhagic *Escherichia coli* 0157:H7 during the manufacture and curing of cheddar cheese. J Food Prot 59(5):460-4.

Smittle RB. 2000. Microbiological safety of mayonnaise, salad dressings, and sauces produced in the United States: a review. J Food Prot 63(8):1144-53.

[USDA] U.S. Dept. of Agriculture, Food Safety and Inspection Service. 1996 [with change 98-01 (Sept 1996 thru Feb 1998)]. Food standards and labeling policy book. Available from: Superintendent of Documents, PO Box 371954, Pittsburgh, PA 15250-7954; (202)512-1800.