SARAH LEWIS HOLLIDAY
Viability of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in Dairy and Non-Dairy Yellow Fat Spreads and Toppings
(Under the Direction of LARRY R. BEUCHAT)

Outbreaks of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* infections associated with dairy products have been documented. Seven dairy and non-dairy yellow fat spreads and toppings were inoculated with *Salmonella*, *Escherichia coli* O157:H7, or *Listeria monocytogenes* and stored at 4.4, 10, or 21°C for up to 94 days to determine survival and growth characteristics. *Salmonella* and *E. coli* O157:H7 did not grow in any of the test products; however, *L. monocytogenes* grew in a food service butter and margarine blend stored at 10 and 21°C. The fate of pathogens inoculated onto the surface of six yellow fat products subjected to temperature abuse and high relative humidity followed by storage at 4.4 or 21°C for up to 21 days was also observed. Pathogens grew in a salted, sweet cream whipped butter held at 21°C but not in the unsalted variety or margarine products.

INDEX WORDS: *E. coli* O157:H7, *Salmonella*, *L. monocytogenes*, Butter, Margarine, Yellow fat, Spreads, Toppings

VIABILITY OF *SALMONELLA, ESCHERICHIA COLI* O157:H7, AND *LISTERIA MONOCYTOGENES* IN DAIRY AND NON-DAIRY YELLOW FAT SPREADS AND TOPPINGS

by

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DEDICATION

To my family

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

LITERATURE REVIEW

Edible yellow fat products such as butter, margarine, and dairy and nondairy spreads are used for many purposes including baking, cooking, and toppings for breads and many other foods (Zijl and Klapwijk, 2000). They act as flavor carriers, lubricants for breads and other baked goods during the chewing process, aid in the feeling of satiety due to the high fat content, and provide other functional properties in foods (Moran, 1993). The major proportion of fat intake by the body is through the use of dairy and nondairy fat spreads (Moran, 1993). Edible fat spreads containing more than 15-20% fat are water-in-oil emulsions, whereas emulsions of oil-in-water normally contain lower fat content (Moran, 1993). Full fat products such as margarine and butter contain at least 80% fat.

Composition, Manufacture, and Stability

Butter

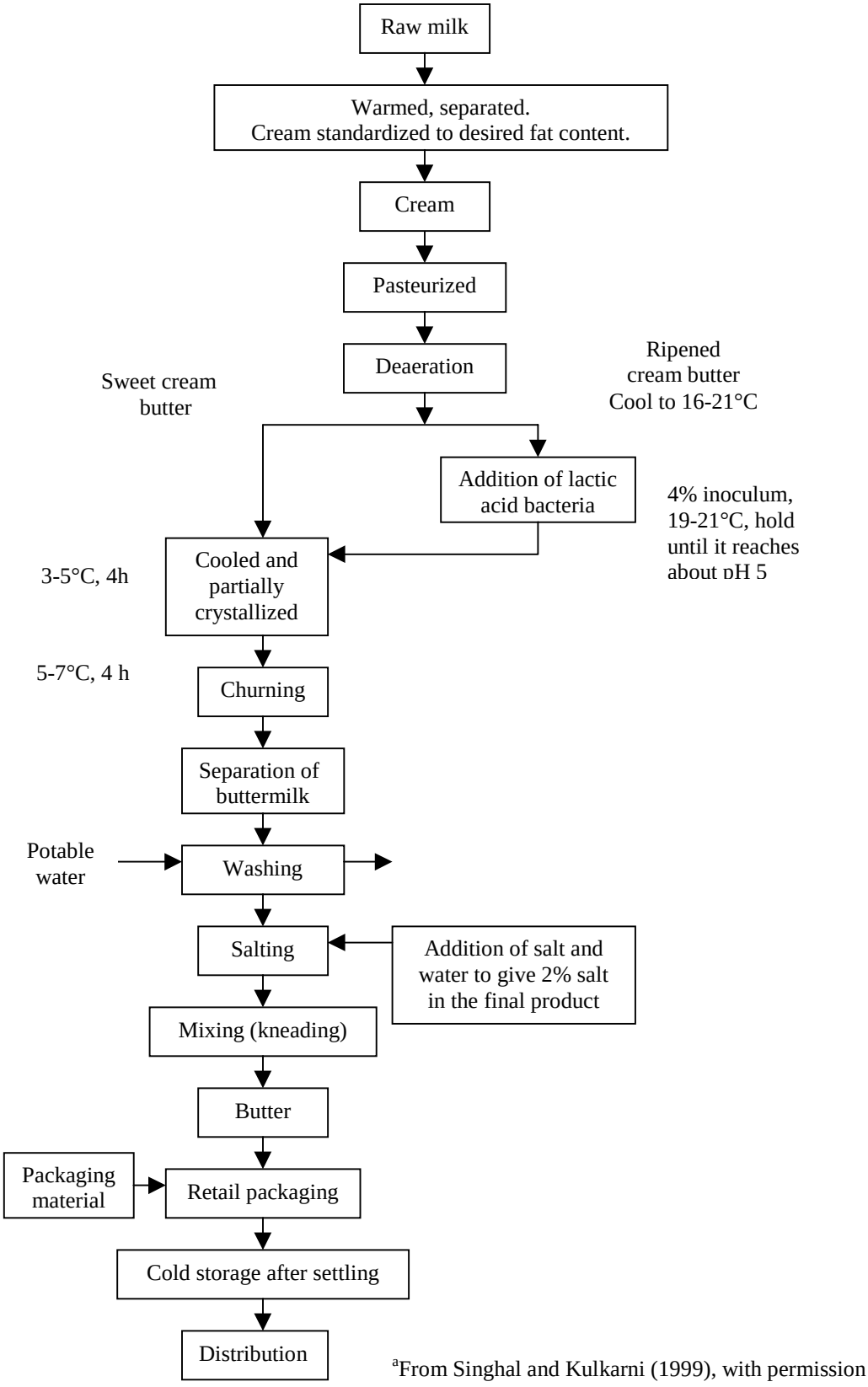
Butter was one of the first dairy products manufactured from cream and has been traded internationally since the 14th century (Kornacki et al., 2001). Mixing salt and starter cultures with concentrated milk fat was originally used to make butter, the oldest yellow fat (from milk) product. Cream, the primary component of butter, is an oil-in-water emulsion that undergoes a phase inversion during the churning step of manufacture, resulting in a water-in-oil emulsion. Before 1800, butter manufacture relied on cream obtained from milk by gravity separation. Large-scale manufacture became possible only after the development of a mechanical cream separator in 1877 (Varnam and Sutherland, 1994; Kornacki et al., 2001). Since factory production began in 1850, the composition of butter has not changed over the years due to strict regulations. In the United States, butter was defined in 1923 by an act of Congress, not by Standard of Identity in the Food, Drug, and Cosmetic Act. Butter is defined by the U.S. Food and Drug Administration (FDA) as follows: “butter shall be understood to mean the food product usually known as butter, and which is made exclusively from milk or cream or

both, with or without common salt, and with or without additional coloring matter, and containing not less than 80 percent fat." In 1998, the United States produced 1.08×10^9 lb of butter while world wide consumption was estimated at 4.84×10^9 lb (Kornacki et al., 2001).

Composition. Butter is composed of 81% milk fat, up to 2% salt, 15% water, and 0.5% milk solids (carbohydrates and protein) (Jay, 2000). All commercial butter is produced from pasteurized cream. Butter may be either sweet cream butter, which may or may not be salted, or ripened cream butter. The aqueous phase contains 10% salt but after dispersion into the continuous phase is up to 2% of the finished product (Jay, 2000). Due to the preservative effect of the salt, the shelf life of refrigerated salted butter is 3-6 months. If the butter is salt free, moisture and fat contents are adjusted slightly higher to compensate. Salt-free butter must be frozen to preserve it for table use due to its short shelf life of about 30 days (Bradley et al., 2000).

Manufacture. The basic steps for butter manufacture are the concentration of the fat phase of milk, crystallization of the fat phase, phase separation of the oil-in-water emulsion, and formation of the platicized water-in-oil emulsion (Varnam and Sutherland, 1994). Butter manufacture is a continuous process in most countries (Zijl and Klapwijk, 2000). The flow chart in Figure 1 shows the major steps in butter manufacture (Singhal and Kulkarni, 1999). Concentration of the fat phase of milk is done by mechanical separation, resulting in cream. The resulting cream with a fat content ranging from 35-45% is then pasteurized for 10-30 sec at 85-95°C (Varnam and Sutherland, 1994). This process kills most of microorganisms, including vegetative cells of pathogens, inactivates enzymes that are naturally present in the cream, and liquefies the milk fat. The cooking process gives the cream a boiled or nutty flavor.

The cream is cooled and tempered to 3-7°C for 4 h or more to promote crystallization of the fat phase (Kornacki and Flowers, 1998). The final temperature and holding time are more important than the cooling rate. Large numbers of very small



[a]From Singhal and Kulkarni (1999), with permission

Figure 1. Butter manufacture[a]

stable fat crystals form an extensive network during the cooling process (Varnam and Sutherland, 1994). During the summer, pasteurized cream must be held 2°C lower than in winter to minimize fat loss due to the higher oleic acid content present in the milk fat (Bradley et al., 2000).

In ripened-cream butter, lactic cultures are added to the pasteurized cream once it is cooled to 16-21°C. Cultures of lactic acid bacteria used in manufacturing ripened cream butter ferment citrate in the cream to produce acetoin and diacetyl that impart stronger flavors to the product. Cultures of homofermentative *Lactococcus lactis* ssp. *lactis* or *L. lactis* ssp. *cremoris* (homofermentative organisms) in combination with heterofermentative *L. lactis* ssp. d*iacetylactis* or *Leuconostoc mesenteroides* ssp. *cremoris* are generally used (Varnam and Sutherland, 1994; Kornacki et al., 2001). Cultures are added and the product is incubated until a pH of 5 is achieved by fermentation. The product is further cooled to 3-5°C (Kornacki et al., 2001).

Consumers in Europe and Asia prefer the bold flavors of butter developed using milk cultures, while Americans prefer the bland but nutty or boiled milk flavor of sweet cream butter (Bradley et al., 2000). The NIZO process, primarily used in western Europe, was developed by the Netherlands Institute for Dairy Research (NIZO). This process yields a sweet buttermilk from sweet cream which has a higher market value than sour buttermilk that is made from traditionally cultured butter (Zijl and Klapwijk, 2000). After churning, a lactic starter permeate that contains high concentrations of lactic acid and one or two aromatic starter cultures rich in diacetyl are worked directly into the butter (Murphy, 1981). This procedure lowers the pH to <5.3 and adds flavor components (Zijl and Klapwijk, 2000).

Phase separation begins with churning, followed by working the product into a water-in-oil emulsion. The churning temperature is related to the fat content and hardness of the fat. Formulae are used to determine the approximate temperature: F + 2T = 56 (summer) or F + 2T = 58 (winter), where F = % fat in the cream and T = cream

temperature in °C (Murphy, 1981). Churning breaks the oil-in-water emulsion by disrupting membranes of milk fat globules, causing the formation of a water-in-oil emulsion. After disruption of the membrane, smaller butter granules are formed and buttermilk is drained. The churning process of ripened cream butter differs from that of sweet cream butter in several ways. The low pH weakens the fat globule membrane so a shorter churning time is needed. The more efficient emulsification and coalescence of the butter granules reduce fat losses in the buttermilk. The churning temperature is slightly higher because of a more viscous cream. Ripened cream butter, usually unsalted, may contain up to 0.5% salt but if this value is exceeded, quality will quickly deteriorate. If the product contains higher levels of salt, the pH should be 6 or above to prevent oxidation (Murphy, 1981). The granules may be washed to remove excess buttermilk but washing is not possible when using a continuous butter manufacturing process (Zijl and Klapwijk, 2000). This step is generally not practiced due to the possibility of microbial contamination. Washing also causes problems by increasing the water content (Varnam and Sutherland, 1994). Salt is added as a slurry or brine after the washing step and first working session, but before the second working session, to prevent loss of the salt. Butter at the salting stage contains 14% moisture. To achieve 2% salt in the final product while not increasing the water content above 16%, a slurry containing a 50:50 mixture of salt and water is added (Murphy, 1981). To inhibit the growth of microorganisms, the salt needs to be distributed evenly, resulting in 1-2% of the final product (Kornacki et al., 2001). Two percent salt in the final product would theoretically give a moisture phase containing 12.5% salt (Murphy, 1981).

After churning, the butter is worked until the desired physical structure is obtained. The butter granules are continually worked using shear forces to produce butter with finely dispersed droplets of moisture. If not properly dispersed, larger droplets with low concentrations of salt provide a suitable environment for growth of spoilage microorganisms. The two goals of working butter are to distribute the water containing

salt evenly through the product and to allow for fat crystal growth. The goal is to obtain butter with small water droplets. The consistency and spreadability of the product are determined at this stage (Varnam and Sutherland, 1994). Underworking and overworking may cause problems such as free water and large course water droplets in the product (Zijl and Klapwijk, 2000). After working is complete, butter is dropped into a hopper where it is pumped to packaging machinery (Bradley et al., 2000).

The spreadability of edible fat products can be improved by several methods. Blending butter with vegetable oils can be effective in increasing spreadability when 15-35% vegetable oil is used. Products that contain oils and fat other than milk fat cannot be called butter. Vegetable oil may be blended with butter fat during any stage of the process. Emulsifiers are often added to maintain stability by reducing interfacial tension between the phases and prevent coalescing during the churning process (Fellows, 1997). Texturization, accomplished by vigorously kneading ready-churned butter, may also be used as a means of increasing spreadability. The kneading process releases liquid fat from the crystal network, making the product spreadable at refrigeration temperatures. Whipping may also reduce the hardness of butter. The ease with which the product is spread is proportional to the amount of gas incorporated during whipping. Nitrogen is often used and is injected into the butter between the pump and mixer. The structure of whipped butter is coarse and spongy with 75% overrun, imparting a more spreadable product (Varnam and Sutherland, 1994).

Microorganisms in butter. The microbial stability of butter is determined by the initial population and type of microorganisms in the cream. Microorganisms in whole milk are largely in the cream because they are carried to the surface as fat globules rise (Jay, 2000). Raw milk contains a predominant population of gram positive microorganisms originating from the cow's hide and udder and the dairy environment (Jay, 2000). *Enterococcus*, *Lactococcus*, *Streptococcus*, *Leuconostoc*, *Lactobacillus*, *Micobacterium*, *Oerskovia*, *Pseudomonas*, *Bacillus*, *Listeria*, and coliforms are generally present in raw milk (Jay, 2000). The raw milk may be separated into cream at the dairy

factory or at the farm, with the latter often resulting in a poorer microbiological quality (Zijl and Klapwijk, 2000). At this stage, souring of the cream due to growth of *Lactococcus lactis* or *Geotrichum candidum*, as well as other molds and yeasts, may occur in countries with less advanced dairy industries (Zijl and Klapwijk, 2000).

In fresh cream held at 5°C, the predominant microorganisms were reported to be *Pseudomonas, Alcaligenes, Acinetobacter, Aeromonas,* and *Achromobacter*, and at 30°C *Corynebacterium, Bacillus, Micrococcus*, *Lactobacillus,* and *Staphylococcus* were predominant (Davis, 1981). *Flavobacterium* and *Pseudomonas* may grow, producing protease and lipase that lead to off-flavors during refrigerated storage of unpasteurized cream (Zijl and Klapwijk, 2000). As microorganisms break down proteins, bitter flavors develop in the cream as a result of the production of polypeptides. Yeasts and molds, *Proteus,* and other gram negative rods are capable of producing bitterness (Davis, 1981).

Cream may also contain pathogens such as *Staphylococcus*, *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* as a result of mishandling, transport, and contamination from the environment (Zijl and Klapwijk, 2000). Cream is pasteurized before butter manufacture to kill microorganisms and to inactivate enzymes that compromise the safety and stability of the product. Bacteria that are capable of surviving pasteurization include thermoduric streptococci, lactobacilli, and spore formers, *Bacillus* and *Clostridium* (Jay, 2000). After processing, butter may be stored and allowed to cool and set. Since the cooling rate of butter is so slow, bacterial growth may occur during the first few days of storage until a temperature below 0°C is reached (Murphy, 1981).

Spoilage. Two types of bacterial spoilage, surface taint and rancidity, are commonly encountered with butter. Surface taint is attributed to the growth of *Alteromonas putrefaciens* and/or *Flavobacterium maloloris* and decomposition of proteins, producing organic acids such as isovaleric acid on the surface of the butter (Zijl and Klapwijk, 2000; Jay, 2000). Growth occurs at 4-7°C within 7-10 days. Rancidity

occurs when butterfat is hydrolyzed, releasing free fatty acids. *Pseudomonas fragi* and *P. fluorescens* are known to cause this type of spoilage. Butter is favorable to long-term survival of some microorganisms. Pseudomonads and other microorganisms are capable of surviving for several months at –10 to 20°C in butter containing no salt (Thomas and Druce, 1971). Heat resistant protease and lipase produced by pseudomonads may spoil butter made from pasteurized cream during storage (Kornacki et al., 2001). Lipase from other sources is also capable of causing this spoilage. Three other less common types of microbial spoilage may also be observed: malty flavor, skunklike odor, and black discoloration caused by *Lactococcus lactis* var. *maltigenes*, *Pseudomonas mephitica*, and *P. nigrifaciens*, respectively (Jay, 2000). Only butter made from sweet cream is capable of supporting the growth of psychrotrophic bacteria (Frank, 1997).

Butter is more susceptible to fungal rather than bacterial spoilage due to its low water content and high fat content (Jay, 2000). Molds, unlike bacteria, have the ability to transit through the oil phase (Delmarre and Batt, 1999). Spoilage by *Penicillium*, *Oospora*, *Mucor*, *Geotrichum*, *Aspergillus*, and *Cladosporium* may result in discoloration, presence of mycelia in and on the product, and off-flavors from the generation of free fatty acids (Jay, 2000; Delmarre and Batt, 1999). Discoloration observed on the surface of butter is attributed to spores produced by molds (Jay, 2000). Yeasts and molds are more capable than bacteria of growth in the presence of higher salt and low pH (Zijl and Klapwijk, 2000). Yeasts such as *Candida lipolytica*, *Torulopsis*, and *Crytococcus* have been implicated in spoilage (Zijl and Klapwijk, 2000). Lipases secreted by these organisms hydrolyze the butter fat, forming free fatty acids from which rancid flavors (such as butyric acid) develop (Zijl and Klapwijk, 2000).

Margarine

Margarine was produced in the 19th century as a less expensive alternative to butter. In 1870, a French chemist, Mege–Mouries, created “oleomargarine” that had a taste and texture resembling butter. As the quality and spreadability of refrigerated

margarine improved, so did acceptability (Varnam and Sutherland, 1994). The United States Code of Federal Regulations (2001) refers to margarine as "The food in plastic form or liquid emulsion containing no less than 80% fat".

Composition. Animal fats were used for margarine production until the early 20th century. They are now replaced with vegetable oils such as soybean and sunflower oil. Margarine products contain 80% oil or fat, 15% moisture, small amounts of milk and milk products, preservatives, acidulants, emulsifiers, and salt (up to 2%). Margarine may also be fortified with vitamins A and D. Differences in formulae determine the products' spreadability, flavor release, and stability (Delmarre and Batt, 1999).

Manufacture. The process of margarine manufacture involves the formation of a water-in-oil emulsion by mixing water-soluble ingredients (salt, protein, etc.) in the aqueous phase with the oil phase that contains oil-soluble emulsifiers and flavors (Delmarre and Batt, 1999). The two phases are metered into an emulsifying unit at 45°C and agitated vigorously (Varnam and Sutherland, 1994). The emulsion is then pumped through a series of scraped-surface heat exchangers. Depending on the product type, blenders and holding tubes may be a part of the system. The first scraped-surface heat exchanger pasteurizes the emulsion by holding the product for 2-3 sec at 80-85°C. The emulsion is then chilled immediately in the scraped-surface heat exchanger to initiate crystallization. The steel cylinders of the scraped-surface freezer are externally cooled by a refrigerant such as freon or ammonia to -10 to -20°C (Varnam and Sutherland, 1994). Dispersion of the aqueous phase and the induction of nucleation and fat crystallization occur as shear forces and high internal pressures effect the product during 10-20 sec in the machine. The supercooled emulsion is then processed according to the type of margarine, whether stick or tub. For stick products, the emulsion is passed through large cooling tubes. Shear conditions created by sieve plates fitted in the tubes continue the crystallization of the fat. The ability of the fat to crystallize is a function of time and temperature that varies depending on the composition of the fats and oils used (Varnam

and Sutherland, 1984). Soft tub margarine is worked a second time to improve the plastic range of the product. The product remains in the pin rotor texturizer for up to 3 min, allowing time for rearrangement of the fat to the β′-form. Melting and recrystallization occur due to the release of heat from rearrangement, and mixing prevents fat crystals from forming an extensive network. The hardness of margarine produced determines whether the product is packaged in tubs or molded into sticks. (Varnam and Sutherland, 1994).

Spoilage. Yeasts and molds such as *Trichoderma viride*, *Geotrichum candidum*, *Aspergillus* spp., *Cladosporium*, *Alternaria*, *Paecylomyces*, *Rhizopus*, *Candida lipolytica* and *Penicillium* spp. are the most frequently encountered spoilage microorganisms in margarine (Delmarre and Batt, 1999). In a study by Hocking (1994), the mold genus that accounted for 95% of margarine spoilage was *Pencillium*. The most common species in low salt varieties was *P. expansum*. Molds are generally confined to products containing dairy ingredients because of the availability of nutrients and products formulated with low salt (Zijl and Klapwijk, 2000). Most spoilage appears on the surface because anaerobic conditions within the product retard the growth of most microorganisms (Delmarre and Batt, 1999).

The development of free moisture on the surface of margarine and temperatures >10°C favor the growth of molds (Zijl and Klapwijk, 2000). These conditions may develop after repeated use or temperature abuse. Many molds produce by-products that impart off flavors; these include ketones (cardboard flavor) and geosmin that is responsible for earthy notes in margarine (Delmarre and Batt, 1999). Contamination of margarine by mycotoxins has not been reported (Delmarre and Batt, 1999). Preservatives such as sorbic acid and benzoate are added as antifungal agents to aid in the stability of the products. In some instances, molds are capable of metabolizing these chemicals, e.g., some *Penicillium* spp. are capable of metabolizing sorbic acid to produce 1,3-pentadiene (Finol et al., 1982). *Paecilomyces variotti* converts sorbic acid into pentadiene that leads

to a “kerosene-like” odor, which can be detected before visible signs of growth. Benzoate may also be degraded to benzaldehyde but to a lesser extent (Zijl and Klapwijk, 2000). Bacterial spoilage of margarine is primarily accomplished by lipolytic bacteria such as *Pseudomonas*, *Flavobacterium*, *Micrococci*, *Zymomonas*, and *Bacillus* (Delmarre and Batt, 1999). *Pseudomonas oleovarans* and *P. fragi* lead to spoilage by lipolysis and proteolysis (Zijl and Klapwijk, 2000).

Table Spreads

With increasing awareness of health risks associated with the consumption of polyunsaturated, saturated fats, and *trans* fatty acids, consumers are looking to reduced fat spreads with healthier lipid profiles (Delamarre and Batt, 1999). Low-fat butter products based on butter and vegetable fat blends are now produced due to consumers’ demand for reduced fat products (Zijl and Klapwijk, 2000). Products containing 50-60% fat are considered as reduced fat products. Low fat products contain 39-41% fat, while any product containing less than 30% fat is considered to be a very low fat product (Varnam and Sutherland, 1994; Kornacki and Flowers, 1998). Fat can be derived from milk fat, non-milk fat, or blends of both (Moran, 1993). Hydrogenated oils used in margarine and spread manufacture may contain *trans* fatty acids that act like saturated fats in the body. Reducing the intake of saturated fats results in lowered blood cholesterol levels and reduces the risk of cardiovascular disease. Products that provide the best health benefits while keeping their original function are in demand by consumers concerned about health issues. Over twenty varieties of spreads are available with different functional properties and fat contents (Moran, 1993).

Composition. Table spreads such as reduced fat spreads contain 3-80% fat. Due to a high water content, stability and microbial safety become an issue. Several ingredients are used to stabilize the spreads and aid in retaining mouth feel characteristics and other organoleptic properties (Delmarre and Batt, 1999). Fat replacers (sugar derivatives, starches, or maltodextrins, and micron-sized milk proteins or

polysaccharides) mimic the taste of fat and provide textural similarities. For physical stability, thickeners such as gelatin, maltodextrins, and alginates (1-3% of the product) are added to spreads containing 30-50% fat. Spreads with 20-30% fat may contain up to 10% carbohydrates. Aqueous gels used in reduced fat products have properties similar to fat (Moran, 1993).

In lower fat spreads, preservatives are used to ensure the microbiological stability. Sorbic acid, benzoic acid, and/or their salts are added at a concentration of 0.1% for spreads containing greater than 60% fat and 0.2% in spreads containing less than 60% fat. Since undissociated sorbic acid is the main antimicrobial substance, the pH needs to be low enough to ensure the presence of undissociated acid (pH 5.0-5.3) (Zijl and Klapwijk, 2000; Sofos and Busta, 1983). Sorbic acid is effective against many yeasts and molds and can inhibit growth of some bacteria, including *Escherichia* and *Salmonella* (Sofos and Busta, 1983). The undissociated benzoic acid molecule is also responsible for its antimicrobial activity. It is generally used as an antifungal agent in acidic foods with a pH below 4.5 (Chipley, 1983). Salt is used in such low concentrations (0-1%) that it does not contribute to the microbial stability of the product (Zijl and Klapwijk, 2000).

Manufacture. There are four major processes for the production of spreads. For fat-continuous products containing at least 40% fat, the traditional churning process with adaptations using air bubbles or shearing forces, or standard margarine type processes using scraped-surface heat exchangers are used. The manufacturing process, shown in Figure 2, was developed to create a stable fat-continuous product with the aqueous phase distributed throughout the product as uniformly as possible (Kornacki et al., 2001). Higher fat margarine products are generally made using a swept-surface heat exchanger (Kornacki et al., 2001). Spreads with a range of fat contents can be manufactured using high speed tubular pin mixers that cause the phase inversion of oil-in-water emulsions to water-in-oil emulsions. The inversion route is best for products containing very low fat. The fourth processing technique is used for very low fat products. This process entails

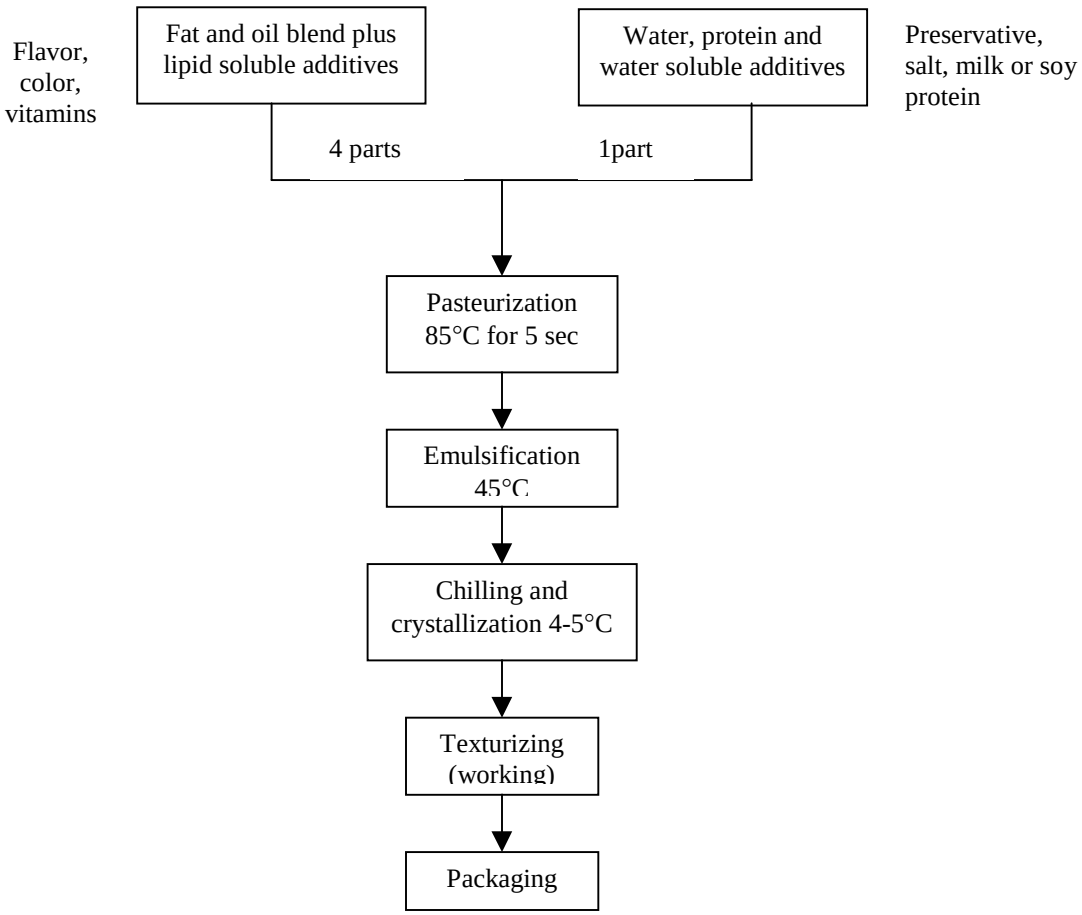


Figure 2. Margarine-type product manufacture[a]

[a]From Kornacki et al. (2001), with permission

cold mixing of aqueous phases or oil-in-water emulsions into preformed continuous fat products.

Factors Influencing Physical and Microbial Stability

Stability and shelf life of butter, margarine, and dairy and nondairy spreads are defined by emulsion characteristics and the process by which the products are created. Fat spreads are composed of liquid oil, fat crystals, water, and other ingredients in the aqueous phase (Juriaanse and Heertje, 1988). Butter and margarine are less suitable substrates to support growth of microorganisms than are milk and cream (Thomas and Druce, 1971). A variety of factors contribute to controlling the growth of microorganisms in butter, margarine, and dairy and non-dairy spreads. Important factors include the fine and uniform distribution of the aqueous phase throughout the lipid phase, uniform distribution of salt throughout the product, low temperature and short storage time, the use of lactic cultures (in ripened butter), presence of preservatives (in margarine and dairy and nondairy spreads), and limited availability of nutrients in the aqueous phase (Kornacki et al., 2001; Varnam and Sutherland, 1994). The aqueous phase of margarine is generally pasteurized so the potential of contamination is small. The composition of margarine and other spreads prevents the growth of many microorganisms. By reducing the fat content of margarine products, the microbiological stability is compromised. With less fat, the emulsion droplets are coarser, providing a larger space for bacteria to grow (Zijl and Klapwijk, 2000). Added ingredients such as acids and water structuring agents are required to stabilize the product. Salt in the aqueous phase aids in the prevention of growth and microorganisms and preservatives aid in preventing spoilage (Kornacki et al., 2001). The addition of acidulants to spreads decreases the pH, in some cases preventing growth. Preservatives also function more effectively in products with low pH (Branen and Davidson, 1983).

Fat functions to provide structure to the product and to stabilize the water droplets. Crystallization of high melting point triacylglycerols determines the texture of edible fat products. Crystallization into four different forms (sub-α, α, β, and β' (most predominant) may occur during manufacture, but the first two forms are unstable and do not exist in spreads (Juriaanse and Heertje, 1988). Large beta crystals may cause products to have a sandy texture. Consistency is determined by the interactions between the fat crystals that form the network. Hardness of the product depends on the amount of fat crystals present, while arrangement of the crystals and strength of interactions depend on the composition of the blends. Slowly crystallizing blends continue to set after packaging, making the product more stable. A high rate crystallization leads to soft products.

In margarine, "shells" of fat may surround water droplets of 2-20 μm in diameter to aid in stabilizing the aqueous phase (Moran, 1993). The shells of fat are interconnected with the three dimensional network of fat crystals. The amount, size, and shape of the fat crystals are related to the level of solid phase in the fat blend, the polymorphic form of the solid phase, and the degree of working and chilling during manufacture (Moran, 1993; Juriaanse and Heertje, 1988). In butter, milk fat globules may still be present in the finished product. Working the product disperses the water droplets, and fat globules are transformed into a crystalline interglobular phase that imparts a hard consistency. The interglobular phase is a mixture of membrane residues, aggregates of fat crystals, and liquid oil. Butter, unlike margarine, may contain continuous water channels formed because of the close proximity of water droplets to the water-containing membranes of fat globules. The presence of fat crystals which form shells around water droplets is a matter of debate (Juriaanse and Heertje, 1988).

Verrips' growth model of water-in-oil emulsions (Verrrips and Zaalberg, 1980) is based on the physical structure of the nutrient-containing aqueous phase, chemical composition, and the initial level of contamination (Charteris, 1996). The ability of

microorganisms to grow in a nutrient dense medium depends on the remaining space in the water droplet (Charteris, 1996). Survival of a microorganism in nutrient poor emulsions is determined by its maintenance energy (Charteris, 1996). The maintenance energy of a microorganism is the amount of energy needed to retain viability of a non-growing cell. Death occurs when energy sources within the water droplet are depleted and become less than the m_e (Verrips and Zaalberg, 1980).

When the water droplet size is smaller than 10 μm, the environment created cannot sustain microbial growth (Delmarre and Batt, 1999). Microorganisms are usually confined to the water droplets where their survival and multiplication depends on the amount of available nutrients. The smaller the water droplet, the more limited area within the droplet and the smaller the quantity of nutrients available for microbial growth (Delmarre and Batt, 1999). The ability of microorganisms to grow is proportional to the availability of nutrients and the size of the water droplets (Verrips, 1989). The average droplet size in full fat margarine is 4-5 μm with a range from 1-20 μm.

Pathogens of Concern

Salmonella

Salmonella is a gram negative, nonsporeforming, short rod-shaped, chemoorganotroph. Most serotypes are motile by peritrichous flagella. They rarely ferment lactose or sucrose, and can utilize citrate as the sole carbon source (El-Gazzar and Marth, 1992). During fermentation of glucose, acid and gas are produced. Salmonellae are oxidase negative and catalase positive (Jay, 2000). They are capable of adapting to extreme environmental conditions (Jay, 2000). Growth temperatures range from 2-45°C with optimum growth at 35-37°C. They exhibit some psychrotrophic characteristics by their ability to grow at refrigeration temperatures. Lag times are increased as the temperature decreases. Matches and Liston (1968) reported that *Salmonella* Typhimurium grew within 12 days when incubated at 6.7°C and 5 days at 7.5°C. *Salmonella* is capable of growth at lower temperatures when on agar (4°C) than in

broth (5.9°C) (D'Aoust, 1991). Growth was observed at 2°C when *S.* Enteritidis was inoculated into minced chicken and when chicken was naturally contaminated with *S.* Typhimurium and held for 2-6 days (Baker et al., 1986).

The various conditions under which *Salmonella* can grow are determined by each strain, but the composition of food is also important in determining survival and growth. Although growth occurs more quickly in a nutrient rich medium, *Salmonella* is not fastidious. *Salmonella* can grow at pH 4-9. The acid present, whether organic (restrictive) or inorganic (permissive), and other growth conditions affect growth of *Salmonella* to varying degrees (Doyle and Cliver, 1990). In an environment with low pH, the number of cells present may affect behavior in that large numbers are capable of growth while fewer than 10 cells per gram are not (Doyle and Cliver, 1990). In a study by Chung and Goepfert (1970), propionic acid used to acidify media (pH 5.5) prevented growth of *Salmonella,* yet growth was observed when media were acidified with HCl or citric acid to a pH of 4.05. Some strains are more acid resistant than others and are capable of surviving at lower pH. The optimal conditions for growth of *Salmonella* are pH of 6.5 -7.5 at 35-37°C. Growth can readily occur under more acidic conditions and lower temperatures. Perales and Garcia (1990) reported that the survival of *S.* Enteritidis inoculated into homemade mayonnaise was dependent on both the temperature and the acidulant.

Most *Salmonella* serotypes can grow in foods with a a_w of 0.945-0.999 (Doyle and Cliver, 1990). As the a_w is reduced to 0.945, other environmental conditions such as pH and temperature need to be optimal for growth to occur. The minimal a_w for growth of salmonellae is 0.90 (El-Gazzar and Marth, 1992). Three to four percent salt (NaCl) in growth media generally inhibits salmonellae. The ability of *Salmonella* to tolerate higher salt concentrations increases as the temperature increases from 10 to 30°C (Jay, 2000). Over twenty strains of *Salmonella* grew at 30°C in tryptic soy broth (TSB) containing 5 or 8% NaCl, but when incubated at 10°C, only one strain grew in TSB containing 5%

NaCl (Alford and Palumbo, 1969). Matches and Liston (1972a) reported that low concentrations of salt added to nutrient broth inhibited the growth of *Salmonella* but as incubation temperature was increased to 37°C, growth was observed in broth containing up to 8% NaCl.

Salmonella is pathogenic to humans and are capable of causing enteric fever, entercolitis, and septicemia (El-Gazzar and Marth, 1992). The infective dose of *Salmonella* is believed to be 10^8 or 10^9 (Doyle and Cliver, 1990), although researchers have reported that fewer than 10 cells of *Salmonella* Typhimurium in Cheddar cheese caused illness (El-Gazzar and Marth, 1992). Foods associated with a low infectious dose, such as, chocolate, cheese, and meat have high fat contents (D'Aoust, 1997). Several factors influencing survival of *Salmonella*, e.g., the composition of the food, the strain, numbers of the pathogen, and the storage temperature, affect the ability of *Salmonella* to cause illness. In 1985, an outbreak of salmonellosis involving 23,000 persons was traced to whole and 2% pasteurized milk (Donnelly, 1990). Outbreaks of salmonellosis involving raw milk and cheese, ice cream, and shredded Mozzarella cheese have been documented (El-Gazzar and Marth, 1992). In a study reported by Sims et al. (1970), butter made from contaminated cream and water used to wash away excess buttermilk from the butter granules supported growth of *Salmonella* at 25°C. Populations decreased when butter was stored at or below 4.4°C. Large decreases in populations of *Salmonella* were also observed in unsalted butter held at –17.8 or 23.3°C (El-Gazzar and Marth, 1992).

***Escherichia coli* O157:H7**

Escherichia coli O157:H7 was first identified as a foodborne pathogen in 1982 when an outbreak was linked to eating ground beef sandwiches (Doyle, 1991). Gastrointestinal illness may lead to other severe syndromes such as hemorrhagic colitis, hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP).

Transmission of *E. coli* O157:H7 may occur through a variety of foods and water, as well as from person to person through the fecal oral route (Shere et al., 1998). Its low infectious dose and ability to survive in high-acid foods have made its presence in food a major public health concern.

The majority of outbreaks of *E. coli* O157:H7 infection have involved ground beef or dairy products. Because of this association, dairy cattle are thought to be a principal reservoir of the pathogen (Shere et al., 1998). Illness caused by *E. coli* O157:H7 has been associated with the consumption of ground beef and raw milk. Unpasteurized milk contaminated with *E. coli* O157:H7 and milk contaminated post-pasteurization have resulted in several outbreaks of gastroenteritis (Wang et al., 1997). In a study reported by Abbar and Mohamed (1987), butter from a single producer in Baghdad was analyzed for the presence of fecal coliforms. About 76% of the samples were considered unacceptable because they contained more than of 100 coliforms/g. *E. coli* was present in 41% of samples, indicating poor sanitation conditions in the processing facility (Abbar and Mohamed, 1987). Outbreaks of *E. coli* O157:H7 infection have also been linked to acidic foods such as salami and apple cider (Deng et al., 1999). In 1993, an outbreak involving 40 people was linked to the consumption of mayonnaise prepared in a restaurant (Zhao and Doyle, 1994).

Enterohemorrhagic *E. coli* O157:H7, is a gram negative, nonsporeforming rod incapable of fermenting sorbitol within 24 h (Doyle et al., 1997). The MUG assay, used as a rapid fluorogenic method for the detection of *E. coli,* will not detect *E. coli* O157:H7 due to its inability to produce β-glucuronidase (Doyle, 1991). Gas is produced as a result of fermentation of lactose. *E. coli* O157:H7 is incapable of growing well or at all when incubated at $\geq$ 44.5°C. Serotype O157:H7 is capable of producing large quantities of potent toxins similar to the Shiga toxin produced by *Shigella dysenteriae* that cause severe damage to the intestinal lining. These toxins, Stx1 and Stx2, are cytotoxic to Vero cells (African green monkey kidney cells). All Stxs are composed of an active A subunit

and multiple B subunits. The toxins bind to the receptor, globotriaosylceramide, and are internalized into the cell. The A subunit binds and releases an adenine residue that inhibits protein synthesis. The B subunits with a single A subunit form pentamers that bind the toxin to glycolipid receptors (Jay, 2000). The pathogen does not possess an unusual resistance to heat and may be eliminated during pasteurization processes. The high fat content of foods such as ground beef provides protection against thermal inactivation (Doyle et al., 1997). Protection supposedly increases heat resistance by directly affecting cell moisture and rate of heat transfer in food (Jay, 2000).

E. coli O157:H7 has been shown to survive in acidic food environments. In a study by Zhao et al. (1993), *E. coli* O157:H7 was capable of surviving for 31 days at 8°C when inoculated into unpasteurized apple cider (pH 3.6-4.0). The addition of 0.1% sodium benzoate increased the rate of inactivation of *E. coli* O157:H7. Zhao and Doyle (1994) reported that *E. coli* O157:H7, when initially present at 6.5×10^3 CFU/g, survived in mayonnaise at 20°C for 21 days and at 5°C for 55 days. The pathogen survived longer when inoculated into real mayonnaise than when inoculated in a reduced-calorie formulation containing less acid (Zhao and Doyle, 1994).

The survival and ability of cells to withstand low pH is influenced by the acidulant. *E. coli* O157:H7 grew in tryptic soy broth (TSB) adjusted with HCl to pH of 4, while growth was not observed in TSB (pH 4.5) when pH was adjusted with lactic acid. *E. coli* O157:H7 was also capable of surviving after storage for 2 months at 4°C in dry fermented sausage with a pH of 4.5 (Glass et al., 1992). In a study by Abdul-Raouf et al. (1993), growth of *E. coli* O157:H7 after incubation at 21°C for 24 h was observed in beef slurries (pH 5.0-5.4) acidified with citric and lactic acid. Decreases in populations were observed when slurries were acidified to a pH less than 4.7 with citric and lactic acid and less than pH 5.4 with acetic acid. In a study reported by Lin et al. (1996), acid resistance, once induced, will persist if cells are held at 4°C.

Listeria monocytogenes

Listeria monocytogenes is a gram positive, facultatively anaerobic, nonsporeforming, hemolytic, motile, rod-shaped bacterium. It is catalase positive and produces lactic acid from glucose and other sugars, with the exception of xylose. Beta hemolysis is observed on blood agar. Four vitamins, biotin, riboflavin, thiamin, and alpha lipoic acid, are required for its growth. Amino acids required for growth are cysteine, glutamine, isoleucine, leucine, and valine. *Listeria monocytogenes* resembles enterococci in that it hydrolyzes esculin but differs in its ability to grow in the presence of 0.02% sodium azide. Among pathogens, it is second to *Staphylococcus* in its ability to grow at a_w less than 0.93 (Jay, 2000). It is able to grow over a wide range of temperatures (3-45°C) with an optimum range of 30-37°C. Its ability of multiplying at refrigeration temperatures (0-7°C) (Walker et al., 1990) increases concern for the safety of refrigerated foods. *Listeria monocytogenes* has been shown to grow at 4°C in fluid dairy products (cream, whole and skim milk) after a 5-day lag phase (Bahk and Marth, 1990). Extended periods of refrigerated storage and improper holding temperatures increase the risk of listeriosis because a few cells have the opportunity to grow (Beuchat, 1998). The pathogen is capable of growth over a pH range of 5 to 9.6 (Donnelly et al., 1992), yet growth was observed at pH 4.5 in TSB when incubated at 30°C (Parish and Higgins, 1989). Parish and Higgins (1989) also reported that low pH had a harmful effect on *L. monocytogenes*, but significant lag periods were observed before population reduction when incubation was at 4°C. The presence of acetic acid in media severely reduces the ability of *L. monocytogenes* to grow (Cheroutre-Vialette et al., 1998). *Listeria* is fairly salt tolerant and capable of growing in a saturated brine solution held at 4°C for 132 days (Mitscherlich and Marth, 1984). In a study done by Cheroutre-Vialette et al. (1998), *L. monocytogenes* tolerated KCl (80 g/L) better than NaCl (80 g/L). The solute used to control a_w can influence growth behavior. Under acidic and osmotic stresses, growth in high-salt media is more affected.

Listeria monocytogenes is ubiquitous in nature and present in the intestinal tract of many animals, including humans. Recent studies have determined that 2-6% of healthy people are carriers of the organism (Meng and Doyle, 1997). *Listeria monocytogenes* causes listeriosis, a disease that most often effects immuno-compromised individuals such as the elderly, AIDS patients, infants, and pregnant women (NACMCF, 1991). A high mortality rate of 30% is associated with listeriosis. Serovar 4b, the most virulent serovar, is most often associated with outbreaks. Serovars 1/2a, 1/2b, and 4b are the most common serovars isolated from foods and humans with listeriosis (Jay, 2000).

The presence of *L. monocytogenes* in feces explains why the organism can be found in sewage and soil (Beuchat, 1998). *Listeria monocytogenes* can easily enter dairy product manufacturing facilities from many different sources. In factories where potentially contaminated raw milk is received, special care should be taken to prevent introduction of the pathogen into the finished product. *Listeria monocytogenes* has been found in numerous foods, including raw meats, raw milk, dairy products, vegetables, eggs, and seafood products (Meng and Doyle, 1997).

Outbreaks of listeriosis have been associated with consumption of coleslaw, meat products, pasteurized milk, and milk products, e.g., soft cheeses (Meng and Doyle, 1997). In 1985, an outbreak of listeriosis was linked to 2% fat and whole-fat pasteurized milk (Donnelly, 1990). Lanciotti et al. (1992) concluded that *L. monocytogenes* is capable of growing in light butter because it may be less affected by space and nutritional limitations of the compartmentalized structure. Parker et al. (1995) stated that growth of *L. monocytogenes* occurs in a colony-like form that prevents access to available nutrients. *Listeria monocytogenes* has been shown to survive during processing of butter (Olsen et al. 1988). An outbreak of *L. monocytogenes* serotype 3a infection linked to butter occurred in Finland in 1999 in which four of the eighteen infected individuals died (Lyytikainen et al., 2000). The pathogen was isolated from environmental samples taken from the small production facility and the packaged product. In a study by Wang and Johnson (1992), fatty acids and monoglycerides in milk were evaluated for inhibitory

activity against *L. monocytogenes*. K-conjugated linoleic acid in whole and skim milk was bacteriostatic and prolonged the lag phase when cells were held at 4°C (Wang and Johnson, 1992).

Research Needs

Milk and milk products are susceptible to contamination by several pathogenic micoorganisms (NACMCF, 1991). *Salmonella*, *Escherichia coli* O157:H7, and *L. monocytogenes* have been isolated from dairy processing environments and processed dairy food products (Wang, and Johnson, 1992; Gabis et al., 1989; Wang, et al., 1996). These pathogens have been responsible for outbreaks of infections involving milk and dairy products (Wang et al., 1997). The major objective of the study reported here was to determine the survival characteristics of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* inoculated into seven yellow fat products as affected by storage temperature.

There is increasing popularity for large multiple-use containers of yellow fat products in food service establishments, restaurants, and the home. The possibility of cross contamination increases as products are taken from containers over longer periods of time. The abuse of products and constant reuse or dipping into products in large containers may increase the opportunity for cross contamination by food service employees with poor hygiene. Sneezing, coughing, spitting, or handling the product with unclean hands may result in contamination of the product.

The constant dipping into products increases risks of contamination by bacteria, molds, and yeasts by unclean utensils. Lack of temperature control, disturbance of the emulsion, and the collection of water as a result of condensation on the surface of yellow fat products may enhance the ability of pathogens to survive and, perhaps, grow. A second objective of this study was to determine the survival characteristics of pathogens surface inoculated onto yellow fat products, including butter, that has been subjected

abuse conditions, namely, elevated temperature, disturbance of the emulsion, and water condensation.

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CHAPTER 2

VIABILITY OF *SALMONELLA, ESCHERICHIA COLI* O157:H7, AND *LISTERIA MONOCYTOGENES* IN YELLOW FAT PRODUCTS AS AFFECTED BY STORAGE TEMPERATURE

ABSTRACT

Yellow fat products are stored at refrigeration temperatures to maintain quality rather than to prevent growth of foodborne pathogens. However, rates of inactivation of pathogens in these products as affected by temperature have not been reported. This study was done to determine the time required for elimination of *Salmonella* (6.23 – 6.55 $\log_{10}$ CFU/3.5-ml or 4g sample), *Escherichia coli* O157:H7 (5.36 - 6.14 $\log_{10}$ CFU/3.5-ml or 4-g sample), and *Listeria monocytogenes* (5.91 - 6.18 $\log_{10}$ CFU/3.5-ml or 4-g sample) inoculated into seven yellow fat products (one margarine, one butter and margarine blend, and five dairy and non-dairy spreads and toppings) and held at 4.4, 10, and 21°C for up to 94 days. Neither *Salmonella* nor *E. coli* O157:H7 grew in any of the test products. *Listeria monocytogenes* did not grow in six of the test products but increased in population between 42 and 63 days in a butter and margarine blend stored at 10°C and between 3 and 7 days when storage was at 21°C. The presence of butter in the margarine and butter blend, as well as emulsion characteristics, apparently provided a more favorable environment for growth. *Escherichia coli* O157:H7 died more rapidly than *Salmonella* and *L. monocytogenes*, regardless of storage temperature. *Salmonella* survived longer in high fat (>60%) products than in products with lower fat content. All three pathogens died most rapidly in a spray topping (pH 3.66) containing 41% fat and 4.12% sodium chloride. Inhibition of growth is attributed to factors such as acidic pH, salt content, presence of preservatives, emulsion characteristics, and nutrient deprivation.

INTRODUCTION

Yellow fat products are used as ingredients in baked and cooked foods as well as toppings for a variety of foods such as vegetables and breads (Zijl and Klapwijk, 2000). Yellow fat products containing more than 15-20% fat are water-in-oil emulsions, while products containing lower fat content are oil-in-water emulsions (Moran, 1993). Yellow fat spread products are made from oil or fat, water, small amounts of milk and milk products, preservatives, acidulants, emulsifiers, and salt (Delmarre and Batt, 1999). Health concerns over consumption of full-fat products such as butter and margarine (80% fat) have led to the development of reduced-fat products and products containing oil with lower amounts of saturated and trans-fatty acids. Products containing less than about 60% fat are considered to be reduced-fat products. Low-fat products contain 39-41% fat, while products containing less than 30% fat are considered as a very low-fat (Varnam and Sutherland, 1994).

As products are developed with high water content, sensory stability and microbial safety may become a concern. The aqueous phase is pasteurized so the presence and growth of microorganisms in unopened commercially processed and packaged products is greatly minimized. Ingredients in margarine and spreads also help to prevent the growth of microorganisms. Sodium chloride (8% in the aqueous phase) and low serum pH help to prevent growth of most bacteria and fungi. Stability and shelf life are also greatly influenced by emulsion characteristics (Charteris, 1996). In reduced-fat products, ingredients such as thickeners are added to retain mouthfeel, physical stability, and other organoleptic properties (Delmarre and Batt, 1999). The ability of a microorganism to grow in an emulsion depends, in part, on the availability of nutrients and the volume of the water droplets in which they are usually confined (Verrips, 1989). Microorganisms are usually confined in water droplets that range from 1-20 μm in diameter. Finer emulsions (droplets smaller than 10 μm) provide an environment in which microorganisms are not capable of growing (Delmarre and Batt, 1999). With less

fat, the water droplets are larger, providing cells increased space and more water-soluble nutrients from added ingredients (Zijl and Klapwijk, 2000). The survival of a microorganism in nutrient poor emulsions is determined by its maintenance energy (m_e) (Charteris, 1996). The maintenance energy of a microorganism is the amount of energy needed to retain viability of a non-growing cell. Death occurs when energy sources within the water droplet are depleted and become less than the m_e (Verrips and Zaalberg, 1980).

Milk and milk products are susceptible to contamination by several pathogenic microorganisms (NACMCF, 1991). *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Salmonella* have been isolated from dairy processing environments and/or processed dairy food products (Wang and Johnson, 1992; Gabis et al., 1989; Wang, et al., 1996). These pathogens have been responsible for outbreaks of infections associated with milk and dairy products. In 1985, an outbreak of salmonellosis involving 23,000 persons was traced to whole and 2% pasteurized milk (Donnelly, 1990). Outbreaks of salmonellosis involving raw milk and cheese, ice cream, and shredded Mozzarella cheese have been documented (El-Gazzar and Marth, 1992). In a study reported by Sims et al. (1970), butter made from contaminated cream and water used to wash away excess buttermilk from the butter granules supported growth of *Salmonella* at 25°C. Populations decreased when butter was stored at or below 4.4°C. Large decreases in populations of *Salmonella* were also observed in unsalted butter held at –17.8 or 23.3°C (El-Gazzar and Marth, 1992).

Unpasteurized milk contaminated with *E. coli* O157:H7 and milk contaminated postpasteurization have resulted in several outbreaks of gastroenteritis (Wang et al. 1997). In a study reported by Abbar and Mohamed (1987), butter from a single producer in Baghdad was analyzed for the presence of fecal coliforms. About 76% of the samples were considered unacceptable because they contained more than 100 coliforms/g. *E. coli*

was present in 41% of samples, indicating poor sanitation conditions in the processing facility.

Listeria monocytogenes is ubiquitous in nature and can enter dairy product manufacturing facilities from many different sources. In factories where potentially contaminated raw milk is received, special care should be taken to prevent introduction of the pathogen into the finished product. In 1985, an outbreak of listeriosis was linked to 2% fat and whole-fat pasteurized milk (Donnelly, 1990). Lanciotti et al. (1992) concluded that *L. monocytogenes* was capable of growing in light butter because it may be less affected by space and nutritional limitations of the compartmentalized structure. *Listeria monocytogenes* has been shown to survive the processing of butter (Olsen et al. 1988). An outbreak of *L. monocytogenes* serotype 3a infection linked to butter occurred in Finland in 1999 in which four of the eighteen infected individuals died. The pathogen was isolated from environmental samples taken from the small production facility and the packaged product (Lyytikainen et al., 2000).

Recognition that pathogens are capable of surviving and growing in products such as butter raises concern over their behavior in all yellow fat products. The introduction of numerous reduced-fat spreads containing high percentages of water, dairy, and non-dairy ingredients heightens the need to determine survival and growth characteristics of pathogens in these products as affected by storage temperature. The objective of this study was to monitor populations of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* inoculated into seven yellow fat products stored at 4.4, 10, and 21°C for up to 94 days.

MATERIALS AND METHODS

Products evaluated. Seven commercially manufactured of yellow fat products representing 95-99% of the U.S. retail market were used (Table 2.1). Products were held at 4°C after receipt from the manufacturers and were inoculated with pathogens within 3 weeks. All products are fat-continuous, except Product A, which is water-continuous spray product.

Table 2.1. Products evaluated in studies designed to determine survival and growth characteristics of *Salmonella* , *E. coli* O157:H7, and *L. monocytogenes*

Product code	Product name	Net weight (oz)	Ingredients	Manufacturer
A	I Can't Believe It's Not Butter® Spray	8	Water, liquid soybean oil, salt, sweet cream buttermilk xanthan gum, soy lecithin, polysorbate 60, lactic acid, potassium sorbate and calcium disodium EDTA added as preservatives, artificial flavor, colored with beta carotene, vitamin A (palmitate) added	Lipton, Englewood Cliffs, N.J.
B	Shedd's Spread Country Crock®	16	Liquid soybean oil and partially hydrogeneated soybean oil, water, whey, salt, vegetable mono and diglycerides, soy lecithin, potassium sorbate, citric acid, and calcium disodium EDTA as preservatives, artificial flavor, colored with beta carotene, vitamin A (palmitate) added	Van Den Bergh Foods Co., Lisle, Ill.
C	Blue Bonnet® Light Margarine	16	Water, liquid soybean oil and partially hydrogenated soybean oil, maltodextrin, salt, vegetable mono-glycerides (emulsifier), potassium sorbate and sodium benzoate and calcium disodium EDTA (to preserve freshness), citric acid (acidulant), artificial flavor, colored with beta carotene (vitamin A), vitamin A palmitate	Beatrice Foods, Indianapolis, Ind.
D	Parkay® Squeeze	16	Liquid soybean oil, water, salt, hydrogenated cotton-seed oil, vegetable monoglycerides and soy lecithin (emulsifiers), potassium sorbate and sodium benzoate (to preserve freshness), artificial flavor, phosphoric acid (acidulant), colored with beta carotene (source of vitamin A), vitamin A palmitate	Beatrice Foods, Downer's Grove, Ill.

Table 2.1. continued

Product code	Product name	Net weight (oz)	Ingredients	Manufacturer
E	Promise® Spread	16	Liquid sunflower oil, sweet dairy whey, water, hydrogenated soybean oil, partially hydrogenated soybean oil, liquid canola oil, salt, vegetable mono- and diglycerides, soy lecithin, potassium sorbate and citric acid (as preservatives), artificial flavor, colored with beta carotene, vitamin A (palmitate) added	Lipton, Englewood Cliffs, N.J.
F	Fleischmann's® Original Stick	4	Liquid corn oil, partially hydrogenated soybean oil, whey, salt, vegetable mono- and diglycerides and soy lecithin (as emulsifiers), sodium benzoate (to preserve freshness), vitamin A palmitate, artificial flavor, vitamin D_3	Beatrice Foods, Downer's Grove, Ill.
G	Food service butter and margarine blend	16	Butter, liquid soybean oil, partially hydrogenated soybean oil, water, salt, whey solids, soy lecithin, vegetable mono- and diglycerides, sodium benzoate (a preservative) artificial butter flavor, beta carotene, vitamin A palmitate	Ventura Foods, City of Industry, Calif.

Pathogens. *Salmonella*, *E. coli* O157:H7, and *Listeria monocytogenes* were studied for their ability to survive in margarine and yellow fat spreads and toppings as affected by temperature during storage for up to 94 days. Five serotypes of *Salmonella* were used: Anatum (isolated from a patient with salmonellosis associated with consuming alfalfa sprouts), Enteritidis E190-88 (human isolate), Montevideo CDC #G4639 (isolated from a patient in an outbreak linked to tomatoes), Muenchen 372 (orange juice isolate), and Typhimurium DT104 (multi-antibiotic resistant).

Five strains of *E. coli* O157:H7 were used: F500 (human isolate associated with a mayonnaise outbreak), E0018 (calf fecal isolate), SEA 13B88 (associated with an unpasteurized apple cider outbreak), 994 (salami isolate), and 932 (human isolate from meat outbreak).

Six strains of *L. monocytogenes* were used: Scott A (serotype 4b) (isolate from a patient in an outbreak associated with milk), 302 (serotype 1) (from Cheddar cheese), 310 (serotype 4) (from goat cheese), G1091 (serotype 4b) (patient isolate associated with eating coleslaw), F8369 (serotype 1/2a) (isolate from corn), and F8255 (serotype 1/2b) (peach/plum isolate).

Growth of cells for preparation of inocula. Stock cultures of *Salmonella* and *E. coli* O157:H7 were inoculated into 10 ml of tryptic soy broth (TSB) (BD Diagnostic Systems, Sparks, Md.) supplemented with 1% glucose (TSBG). Stock cultures of *L. monocytogenes* were inoculated into 10 ml of tryptose phosphate broth (TPB) (BD Diagnostic Systems) supplemented with 1% glucose (TPBG).

Microorganisms were cultured in 10 ml of TSBG or TPBG at 37°C for 28 h. All strains were transferred (1 loopful to 10 ml) two times at 28-h intervals. These cultures were used to prepare inocula for liquid (spray or squeeze) products (product codes A and D, respectively). Sterile plastic Vitek sealer plugs (3 x 3 x 27 mm) (bioMerieux, Hazelwood, Mo.) were used as vehicles for inocula for non-liquid products (B, C, E, F, and G). Twenty-eight hours preceding inoculation of the plugs, each serotype or strain

was transferred to 100 ml of TSBG (*Salmonella* and *E. coli* O157:H7) or TPBG (*L. monocytogenes*) in 250-ml Erlenmeyer flasks. Cultures were incubated at 37°C for 28 h.

Cultures were confirmed to be pure by streaking on Hektoen Enteric (HE) (BD Diagnostic Systems) agar for *Salmonella*, sorbitol MacConkey (SMAC) (Oxoid, Basingstroke, U.K.) for *E. coli* O157:H7, and modified Oxford medium (MOX) (Oxoid) for *L. monocytogenes*. Plates were incubated at 37°C for 24 h. Selected colonies were confirmed using the *Salmonella* latex agglutination test (Oxoid), *E. coli* O157 latex agglutination test (Oxoid), and API *Listeria* assay (bioMerieux).

Preparation of inocula for liquid products. To prepare cells for inoculation into liquid products (A and D), cultures were centrifuged (2000 x *g*, 15 min, 21°C) and pelleted cells were washed in sterile potassium phosphate-buffered (0.05 M, pH 6.5) saline (0.85% NaCl) (PBS). Pellets of each serotype or strain were resuspended in 10 ml of PBS. Five milliliters of each serotype or strain of each pathogen were combined to give mixed-serotype or mixed-strain suspensions containing $10^8 – 10^9$ CFU/ml. Each single-pathogen mixture contained approximately equal populations of serotypes or strains.

Preparation of inocula for non-liquid products. Inoculum for non-liquid products also consisted of a mixture of cells of five or six serotypes or strains from 28-h cultures of pathogens. Cultures were centrifuged (2000 x *g*, 10 min, 4°C) and the supernatant was decanted. Pellets collected from *Salmonella* and *E. coli* O157:H7 cultures were resuspended in sterile TSBG; *L. monocytogenes* was resuspended in sterile TPBG. Populations in 28-h cultures were concentrated five-fold by resuspending cells harvested from 600 ml of each *Salmonella* or *E. coli* O157:H7 culture in 120 ml of TSBG, or 600 ml of each *L. monocytogenes* culture in 120 ml of TPBG. Cell suspensions (120 ml of each serotype or strain) of individual pathogens were combined for use as inocula for plugs. Single-pathogen inocula contained approximately equal populations of each serotype or strain.

Inoculation of plugs. Vitek API plugs were used as carriers to inoculate non-liquid products. Plugs were submerged in 70% ethanol for 1 h to assure sterility, then air dried in a laminar flow hood and stored in sterile Petri dishes. Approximately 1000 plugs were immersed in mixed-serotype or mixed-strain inocula for 1 min while being constantly agitated. The inoculum was drained and plugs were individually placed on end in sterile Petri dishes using sterile forceps and dried for 16-18 h in a laminar flow hood. Plugs were propped on the upper rim of the dish to facilitate uniform drying and minimize contact with the dish.

Inoculation of products. Twelve bottles of each liquid product (Products A [8-oz] and D [16 oz]) were inoculated with mixed-serotype or mixed-strain suspensions of *Salmonella*, *E. coli* O157:H7, or *L. monocytogenes*. Cell suspensions (0.1 ml per 100 ml of product) were inoculated into these products to give a population of 10^5 - 10^6 CFU/ml; 0.25 ml of cell suspension was inoculated into 226 ml of the light spray product (Product A) and 0.50 ml of the cell suspension was inoculated into the 453 ml of the squeeze product (Product D). The containers were sealed, shaken vigorously in a 1-m horizontal plane for 30 sec, and incubated at 4.4, 10, or 21°C for up to 94 days before samples were analyzed for populations of pathogens.

Inoculated plugs were used to inoculate non-liquid products (B, C, E, F, and G). Plugs were gently pushed into the products to mimic incidental contamination by insertion of a cooking or serving utensil in a food service or home setting. The foil or wax paper covering 4-oz sticks of Products F and 16-oz blocks of Product G, respectively, were removed using a sterile scalpel. The plugs were inserted ca. 2 cm apart into 16-oz tubs of Products B, C, and E, 4-oz sticks of Product F, or 16-oz blocks of Product G as gently as possible to prevent disturbance of the emulsion. Seven plugs were inserted into each product packaged in 16-oz tubs. Five plugs were inserted into each stick of Product F and seven plugs were inserted into each block of the food service butter and margarine blend (Product G). Three inoculated sticks of Product F were then placed in shallow

plastic trays (23.5 long x 13.5 wide x 5 cm deep) and three inoculated blocks of Product G were placed in deep plastic trays (24.5 x 24.5 x 11 cm) and sealed. All other inoculated products were sealed by applying lids and stored in the containers in which they were commercially packaged. Products were incubated at 4.4, 10, or 21°C. Inoculated plugs placed individually in stomacher 80 bags (Seward Medical Ltd., London, U.K.) heat-sealed and stored at 4.4, 10, and 21°C to determine survival characteristics of pathogens on plugs.

Microbiological analyses. Populations in single-serotype or single-strain cultures and in the mixed-serotype or mixed-strain suspensions were determined by serially diluting each cell suspensions in sterile 0.1% peptone and surface plating (0.1 ml) in duplicate on HE and tryptic soy agar (TSA) (BD Diagnostic Systems) (*Salmonella),* SMAC and TSA (*E. coli* O157:H7), and MOX and tryptose phosphate agar (TPA) (BD Diagnostic Systems) (*L. monocytogenes*). Plates were incubated at 37°C for 24 h for *Salmonella* and *E. coli* O157:H7 and 48 h for *L. monocytogenes* before colonies were counted.

Inoculated non-liquid products (Products B, C, E, F, and G) were analyzed for numbers (CFU/plug plus 4 g of product surrounding the plug) and presence (by enrichment) of each pathogen after storage for 0 (within 30 min of inoculation), 3, 7, 14, 21, 28, 42, 63, 78, and 94 days at 4.4, 10, and 21°C. Samples of inoculated liquid (spray or squeeze) products (Products A and D) were analyzed for populations (CFU/ml or CFU/g, respectively) and presence (by enrichment) of each pathogen after storage for 0 (within 30 min of inoculation), 1, 3, 5, 7, 10, 14, 21, 28, 42, 63, 78, and 94 days at 4.4, 10, and 21°C. Products stored at a given temperature in which a pathogen was not detected at two or more consecutive sampling times were not subjected to further analysis.

At each sampling time, the inoculated plug and 4 g of Products B, C, E, F, and G immediately surrounding the plug were removed using a sterile wooden splint. Care was

taken to remove from the stick, block, or tub all of the product in immediate contact with the plug. The plug and 4-g sample were placed in a stomacher 80 bag. The splint was placed inside the bag and product was removed by grasping the splint with two fingers on the outside of the bag and sliding the fingers downward.

Liquid products (A and D) were shaken in containers for 30 sec before removal of the samples. A 5-ml pipette was warmed by drawing in sterile water (40°C) and discharging before drawing in samples of Product A. Samples of Product A (3.5 ml) and Product D (4.0 g) were placed in stomacher 80 bags. Samples were placed in a water bath at 40°C for 5 min. Separation of the fat and serum fractions was minimal. Forty milliliters of warm (40°C) lactose broth (Difco), modified TSB (Padhye and Doyle, 1992), or *Listeria* enrichment broth (Difco) was combined with samples inoculated with *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*, respectively, and the mixtures were stomached at high speed for 1 min. A warm (40°C) pipette was used to withdraw samples of stomachate for direct plating on enumeration media or to serially dilute in sterile 0.1% peptone. Samples (avoiding the fat layer) of the stomachate were surface plated (0.25 ml in quadruplicate and 0.1 ml in duplicate) on non-selective media (TSA for *Salmonella* and *E. coli* O157:H7, and TPA for *L. monocytogenes*) and on the appropriate selective media (HE agar, SMAC agar, and MOX agar, respectively). Samples serially diluted in 0.1% peptone were also surface plated (0.1 ml in duplicate) on non-selective and selective media. Plates were incubated at 37°C for 24 h (*Salmonella* and *E. coli* O157:H7) or 48 h (*L. monocytogenes*) before counting presumptive colonies. Colonies were subjected to confirmation tests as described above. Stomachates were also incubated at 37°C for 24 h (*Salmonella* and *E. coli* O157:H7) or 48 h (*L. monocytogenes*), then streaked, respectively, on HE agar, SMAC agar, and MOX agar. Samples enriched in lactose broth (1 ml) were inoculated into 10 ml of selenite cystine broth (Difco), incubated for 24 h at 37°C, and streaked on HE agar. After incubating plates for 24 h at 37°C, presumptive colonies of *Salmonella* were confirmed as described

above. Presumptive colonies of *E. coli* O157:H7 and *L. monocytogenes* formed on SMAC agar and MOX agar, respectively, were also subjected to confirmation tests.

Populations of pathogens on inoculated plugs not inserted into test products were determined. Each plug was placed in 40 ml of warm (40°C) enrichment broth (lactose broth, modified TSB, or *Listeria* enrichment broth for plugs inoculated with *Salmonella, E. coli* O157:H7, and *L. monocytogenes,* respectively) and stomached at high speed for 1 min. Undiluted samples of broth (0.25 ml in quadruplicate and 0.1 ml in duplicate) and samples (0.1 ml in duplicate) serially diluted in sterile 0.1% peptone were surface plated on non-selective media (TSA for *Salmonella* and *E. coli* O157:H7, and TPA for *L. monocytogenes*) and on appropriate selective media. Plates were incubated for 24 h at 37°C (*Salmonella* and *E. coli* O157:H7) or 48 h (*L. monocytogenes*) before presumptive colonies were counted. Colonies were confirmed as described above.

Statistical analysis. All experiments were replicated three times. Trials for each pathogen were conducted in a randomized complete block using a full-factorial design. Analysis of variance was conducted (PROC GLM) with mean separation using Fisher's LSD. The full model was analyzed, but due to main effect interactions, the model was reduced.

RESULTS AND DISCUSSION

Table 2.2 lists the distribution of size (diameter, μm) of water droplets in dairy and non-dairy yellow fat spreads and toppings used in the study. The majority of water droplets in Products C (97.5%), D (62.1%), and F (99.8%) were ≤ 5 μm diameter. Thirty percent of the water droplets in product D were between 5 and 10 μm, indicating a courser emulsion compared to the solid products. Nearly 8% of the water droplets in Product D were 10 - 50 μm in diameter, theoretically providing better conditions for bacterial growth (Delamarre and Batt, 1999).

The proximate composition of products used in the study is listed in Table 2.3. Products ranged in fat content from 29.6 (Product C) to 81.6 % (Product F). Less fat can adversely

Table 2.2. Water droplet size distribution of test products[a]

Product code[b]	Percentage of water droplets with diameter (μm)				
	<1	1 to 5	5 to 10	10 to 50	>50
B	ND[c]	ND	ND	ND	ND
C	4.74	92.77	2.47	0.02	0
D	1.19	60.90	30.01	7.90	0
E	ND	ND	ND	ND	ND
F	12.50	87.27	0.23	0	0
G	ND	ND	ND	ND	ND

[a]Data were provided by the manufactures of test products

[b] B = Country Crock Shedd Spread; C = Blue Bonnet Light; D= Parkay Squeeze; F = Fleischmann's stick; and G = Foodservice butter and margarine blend

[c]ND = Not Determined

Table 2.3. Compositional analysis of test products[a]

Product		Moisture	Fat	Protein	Ash	Salt	Sodium benzoate	Potassium Sorbate		Titratable[e]
Code[b]	Lot[c]	(%)	(%)	(%)	(%)	(%)	(ppm)	(ppm)	pH	acidity
A	1	55.76±0.09	41.22±0.18	0.32	3.75	4.12	ND[d]	1155	3.66	0.110
	2	55.54 ± 0.01	40.67 ± 0.11	0.31	3.32	3.84	ND	1005	3.82	0.100
B	1	49.65 ± 0.42	48.62 ± 0.16	0.20	1.62	2.07	ND	957	4.85	0.050
	2	49.96 ± 0.07	48.83 ± 0.05	0.21	1.28	1.84	ND	961	4.89	0.050
C	1	64.65 ± 0.05	29.62 ± 0.05	<.10	1.90	1.67	590	540	5.00	0.026
	2	61.85 ± 0.05	30.84 ± 0.05	<.10	1.70	1.68	590	540	5.10	0.022
D	1	37.40 ± 0.05	62.60 ± 0.05	<.10	2.10	2.06	470	400	5.20	0.009
	2	37.65 ± 0.05	62.30 ± 0.05	<.10	2.00	1.97	470	400	5.40	0.009
E	1	38.00 ± 0.29	61.22 ± 0.07	0.31	1.17	1.49	ND	745	5.01	0.080
	2	38.03 ± 0.11	60.30 ± 0.17	0.29	0.66	1.30	ND	1225	4.82	0.100
F	1	16.42 ± 0.05	80.88 ± 0.05	<.10	2.20	2.00	470	< 20	5.60	0.027
	2	15.88 ± 0.05	81.58 ± 0.05	<.10	2.20	2.00	470	< 20	5.60	0.029
G	1	22.98 ± 0.13	77.09 ± 0.31	0.25	1.65	1.88	1325	ND	6.66	0.020

[a]Data were provided by manufacturers of test products

[b] A = I Can't Believe It's Not Butter Spray; B = Country Crock Shedd Spread; C = Blue Bonnet Light; D = Parkay Squeeze; E = Promise; F = Fleischmann's Stick; and G = Food service butter blend

[c] Lot 1 was used in *Salmonella* studies; lot 2 (codes A - F) and lot 1 (code G) were used in *E. coli* O157:H7 and *L. monocytogenes* studies

[c]None detected

[e]Titratable acidity for product A is based on lactic acid, product D is based on phosphoric acid, and products B, C, E, and G are based on citric acid.

affect retention of quality of products, necessitating changes in formulation, e.g., addition of water structuring agents, preservatives and/or decreasing pH, to maintain stability. Acidulants such as citric, lactic, or phosphoric acids were present in all reduced and low-fat products (A, B, C, D, and E) to aid in maintaining stability (Table 2.1). Products not containing acidulants, (Products F and G), had the highest pH among the seven test products. Products A, B, and E, contained more protein than other products (Table 2.3). Two of seven products (C and D) tested did not contain dairy ingredients such as whey, whey solids, or sweet cream buttermilk (Table 2.1). Preservatives such as sodium benzoate and/or potassium sorbate were used in all products at varying concentrations.

Populations of pathogens in inocula. Populations of $\log_{10}$ CFU/ml in each serotype or strain and populations present in mixed-serotype and -strain suspensions used to inoculate liquid products and plugs are presented in Table 2.4. Approximately equal populations of each serotype or strain were present in suspensions; however, suspensions used to inoculate plugs contained higher populations of all serotypes or strains compared to suspensions of respective pathogens used to inoculate liquid products.

Products inoculated with *Salmonella*. Results of microbiological examination of products inoculated with *Salmonella* are shown in Tables 2.5 - 2.7. Initial populations were 6.23 - 6.55 $\log_{10}$ CFU/3.5-ml or 4-g sample. Populations and presence of *Salmonella* in products stored at 4.4, 10, and 21°C, are shown in Tables 2.5, 2.6, and 2.7, respectively; data are presented graphically in Figures 2.1, 2.2, and 2.3. Higher populations of pathogens were recovered on TSA compared to HE agar. Differences of up to 0.74 $\log_{10}$ CFU/sample (Product F) inoculated with *Salmonella* were recovered on TSA and HE on day 0 of the study. As the storage time progressed, differences in counts of up to 2.38 $\log_{10}$ CFU/sample (Product D, 7 days at 4.4°C) occurred between the two media (Table 2.5). This observation is attributed to the inability of stressed cells to resuscitate and form colonies on HE agar. Cells may have become stressed as a result of

Table 2.4. Populations of microorganisms in inocula

Microorganism	Serotype or strain	Population (log_{10} CFU/ml)		
		Suspension[a]	Plug 1[b]	Plug 2[c]
Salmonella	Anatum	8.52	9.54	9.01
	Montevideo	8.65	9.77	8.99
	Typhimurium	8.69	9.83	9.08
	Muenchen	8.74	9.42	9.19
	Enteritidis	8.60	9.55	9.17
	Five-serotype mixture	8.64	9.43	8.98
E. coli O157:H7	932	8.53	9.50	
	SEA 13B88	8.82	9.73	
	E0018	8.70	9.60	
	994	8.63	9.61	
	F500	8.69	9.33	
	Five-strain mixture	8.49	9.14	
L. monocytogenes	G1091	8.56	8.32	
	F8369	7.91	8.99	
	310	8.78	9.30	
	Scott A	8.42	8.89	
	F8255	8.56	8.32	
	302	8.48	9.04	
	Five-strain mixture	8.59	9.75	

[a] Mixed-serotype or mixed-strain suspensions were used to inoculate Products A and D.

[b] Mixed-serotype or mixed-strain suspensions were used to inoculate plugs that were used to inoculate Products B, C, E, and F (*Salmonella*) and Products B, C, E, F, and G (*E.coli* O157:H7 and *L. monocytogenes*).

[c] A mixed-serotype suspension of *Salmonella* was used to inoculate plugs that were used to inoculate Product G.

Table 2.5. Populations of *Salmonella* recovered from products stored at 4.4°C

Storage time (days)	Recovery medium[c]	Product codes[a] and population (log_{10} CFU/sample)[b] recovered								
		P1	P2	A	B	C	D	E	F	G
		e[d]	e	e	e	e	e	e	e	e
0	TSA	ab 6.47 a	a 6.55 a	cd 5.68 a	d 5.37 a	cd 5.69 a	cb 6.05 a	d 5.56 a	d 5.32 a	ab 6.23 a
	HE	b 5.52 a	a 6.07 a	bc 5.19 a	cd 4.75 a	bc 5.17 a	b 5.58 a	cd 4.96 a	d 4.58 a	ab 5.63 a
1	TSA			b 4.83 b			a 6.51 a			
	HE			b 4.09 b			a 5.68 a			
3	TSA	c 4.57 b	bc 4.84 bc	d 3.25 c	c 4.64 a	a 5.70 a	ab 5.36 b	a 5.55 a	c 4.17 b	ab 5.51 b
	HE	b 3.69 b	ab 4.08 b	c 1.39 c	b 3.56 b	a 5.03 a	ab 4.29 b	a 4.81 a	b 3.48 b	a 4.97 ab
5	TSA			b 1.60 d 3			a 4.47 c			
	HE			b <1.60 d			a 2.46 c			
7	TSA	bc 4.10 b	a 5.29 ab	d 0.53 e 2	c 3.52 b	ab 4.84 a	c 3.78 d	a 5.18 ab	bc 4.20 b	a 5.32 bc
	HE	cd 3.29 bc	ab 4.66 b	f <1.60 d	d 2.60 b	abc 3.87 b	e 1.40 d	ab 4.47 ab	bc 3.65 ab	a 4.71 b
10	TSA			b <1.60 e 1			a 3.06 e			
	HE			a <1.60 d			a 1.07 d			
14	TSA	bc 3.80 b	bc 4.08 bc	f <1.60 e 0	cd 3.15 b	de 2.53 b	e 1.90 f 3	ab 4.74 bc	cd 3.46 c	a 5.33 bc
	HE	bc 2.67 c	b 2.98 c	e <1.60 d	cd 1.55 c	de 0.97 c	e <1.60 e	ab 3.72 bc	b 3.01 bc	a 4.44 b
21	TSA	b 2.40 c	b 3.38 c	c <1.60 e 0	c 0.53 c 3	b 2.52 b	c 1.07 g 3	a 4.85 bc	b 3.14 cd	a 4.83 cd
	HE	de 1.46 d	bc 2.69 c	f <1.60 d	f <1.60 d	ef 0.53 c	f <1.60 e	ab 3.63 c	cd 2.35 c	a 4.29 b
28	TSA	c 2.27 c	b 3.32 de		d <1.60 c 1	d <1.60 c 3	d <1.60 h 0	a 4.45 c	b 3.13 cd	a 4.39 de
	HE	b 1.83 d	b 2.21 c		c <1.60 d	c <1.60 c	c <1.60 e	a 3.19 cd	b 2.44 c	a 3.51 c

Table 2.5., continued

Storage time (days)	Recovery medium[c]	Product codes[a] and population (log_{10} CFU/sample)[b] recovered: P1		P2		A		B		C		D		E		F		G	
			e[d]		e		e		e		e		e		e		e		e
42	TSA	cd 1.50 c		de 0.77 ef				e <1.60 c	0	cd 1.51 b	2	e <1.60 h	0	a 4.42 c		bc 2.79 de		ab 4.06 e	
	HE	b <1.60 e		b 0.53 d				b <1.60 d		b <1.60 c		b <1.60 e		a 2.81 d		b 0.63 d		a 3.11 c	
63	TSA	d <1.60 d	3	c 1.80 de	3			d <1.60 c	0	d <1.60 c	0	d <1.60 h	0	ab 2.85 d		bc 2.41 ef		a 3.51 f	
	HE	c <1.60 e		c <1.60 d				c <1.60 d		c <1.60 c		c <1.60 e		b 1.17 e		bc 0.69 d		a 2.32 d	
78	TSA	d <1.60 d	3	cd 0.93 ef	3					d <1.60 c	1			ab 3.33 d		bc 2.04 f	3	a 3.39 f	
	HE	b <1.60 e		b <1.60 d						b <1.60 c				b 0.63 ef		b <1.60 d		a 1.93 d	
94	TSA	b <1.60 d	3	b <1.60 f	3					b <1.60 c	1			a 2.75 d	3	a 2.66 de	3	a 2.79 g	
	HE	a <1.60 e		a <1.60 d						a <1.60 c				a <1.60 f		a <1.60 d		a 0.53 e	

[a] P1 = Plug 1 used to inoculate products B,C,E and F; P2 = Plug 2 used to inoculate Product G; A = I Can't Believe It's Not Butter Spray; B = Country Crock Shedds Spread; C = Blue Bonnet Light; D =Parkay Squeeze; E = Promise; F = Fleischmann's stick; G = Foodservice butter and margarine blend

[b] Samples consisted of 3.5 ml of Product A, 4 g of Product D, 4 g of products B, C, E, F, or G plus the plug, or the plug (P). Within recovery medium, mean values in the same column that are not followed by the same letter are significantly different ($P \leq 0.05$). Mean values in the same row that are not preceded by the same letter are significantly different ($P \leq 0.05$).

[c] Tryptic soy agar (TSA) and Hektoen Enteric (HE) agar were used to recover *Salmonella*

[d] Number out of three replicate samples that were positive for *Salmonella*

FIGURE 2.1. Populations of *Salmonella* recovered on TSA from products stored at 4.4°C for up to 94 days.

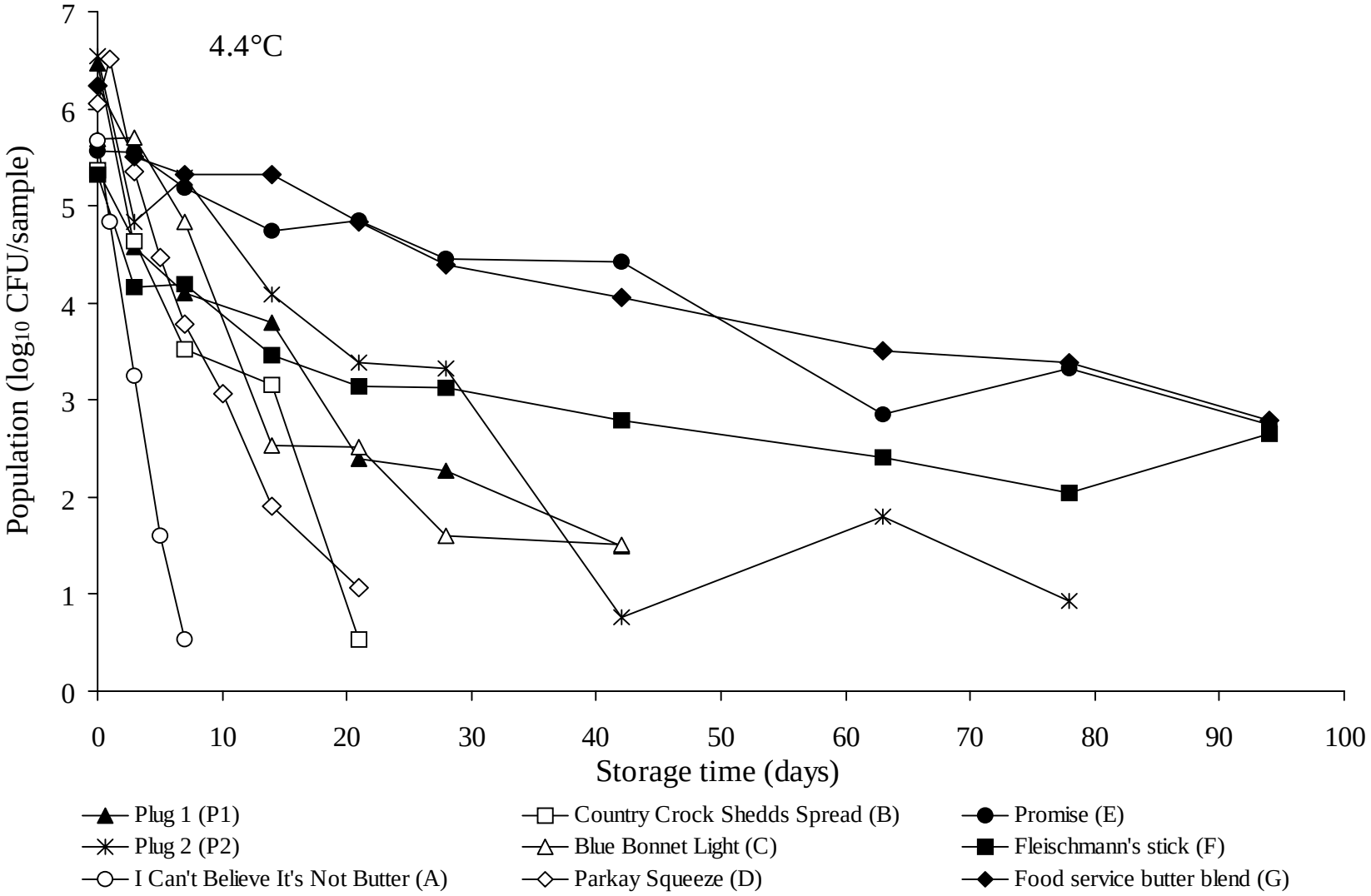

4.4°C
Population (log10 CFU/sample)
Storage time (days)
0
1
2
3
4
5
6
7
0
10
20
30
40
50
60
70
80
90
100
Plug 1 (P1)
Plug 2 (P2)
I Can't Believe It's Not Butter (A)
Country Crock Shedds Spread (B)
Blue Bonnet Light (C)
Parkay Squeeze (D)
Promise (E)
Fleischmann's stick (F)
Food service butter blend (G)

Table 2.6. Populations of *Salmonella* recovered from products stored at 10°C

Storage time (days)	Recovery medium[c]	Product codes[a] and population (log_{10} CFU/sample)[b] recovered								
		P1	P2	A	B	C	D	E	F	G
0	TSA	ab 6.47 a[d]	a 6.55 a	cd 5.68 a	d 5.37 a	cd 5.69 a	bc 6.05 a	d 5.56 a	d 5.32 a	ab 6.23 a
	HE	b 5.52 a	a 6.07 a	bc 5.19 a	cd 4.75 a	bc 5.17 a	b 5.58 a	cd 4.96 a	d 4.58 a	ab 5.63 a
1	TSA			a 4.84 b			a 5.95 a			
	HE			a 4.32 b			a 5.31 a			
3	TSA	cd 4.33 b	a 5.70 ab	e 3.39 c	bc 4.86 ab	bcd 4.74 ab	bc 4.84 b	ab 5.31 ab	de 4.07 b	ab 5.33 b
	HE	de 3.45 b	a 5.12 a	e 2.69 c	cd 3.90 ab	bcd 4.27 ab	d 3.63 b	abc 4.70 ab	d 3.57 b	ab 4.80 ab
5	TSA			b 0.63 d 3			a 4.26 bc			
	HE			b <1.60 d			a 2.79 bc			
7	TSA	bc 4.50 b	a 6.04 ab	e <1.60 e 3	bcd 4.16 b	bc 4.54 ab	d 3.46 cd	b 5.08 abc	cd 3.68 b	b 4.89 c
	HE	bc 3.75 b	a 5.45 a	e <1.60 d	cd 3.03 b	bcd 3.31 bc	d 2.20 c	b 4.28 ab	cd 2.94 bc	ab 4.38 bc
10	TSA			b <1.60 e 2			a 2.58 d 3			
	HE			b <1.60 d			a <1.60 d			
14	TSA	bc 4.37 b	a 5.90 ab	d <1.60 e 2	d 1.39 c 3	bc 3.82 bc	d 1.39 e 3	ab 4.79 bcd	c 3.25 b	ab 4.79 cd
	HE	bc 3.30 b	a 5.32 a	d <1.60 d	d 0.53 c	c 2.51 cd	d <1.60 d	bc 3.63 bc	c 2.49 c	ab 4.30 bc
21	TSA	ab 4.55 b	a 5.43 b	d <1.60 e 0	d <1.60 d 0	c 2.88 cd	d 0.53 ef 1	b 4.39 cde	c 2.41 c	b 4.50 de
	HE	bc 3.51 b	a 4.87 a	e <1.60 d	e <1.60 c	d 1.27 de	e <1.60 d	c 2.83 cd	d 1.30 d	ab 4.02 bcd
28	TSA	ab 4.11 b	a 5.39 b	d <1.60 e 2	d <1.60 d 0	c 2.15 d 3	d <1.60 f 0	ab 4.20 de	bc 3.28 b	ab 4.48 de
	HE	bc 3.01 bc	a 4.78 a	e <1.60 d	e <1.60 c	de 0.73 e	e <1.60 d	cd 1.94 de	c 2.36 c	ab 3.86 cd

Table 2.6., continued

Storage time (days)	Recovery medium[c]	Product codes[a] and population (log_{10} CFU/sample)[b] recovered								
		P1	P2	A	B	C	D	E	F	G
42	TSA	b 4.47 b e[d]	a 6.00 ab e	e <1.60 e 2	e <1.60 d 1	e <1.60 e 2	e <1.60 f 0	c 3.85 e	d 2.31 c	b 4.28 e
	HE	b 3.37 b e	a 5.34 a e	d <1.60 d e	d <1.60 c e	d <1.60 e e	d <1.60 d e	c 1.07 ef e	cd 0.53 de e	b 3.36 de e
63	TSA	bc 3.10 c e	a 5.86 ab e	e <1.60 e 0	e <1.60 d 0	e <1.60 e 0	e <1.60 f 0	c 2.94 f e	d 1.90 c 3	b 3.89 f e
	HE	bc 1.76 cd e	a 5.19 a e	d <1.60 d e	d <1.60 c e	d <1.60 e e	d <1.60 d e	cd 0.63 f e	d <1.60 e e	b 2.65 ef e
78	TSA	bc 2.80 c e	a 5.81 ab e	d <1.60 e 0	d <1.60 d 0	d <1.60 e 1		c 2.19 fg 3	d 0.53 d 3	b 3.23 g e
	HE	b 1.27 d e	a 4.73 ab e	c <1.60 d e	c <1.60 c e	c <1.60 e e		c <1.60 fg e	c <1.60 e e	b 1.39 g e
94	TSA	ab 3.00 c e	a 3.54 c e	d <1.60 e 0	d <1.60 d 0	d <1.60 e 0		bc 2.02 g 3	cd 1.07 d 3	a 3.47 g e
	HE	ab 1.48 d e	a 2.36 b e	b <1.60 d e	b <1.60 c e	b <1.60 e e		b <1.60 f e	b <1.60 e e	a 2.04 fg e

[a] P1 = Plug 1 used to inoculate products B, C, E, and F; P2 = Plug 2 used to inoculate Product G; A = I Can't Believe It's Not Butter Spray; B = Country Crock Shedd Spread; C = Blue Bonnet Light; D = Parkay Squeeze; E = Promise; F = Fleischmann's stick; G = Food service butter and margarine blend

[b] Samples consisted of 3.5 ml of product A, 4 g of Product D, 4 g of products B, C, E, F, or G plus the plug, or the plug (P). Within recovery medium, mean values in the same column that are not followed by the same letter are significantly different ($P \leq 0.05$). Mean values in the same row that are not preceded by the same letter are significantly different ($P \leq 0.05$).

[c] Tryptic soy agar (TSA) and Hektoen Enteric (HE) agar were used to recover *Salmonella*

[d] Number out of three replicate samples that were positive for *Salmonella*

FIGURE 2.2. Populations of *Salmonella* recovered on TSA from products stored at 10°C for up to 94 days.

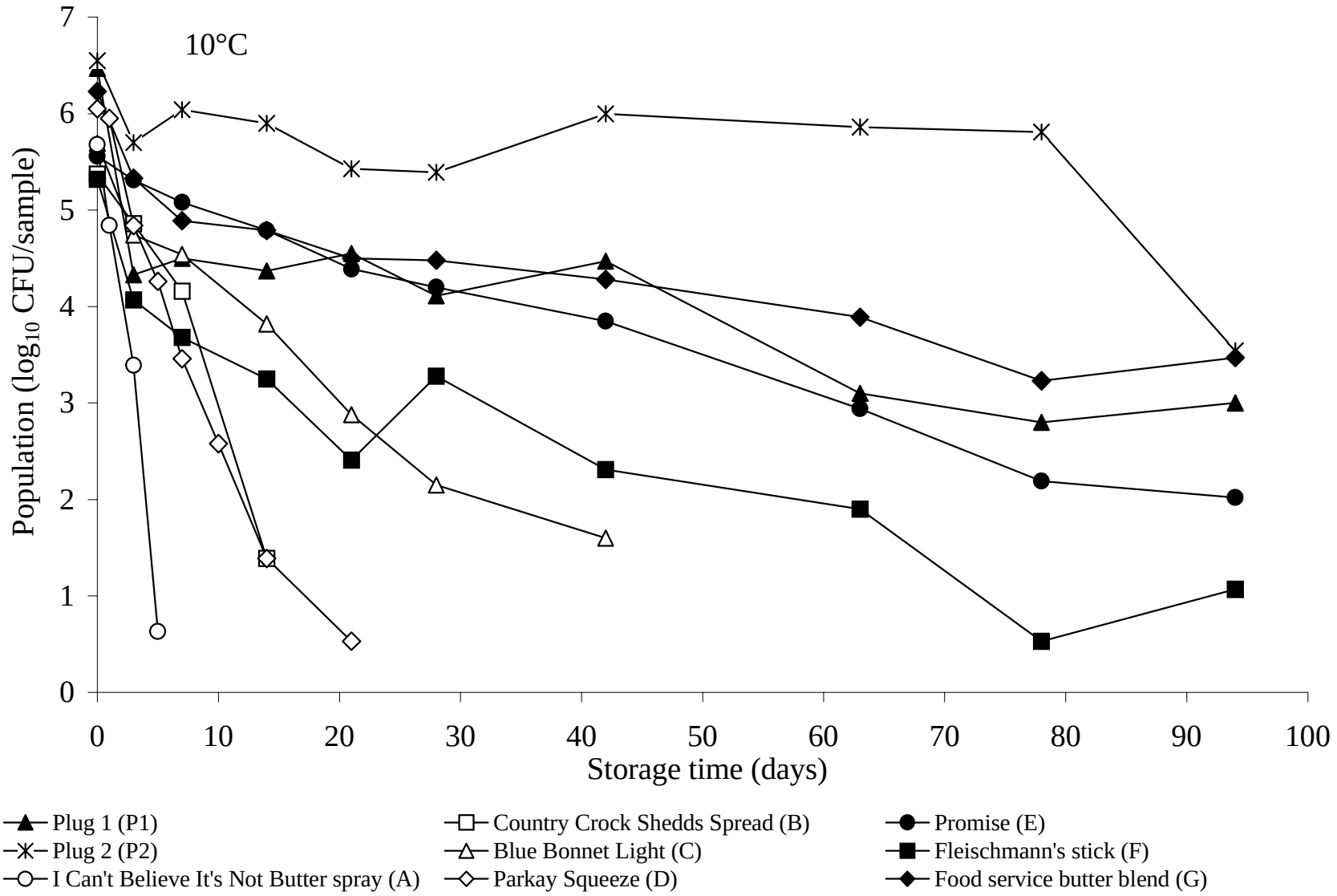

10°C
Population (log10 CFU/sample)
Storage time (days)
0
1
2
3
4
5
6
7
0
10
20
30
40
50
60
70
80
90
100
Plug 1 (P1)
Plug 2 (P2)
I Can't Believe It's Not Butter spray (A)
Country Crock Shedds Spread (B)
Blue Bonnet Light (C)
Parkay Squeeze (D)
Promise (E)
Fleischmann's stick (F)
Food service butter blend (G)

Table 2.7. Populations of *Salmonella* recovered from products stored at 21°C

Storage time (days)	Recovery medium[c]	Product codes[a] and population (log_{10} CFU/sample)[b]								
		P1	P2	A	B	C	D	E	F	G
0	TSA	ab 6.47 a e[d]	a 6.55 a e	cd 5.68 a e	d 5.37 a e	cd 5.69 a e	bc 6.05 a e	d 5.56 a e	d 5.32 a e	ab 6.23 a e
	HE	b 5.52 a	a 6.07 a	bc 5.19 a	cd 4.75 a	bc 5.17 a	b 5.58 a	cd 4.96 a	d 4.58 a	ab 5.63 a
1	TSA			b 4.26 b			a 5.81 a			
	HE			b 3.65 b			a 5.06 a			
3	TSA	a 6.13 ab	a 5.89 a	c 2.54 c	c 2.53 b 3	a 5.47 a	bc 3.40 b	ab 4.82 abc	bc 3.37 b	a 5.15 b
	HE	a 5.16 ab	a 5.09 b	c 1.17 c	c 0.95 b	a 4.71 a	b 3.16 b	ab 4.05 b	b 2.83 b	a 4.81 b
5	TSA			a 0.53 d 1			a 3.18 b			
	HE			a <1.60 d			a 1.71 c			
7	TSA	a 5.63 bc	a 5.83 a	d <1.60 d 0	d <1.60 c 0	b 3.79 b	c 2.08 c 3	a 5.04 ab	b 3.31 b	a 5.21 b
	HE	a 4.74 ab	a 5.07 b	e <1.60 d	e <1.60 b	cd 1.95 b	de 0.53 d	ab 4.00 b	bc 2.48 b	a 4.81 b
10	TSA			a <1.60 d 0			a 0.63 d 3			
	HE			a <1.60 d			b <1.60 d			
14	TSA	ab 4.93 cd	a 5.63 ab	e <1.60 d 0	e <1.60 c 0	d 1.63 c 3	e 0.53 d 1	b 4.20 bc	c 2.87 bc	ab 4.95 b
	HE	a 3.89 b	a 4.59 ab	d <1.60 d	d <1.60 b	cd 0.53 c	d <1.60 d	b 2.63 c	c 1.17 c	a 4.36 bc
21	TSA	a 5.27 bcd	ab 4.70 bc		d <1.60 c 0	d 0.87 cd 1	d <1.60 d 0	b 3.99 c	c 2.10 cd 3	ab 4.48 c
	HE	a 4.39 ab	a 3.58 c		c <1.60 b	c <1.60 c	c <1.60 d	b 1.67 d	c <1.60 d	a 3.83 cd
28	TSA	a 4.41 d	a 4.12 c			c <1.60 d 0	c <1.60 d 0	b 2.03 d 2	b 1.43 d 3	a 4.17 d
	HE	a 3.98 b	a 3.04 c			b <1.60 c	b <1.60 d	b <1.60 e	b <1.60 d	a 3.33 de

Table 2.7., continued

Storage time (days)	Recovery medium[c]	Product codes[a] and population (log_{10} CFU/sample)[b] P1		P2		A		B		C		D		E		F		G	
			e[d]		e		e		e		e		e		e		e		e
42	TSA	b	2.32 e	c	0.63 d					c	<1.60 d	c	<1.60 d	c	<1.60 e	c	<1.60 e	a	3.93 d
	HE	b	1.23 c	c	<1.60 d					c	<1.60 c	c	<1.60 d	c	<1.60 e	c	<1.60 d	a	3.05 e
63	TSA	b	1.70 e	c	<1.60 d					c	<1.60 d	c	<1.60 d	c	<1.60 e	c	<1.60 e	a	3.20 e
	HE	b	<1.60 c	b	<1.60 d					b	<1.60 c	b	<1.60 d	b	<1.60 e	b	<1.60 d	a	2.04 f
78	TSA	b	1.27 e	c	<1.60 d					c	<1.60 d	c	<1.60 d	c	<1.60 e	c	<1.60 e	a	2.48 f
	HE	a	<1.60 c	a	<1.60 d					a	<1.60 c	a	<1.60 d	a	<1.60 e	a	<1.60 d	a	0.53 g
94	TSA	a	1.83 e	b	<1.60 d													b	<1.60 g
	HE	a	1.23 c	b	<1.60 d													b	<1.60 g

Storage time (days)	Recovery medium[c]	G
		e[d]
42	TSA	3
	HE	
63	TSA	0
	HE	
78	TSA	0
	HE	
94	TSA	3
	HE	

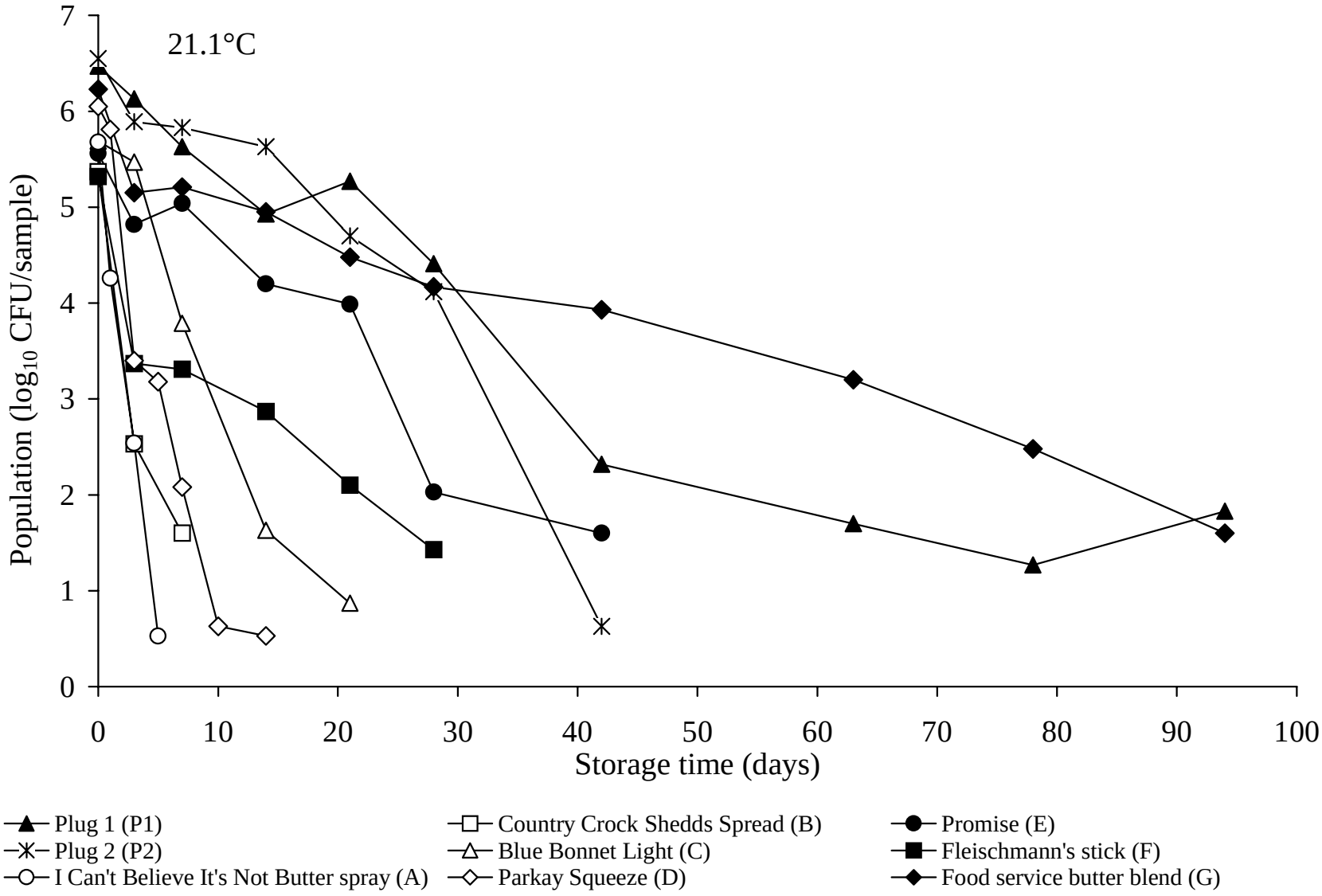
[a] P1 = Plug 1 used to inoculate Products B, C, E, and F; P2 = Plug 2 used to incoulate Product G; A = I Can't Believe It's Not Butter Spray; B = Country Crock Shedd Spread; C = Blue Bonnet Light; D = Parkay Squeeze; E = Promise;F = Fleischmann's stick; G = Food service butter and margarine blend

[b] Samples consisted of 3.5 ml of Product A, 4 g of Product D, 4 g of products B, C, E, F, or G plus the plug, or the plug (P). Within recovery medium, mean values in the same column that are not followed by the same letter are significantly different ($P \leq 0.05$). Mean values in the same row that are not preceded by the same letter are significantly different ($P \leq 0.05$).

[c] Tryptic soy agar (TSA) and Hektoen Enteric (HE) agar were used to recover *Salmonella*

[d] Number out of three replicate samples that were positive for *Salmonella*

FIGURE 2.3. Populations of *Salmonella* recovered on TSA from products stored at 21°C for up to 94 days.


21.1°C
Population (log10 CFU/sample)
Storage time (days)
0
1
2
3
4
5
6
7
0
10
20
30
40
50
60
70
80
90
100
Plug 1 (P1)
Plug 2 (P2)
I Can't Believe It's Not Butter spray (A)
Country Crock Shedds Spread (B)
Blue Bonnet Light (C)
Parkay Squeeze (D)
Promise (E)
Fleischmann's stick (F)
Food service butter blend (G)

products, rendering them unable to resuscitate and grow on HE agar. Non-selective TSA appears to have supported repair of a portion of the stressed cells.

Viable cells were detected on plugs not used to inoculate products, either by direct plating or by enrichment, throughout the 94-day storage period, regardless of storage temperature. *Salmonella* survived longer on control plugs stored at 10°C than on plugs stored at 4.4°C or 21°C.

The lowest populations of *Salmonella* shown in Figures 2.1-2.3 represent the lowest number (<1.6 $\log_{10}$ CFU/4-g or 3.5-ml sample) of colonies detected on TSA. Points were not plotted after reaching the lower limit of detection by direct plating, even if the pathogen may have been detected by enrichment at a later sampling time. Growth was not observed in any of the products stored at 4.4, 10, or 21°C for up to 94 days.

Whether stored at 4.4, 10, or 21°C, death of *Salmonella* occurred most rapidly in Product A. When stored at 4.4 or 21°C, cells were not detected by enrichment of Product A stored for 14 and 7 days or longer, respectively. Enrichment of samples stored at 10°C revealed that the pathogen remained viable in Product A for 42 days. Rapid death is attributed to the higher salt content (4.12%) and lower pH (3.66) of Product A compared to other products. A pH of 4.05 has been reported to be the minimum for growth of *Salmonella* in broth acidified with hydrochloric or citric acid (Jay, 2000). The combination of salt, low pH, and nutrient-limiting conditions likely contributed to the lethality of *Salmonella* in test products.

Populations of *Salmonella* in Products D, B, and C, in that order, had the next most rapid rate of decline. The pathogen was not detected in Product D stored for 28 days or longer at 4.4 or 10°C. When stored at 21°C, the pathogen was detected by enrichment of 1 of 3 samples of Product D stored for 42 days. Rates of decline in Products B and C stored at 21°C were more rapid compared to rates of decline at 4.4 or 10°C. *Salmonella* survived longer in Product C stored at 4.4°C (94 days) than at 10°C (78 days), while cells in Product B survived longest (42 days) when held at 10°C. Populations in Product B

decreased to undetectable levels by direct plating more rapidly than in Product C, regardless of storage temperature.

Products G, E, and F, in that order, retained the highest populations of *Salmonella* in products stored at 10 and 21°C. This may be due to the higher fat content in these products (Table 2.3). The initial population (6.55 $\log_{10}$ CFU/sample) in Product G was reduced to 3.47 $\log_{10}$ CFU/sample during storage at 10°C for 94 days. At a given storage time, the highest population was observed in Product G, regardless of storage temperature. This is attributed, in part, to the composition of Product G (80% fat, of which 33% is from butter). In a study by Sims et al. (1970), butter readily supported growth of *Salmonella* at 24°C. Neither refrigeration nor freezing eliminates the pathogen from the butter (El-Gazzar and Marth, 1992).

Populations of *Salmonella* in Products E and F stored for 94 days at 4.4°C were higher than those in the same products stored at 10 or 21°C. Populations of 2.75 and 2.66 $\log_{10}$ CFU/sample (Products E and F, respectively, at 4.4°C) and 2.02 and 1.07 $\log_{10}$ CFU/sample (Products E and F, respectively, at 10°C) were detected on day 94. Direct plating and enrichment failed to detect the pathogen in these products stored for 63 days or longer at 21°C. Our findings follow the same trends reported by Jensen et al. (1983), who reported that the number of bacteria in salted butter is reduced during storage, irrespective of storage temperature or pH of the serum.

Products inoculated with *E. coli* O157:H7. Populations of *E. coli* O157:H7 recovered from inoculated margarine and yellow fat products are listed in Tables 2.8, 2.9, and 2.10 and presented graphically in Figures 2.4, 2.5, and 2.6, respectively. Initial populations of *E. coli* O157:H7 were 5.36 – 6.14 $\log_{10}$ CFU/3.5-ml or 4-g sample. Higher populations were recovered on TSA than on SMAC, regardless of the test product, storage temperature, and storage time. Higher populations of up to 3.56 $\log_{10}$ CFU/sample (Product D, 14 days at 4.4°C) (Table 2.8) were recovered on TSA compared to SMAC.

Table 2.8. Populations of *Escherichia coli* O157:H7 recovered from products stored at 4.4°C

Storage time (days)	Recovery medium[c]	Product codes[a] and population (log_{10} CFU/sample)[b]							
		P	A	B	C	D	E	F	G
0	TSA	b 5.36 a e[d]	a 6.36 a e	de 3.63 a e	bc 4.65 a e	a 6.26 ab e	c 4.46 a e	e 3.03 a e	cd 4.29 a e
	SMA	b 5.19 a e	a 6.21 a e	de 3.39 a e	bc 4.49 a e	a 6.20 a e	cd 4.16 a e	e 2.63 a e	cd 3.98 a e
1	TSA		a 6.16 b e			a 6.36 a e			
	SMA		a 6.06 a e			a 6.26 a e			
3	TSA	c 2.65 b e	a 5.83 c e	c 2.52 ab e	b 4.24 ab e	a 6.15 b e	c 2.86 b e	d 0.69 bc e	bc 3.26 ab e
	SMA	c 2.18 b e	a 5.51 b e	c 2.08 b e	b 4.02 ab e	a 5.95 a e	c 2.53 b e	d 0.53 b e	c 2.86 b e
5	TSA		b 5.36 d e			a 5.97 c e			
	SMA		b 4.67 c e			a 5.72 a e			
7	TSA	d 0.53 c 3	ab 4.26 e e	bc 2.64 ab e	b 3.76 bc e	a 5.71 d e	bc 2.98 b e	cd 1.58 b 3	cd 1.90 bcd 3 e
	SMA	e <1.60 c e	b 3.17 d e	cd 1.32 bc e	b 3.10 b e	a 5.32 ab e	bc 2.50 b e	de 0.53 b e	bc 2.16 b e
10	TSA		b <1.60 f 2 e			a 5.29 e e			
	SMA		b <1.60 e e			a 4.51 b e			
14	TSA	d <1.60 c 0	d <1.60 f 0 e	bc 2.20 b e	b 2.62 d e	a 4.79 f e	bcd 1.53 cd e	cd 0.73 bc 1	bc 2.40 bc 3 e
	SMA	a <1.60 c e	a <1.60 e e	a 0.53 cd e	a 1.27 c e	a 1.23 c e	a <1.60 c e	a <1.60 b e	a 0.69 c e
21	TSA	e <1.60 c 0	e <1.60 f 0 e	e <1.60 c 0 e	ab 3.44 c e	a 3.91 g e	bc 2.40 bc e	de 1.17 bc 2	cd 1.33 cde 2 e
	SMA	c <1.60 c e	c <1.60 e e	c <1.60 d e	b 1.17 c e	a 2.39 c e	c <1.60 c e	c <1.60 b e	c <1.60 c e
28	TSA	c <1.60 c 0	c <1.60 f 0 e	c <1.60 c 0 e	c <1.60 e 1 e	a 2.91 h e	b 1.07 de 2 e	c <1.60 c 0	b 1.27 cde 2 e
	SMA	b <1.60 c e	b <1.60 e e	b <1.60 d e	b <1.60 d e	a 1.33 c e	b <1.60 c e	b <1.60 b e	b <1.60 c e

Table 2.8., continued

Storage time (days)	Recovery medium[c]	Product codes[a] and population (log_{10} CFU/sample)[b]							
		P	A	B	C	D	E	F	G
		e[d]	e	e	e	e	e	e	e
42	TSA	b <1.60 c 0		b <1.60 c 0	b <1.60 e 0	b <1.60 i 1	a 1.07 de 0	b <1.60 c 0	ab 0.53 de 0
	SMA	a <1.60 c		a <1.60 d	b <1.60 d	a <1.60 d	a <1.60 c	a <1.60 b	a <1.60 c
63	TSA	a <1.60 c 0		a <1.60 c 0	a <1.60 e 0	a <1.60 i 1	a <1.60 e 0	a <1.60 c 0	a <1.60 e 0
	SMA	a <1.60 c		a <1.60 d	a <1.60 d	a <1.60 d	a <1.60 c	a <1.60 b	a <1.60 c
78	TSA	a <1.60 c 0			a <1.60 e 0	a <1.60 i 0	a <1.60 e 0	a <1.60 c 0	a <1.60 e 0
	SMA	a <1.60 c			a <1.60 d	a <1.60 d	a <1.60 c	a <1.60 b	a <1.60 c
94	TSA	a <1.60 c 0			a <1.60 e 0	a <1.60 i 0	a <1.60 e 0		a <1.60 e 0
	SMA	a <1.60 c			a <1.60 d	a <1.60 d	a <1.60 c		a <1.60 c

[a] P = Plug used to inoculate Products B, C, E, F, and G; A = I Can't Believe It's Not Butter Spray; B = Country Crock Shedd Spread; C = Blue Bonnet Light; D = Parkay Squeeze; E = Promise;F = Fleischmann's stick; G = Food service butter and margarine blend

[b] Samples consisted of 3.5 ml of Product A, 4 g of Product D, 4 g of Products B, C, E, F, or G plus the plug, or the plug (P). Within recovery medium, mean values in the same column that are not followed by the same letter are significantly different ($P \leq 0.05$). Mean values in the same row that are not preceded by the same letter are significantly different ($P \leq 0.05$).

[c] Tryptic soy agar (TSA) and Sorbitol MacConkey agar (SMA) were used to recover *E. coli* O157:H7.

[d] Number out of three replicate samples that were positive for *E. coli* O157:H7.

FIGURE 2.4. Populations of *E. coli* O157:H7 recovered on TSA from products stored at 4.4°C for up to 94 days.

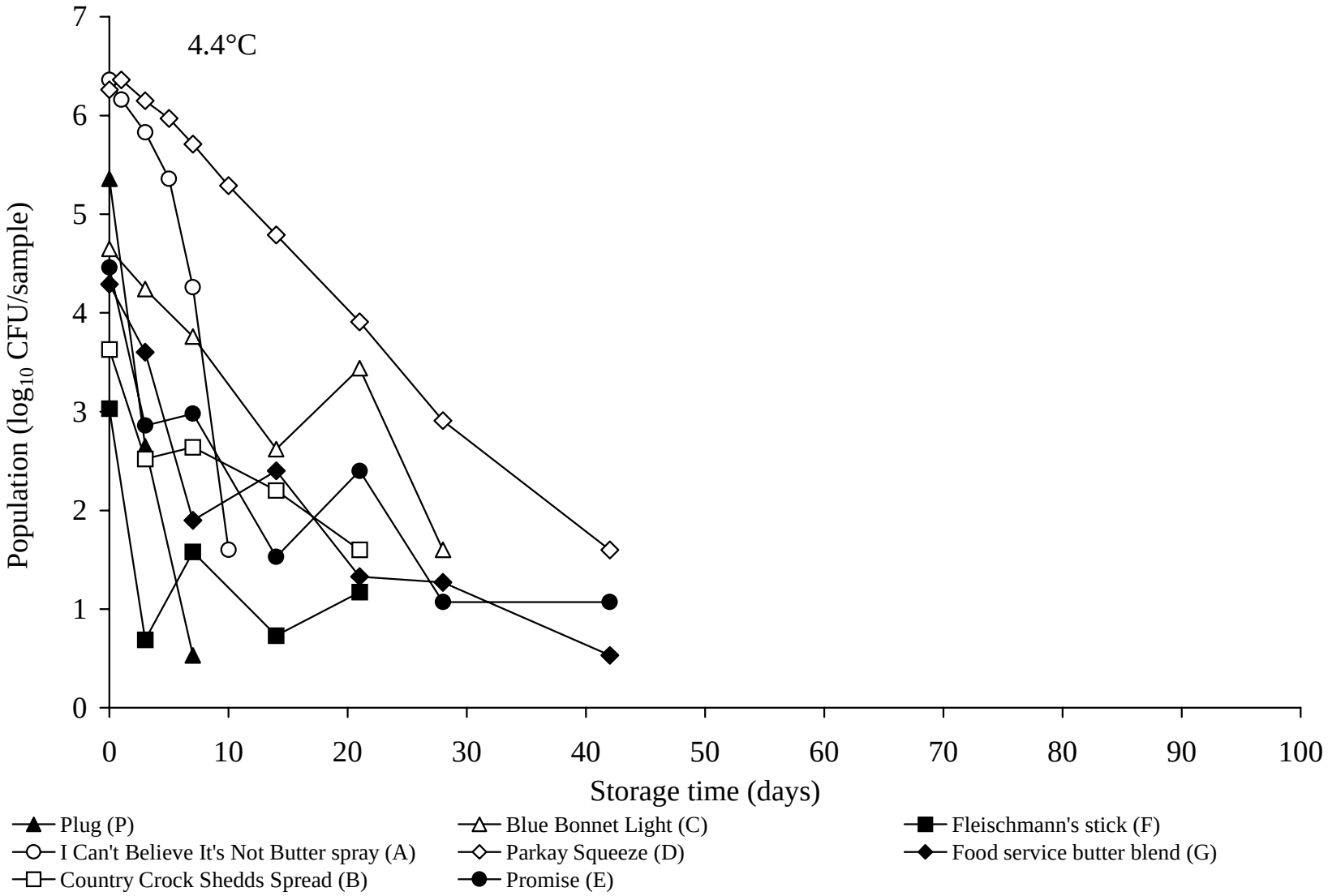

4.4°C
Population (log10 CFU/sample)
Storage time (days)
0
1
2
3
4
5
6
7
0
10
20
30
40
50
60
70
80
90
100
Plug (P)
Blue Bonnet Light (C)
Fleischmann's stick (F)
I Can't Believe It's Not Butter spray (A)
Parkay Squeeze (D)
Food service butter blend (G)
Country Crock Shedds Spread (B)
Promise (E)

Table 2.9. Populations of *Escherichia coli* O157:H7 recovered from products stored at 10°C

Storage time (days)	Recovery medium[c]	Product codes[a] and populations (log_{10} CFU/sample)[b]							
		P	A	B	C	D	E	F	G
0	TSA	b 5.36 a	a 6.36 a	de 3.63 a	bc 4.65 a	a 6.26 a	c 4.46 a	e 3.03 a	cd 4.29 a
	SMA	b 5.19 a	a 6.21 a	de 3.39 a	bc 4.49 a	a 6.2 a	cd 4.16 a	e 2.63 a	cd 3.98 a
1	TSA		a 6.18 a			a 6.39 a			
	SMA		a 6.06 a			6.21 a			
3	TSA	b 4.58 ab	a 5.59 b	bc 4.27 a	b 4.54 ab	a 5.86 ab	cd 3.35 ab	e 0.79 b	d 3.14 b
	SMA	bc 4.84 ab	ab 5.19 b	dc 3.34 a	bc 4.07 a	a 5.63 ab	de 2.58 b	f <1.60 b	e 1.97 b
5	TSA		b 4.48 c			a 5.63 abc			
	SMA		b 3.69 c			5.32 b			
7	TSA	ab 4.26 abc	cd 2.62 d	d 1.88 b	ab 4.34 ab	a 5.26 bc	bc 3.78 ab	e <1.60 b	cd 2.85 bc
	SMA	ab 3.95 bc	de 0.53 d	de 1.17 b	ab 3.62 ab	a 4.23 c	bc 2.73 b	e <1.60 b	cd 1.46 b
10	TSA		b <1.60 e			a 4.95 cd			
	SMA		b <1.60 d			a 3.75 cd			
14	TSA	a 3.34 cd	d <1.60 e	dc 1.27 b	ab 3.67 bc	a 4.19 d	ab 3.03 bc	d <1.60 b	bc 2.30 cd
	SMA	a 3.34 cd	c <1.60 d	c <1.60 c	ab 2.36 bc	ab 3.10 d	abc 1.59 c	c <1.60 b	bc 1.32 b
21	TSA	a 3.02 de	c <1.60 e	c <1.60 c	a 3.10 cd	a 3.27 e	b 2.02 cd	c <1.60 b	b 2.04 d
	SMA	a 2.55 d	b <1.60 d	b <1.60 c	a 1.62 cd	a 1.89 e	b <1.60 d	b <1.60 b	b <1.60 c
28	TSA	a 3.68 bcd	d <1.60 e	d <1.60 c	ab 2.62 d	c 1.30 f	bc 1.53 de	d <1.60 b	bc 1.90 d
	SMA	a 2.70 d	b <1.60 d	b <1.60 c	b 0.63 de	b 0.53 f	bc <1.60 d	b <1.60 b	b <1.60 c

Table 2.9., continued

Storage time (days)	Recovery medium[c]	Product codes[a] and populations (log_{10} CFU/sample)[b] P	A	B	C	D	E	F	G
42	TSA	a 3.82 bcd e[d]	b <1.60 c e	0	b <1.60 e e	0	b 0.63 fg e	1	b 0.53 ef e	0	b <1.60 b e	0	b 0.53 e e	0
	SMA	a 2.63 d	b <1.60 c		b <1.60 e		b <1.60 f		b <1.60 d		b <1.60 b		b <1.60 c	
63	TSA	a 2.16 ef e	1	b <1.60 c e	0	b <1.60 e e	0	b <1.60 g e	1	b <1.60 f e	0		b <1.60 e e	0
	SMA	a 0.53 ef	a <1.60 c		a <1.60 e		a <1.60 f		a <1.60 d				a <1.60 c	
78	TSA	a 1.47 f e	3		b <1.60 e e	0	b <1.60 g e	0	b <1.60 f e	0			b <1.60 e e	0
	SMA	a <1.60 e			a <1.60 e		a <1.60 f		a <1.60 d				a <1.60 c	
94	TSA	a <1.60 g e	0		a <1.60 e e	0	a <1.60 g e	0	a <1.60 f e	0			a <1.60 e e	0
	SMA	a <1.60 e			a <1.60 e		a <1.60 f		a <1.60 d				a <1.60 c	

[a] P = Plug used to inoculate Products B, C, E, F, and G; A = I Can't Believe It's Not Butter Spray; B = Country Crock Shedd Spread; C = Blue Bonnet Light; D = Parkay Squeeze; E = Promise; F = Fleischmann's stick; G = Food service butter and margarine blend

[b] Samples consisted of 3.5 ml of Product A, 4 g of Product D, 4 g of Products B, C, E, F, or G plus the plug, or the plug (P). Within recovery medium, mean values in the same column that are not followed by the same letter are significantly different ($P \leq 0.05$). Mean values in the same row that are not preceded by the same letter are significantly different ($P \leq 0.05$).

[c] Tryptic soy agar (TSA) and Sorbitol MacConkey agar (SMA) were used to recover *E. coli* O157:H7.

[d] Number out of three replicate samples that were positive for *E. coli* O157:H7.

FIGURE 2.5. Populations of *E. coli* O157:H7 recovered on TSA from products stored at 10°C for up to 94 days.

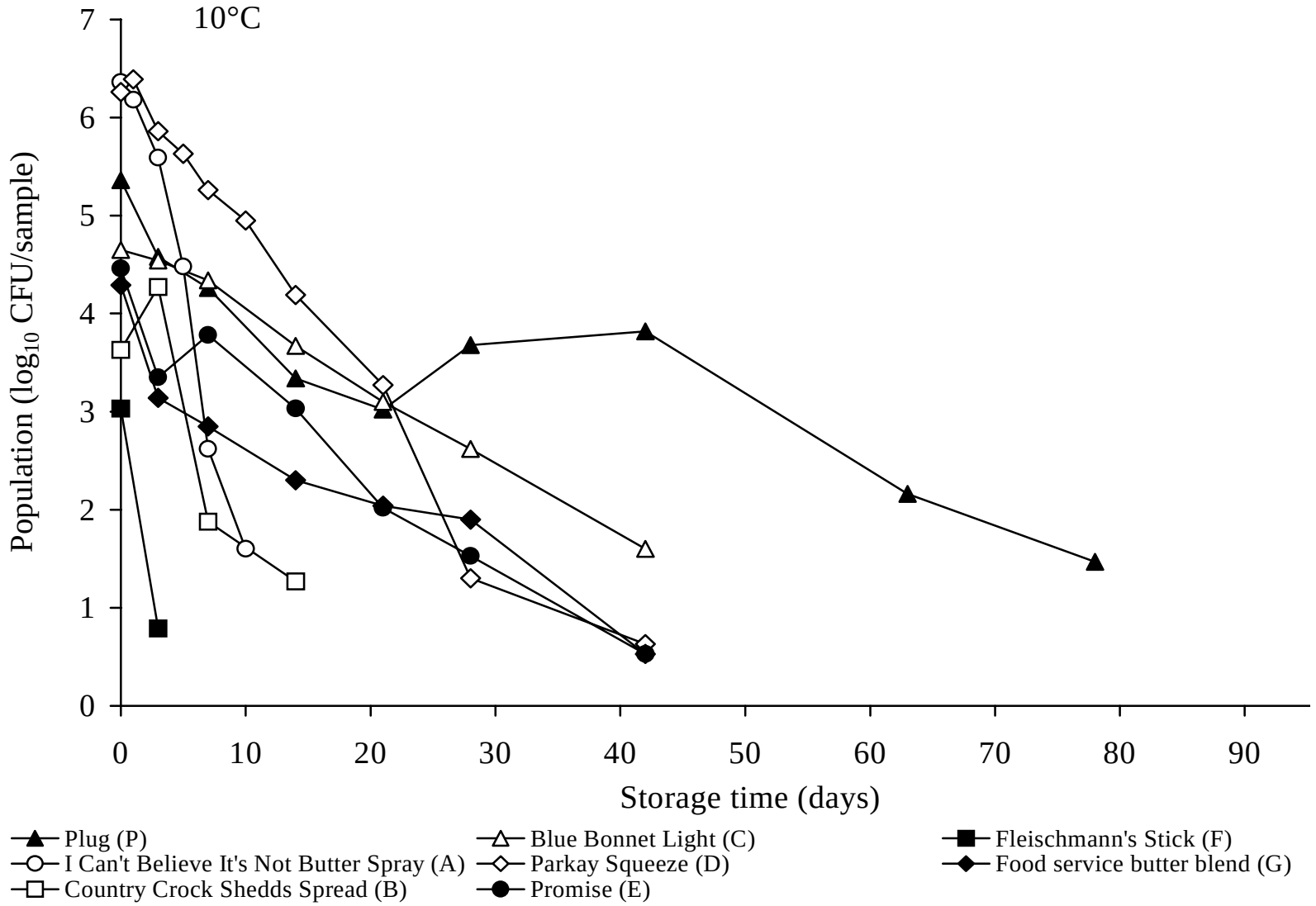

10°C
Population (log10 CFU/sample)
Storage time (days)
0
1
2
3
4
5
6
7
0
10
20
30
40
50
60
70
80
90
Plug (P)
Blue Bonnet Light (C)
Fleischmann's Stick (F)
I Can't Believe It's Not Butter Spray (A)
Parkay Squeeze (D)
Food service butter blend (G)
Country Crock Shedds Spread (B)
Promise (E)

Table 2.10. Populations of *Escherichia coli* O157:H7 recovered from products stored at 21°C

Storage time	Recovery	Product codes[a] and populations (log_{10} CFU/sample)[b]							
		P	A	B	C	D	E	F	G
(days)	medium[c]	e[d]	e	e	e	e	e	e	e
0	TSA	b 5.36 a	a 6.36 a	de 3.63 a	bc 4.65 ab	a 6.26 a	c 4.46 a	e 3.03 a	cd 4.29 a
	SMA	b 5.19 a	a 6.21 a	de 3.39 a	bc 4.49 a	a 6.20 a	cd 4.16 a	e 2.63 a	cd 3.98 a
1	TSA		b 5.70 a			a 6.15 a			
	SMA		b 5.46 b			a 5.99 ab			
3	TSA	bc 4.29 b	e 0.69 b 3	c 3.91 a	ab 4.91 a	a 5.65 b	bc 3.98 a	e 0.53 b 2	d 2.67 b 3
	SMA	bc 4.18 b	e <1.60 c	c 3.26 a	ab 4.53 a	a 5.31 bc	c 3.36 ab	e 0.63 b	d 2.11 b
5	TSA		b <1.60 c 0			a 5.37 b			
	SMA		b <1.60 c			5.01 cd			
7	TSA	b 3.70 c	d <1.60 c 0	b 3.26 a	a 4.58 ab	a 4.78 c	a 4.35 a	d <1.60 b 0	c 2.46 b 3
	SMA	b 3.24 c	d <1.60 c	c 1.40 b	ab 4.07 ab	a 4.37 de	b 3.31 b	d <1.60 b	c 2.20 b
10	TSA		b <1.60 c 0			a 4.23 d			
	SMA		b <1.60 c			a 3.70 e			
14	TSA	c <1.60 d 0	c <1.60 c 0	c 0.92 b 1	a 3.90 b	ab 3.27 e	ab 3.20 b	c <1.60 b 0	b 2.29 b 3
	SMA	c <1.60 d	c <1.60 c	c <1.60 c	a 3.19 bc	a 2.43 f	b 1.43 c	c <1.60 b	c <1.60 c
21	TSA	c <1.60 d 0		c <1.60 b 0	a 3.95 b	b 2.04 f	c <1.60 c 0	c <1.60 b 0	b 1.23 c 3
	SMA	b <1.60 d		b <1.60 c	a 2.64 c	b 0.53 g	b <1.60 d	b <1.60 b	b 0.69 c
28	TSA	b <1.60 d 0		b <1.60 b 0	a 2.64 c	b <1.60 g 1	b <1.60 c 0	b <1.60 b 0	b 0.69 cd 1
	SMA	a <1.60 d		a <1.60 c	a 0.63 d	a <1.60 g	a <1.60 d	a <1.60 b	a <1.60 c

Table 2.10., continued

Storage time (days)	Recovery medium[c]	Product codes[a] and populations (log_{10} CFU/sample)[b]							
		P	A	B	C	D	E	F	G
		e[d]	e	e	e	e	e	e	e
42	TSA	a <1.60 d 0		a <1.60 b 0	a <1.60 d 0	a <1.60 g 0	a <1.60 c 0	a <1.60 b 0	a <1.60 d 0
	SMA	a <1.60 d		a <1.60 c	a <1.60 d	a <1.60 g	a <1.60 d	a <1.60 b	a <1.60 c
63	TSA	a <1.60 d 0		a <1.60 b 0	a <1.60 d 0	a <1.60 g 0	a <1.60 c 0		a <1.60 d 0
	SMA	a <1.60 d		a <1.60 c	a <1.60 d	a <1.60 g	a <1.60 d		a <1.60 c
78	TSA	a <1.60 d 0			a <1.60 d 0	a <1.60 g 0	a <1.60 c 0		a <1.60 d 0
	SMA	a <1.60 d			a <1.60 d	a <1.60 g	a <1.60 d		a <1.60 c
94	TSA	a <1.60 d 0			a <1.60 d 0	a <1.60 g 0			a <1.60 d 0
	SMA	a <1.60 d			a <1.60 d	a <1.60 g			a <1.60 c

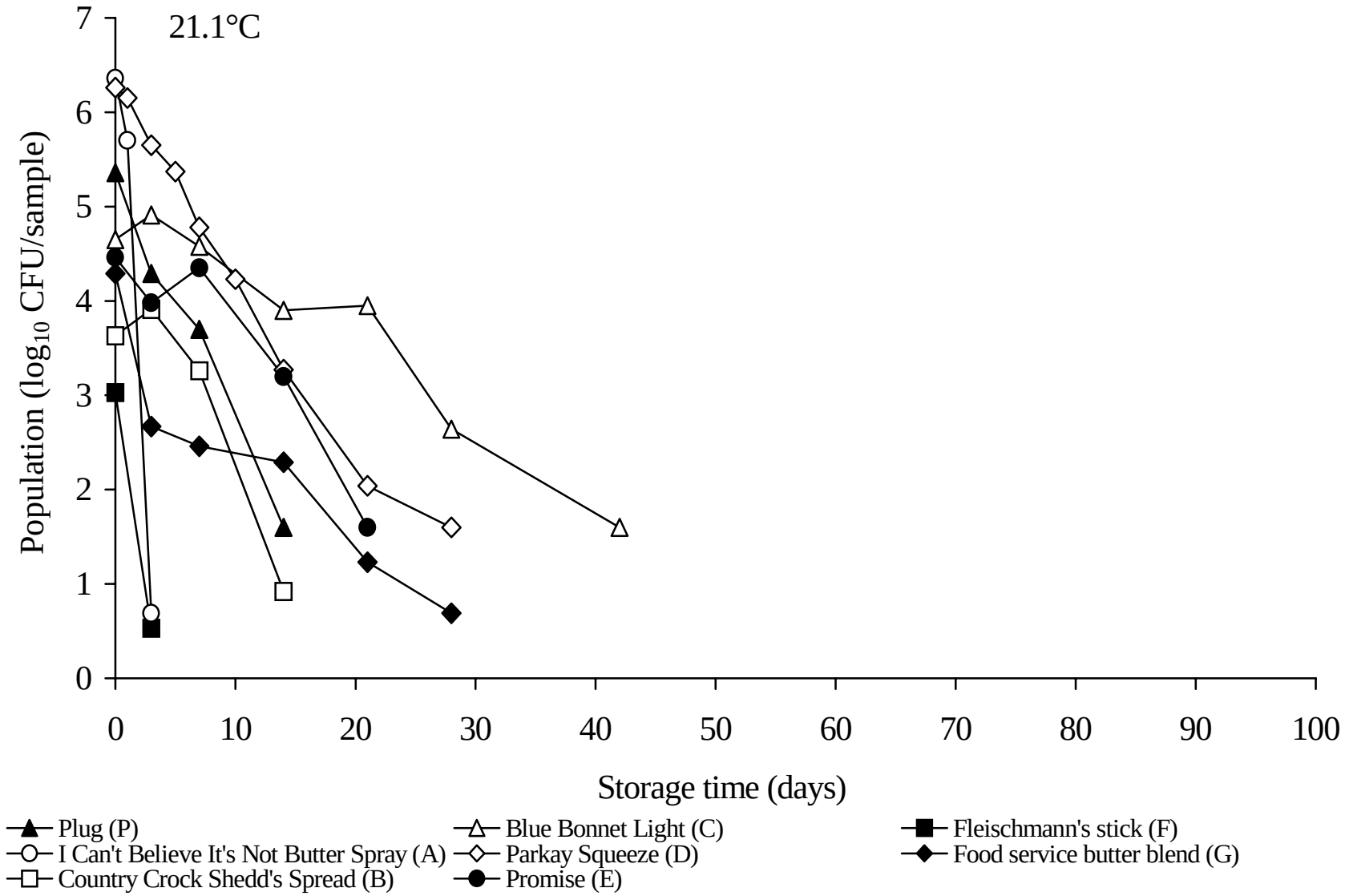
[a] P = Plug used to inoculate Products B, C, E, F, and G; A = I Can't Believe It's Not Butter Spray; B = Country Crock Shedd Spread; C = Blue Bonnet Light; D = Parkay Squeeze; E = Promise;F = Fleischmann's stick; G = Food service butter and margarine blend

[b] Samples consisted of 3.5 ml of Product A, 4 g of Product D, 4 g of Products B, C, E, F, or G plus the plug, or the plug (P). Within recovery medium, mean values in the same column that are not followed by the same letter are significantly different ($P \leq 0.05$). Mean values in the same row that are not preceded by the same letter are significantly different ($P \leq 0.05$).

[c] Tryptic soy agar (TSA) and Sorbitol MacConkey agar (SMA) were used to recover *E. coli* O157:H7.

[d] Number out of three replicate samples that were positive for *E. coli* O157:H7.

FIGURE 2.6. Populations of *E. coli* O157:H7 recovered on TSA from products stored at 21°C for up to 94 days.


21.1°C
Population (log10 CFU/sample)
0
1
2
3
4
5
6
7
0
10
20
30
40
50
60
70
80
90
100
Storage time (days)
Plug (P)
Blue Bonnet Light (C)
Fleischmann's stick (F)
I Can't Believe It's Not Butter Spray (A)
Parkay Squeeze (D)
Food service butter blend (G)
Country Crock Shedd's Spread (B)
Promise (E)

Growth of *E. coli* O157:H7 did not occur in any of the test products. The pathogen did not survive as long as *Salmonella* in products stored under the same conditions. None of the products stored for 78 days at 4.4 or 10°C, or 42 days at 21°C were positive for *E. coli* O157:H7. At a given storage time, populations of *E. coli* O157:H7 recovered from the plugs not used to inoculate products were higher when plugs were stored at 10°C compared to 4.4 and 21°C, as was observed with plugs inoculated with *Salmonella*.

The same trend in survival of *E. coli* O157:H7 in Product A was observed with *Salmonella. E. coli* O157:H7 died more rapidly in Products A and F than in other products and the rate of reduction increased as the storage temperature increased. The pathogen was not detected by enrichment of Product A stored for 14 or more days at 4.4 or 10°C or 5 or more days at 21°C. Cells inoculated into Products C, D, E, and G survived longest at all storage temperatures. Death was enhanced as the storage temperature was increased. In products in which *E. coli* O157:H7 retained highest viability (C, D, and G), the pathogen was not detected by enrichment of samples stored at 21°C for 42 days or more. In a study reported by Hathcox et al. (1995), *E. coli* O157:H7 did not grow in lite or regular mayonnaise, and an increase in storage temperature from 5°C to 20°C also resulted in a dramatic increase in rate of inactivation.

Products inoculated with *L. monocytogenes.* Populations of *L. monocytogenes* in test products stored at 4.4, 10, and 21°C for up to 94 days are shown in Tables 2.11, 2.12, and 2.13 and graphically in Figures 2.7, 2.8, and 2.9, respectively. Initial populations were 5.91 – 6.18 $\log_{10}$ CFU/3.5-ml or 4-g sample. TPA was superior to MOX in recovering of *L. monocytogenes*. A higher population of up to 1.83 $\log_{10}$ CFU/sample (Product D, 28 days at 4.4°C) (Table 2.11) was recovered on TPA versus MOX, indicating that a portion of stressed cells did not form colonies on MOX agar.

The rate of inactivation of *L. monocytogenes* on plugs not used to inoculate products was similar to that observed for *Salmonella* and *E. coli* O157:H7. Retention of

Table 2.11. Populations of *Listeria monocytogenes* recovered from products stored at 4.4°C

Storage time	Recovery	Product codes[a] and population (log_{10} CFU/sample)[b]							
		P	A	B	C	D	E	F	G
(days)	medium[c]	e[d]	e	e	e	e	e	e	e
0	TPA	b 5.91 a	ab 6.02 a	d 5.29 a	bcd 5.62 a	a 6.34 a	bc 5.76 a	bcd 5.62 a	cd 5.48 a
	MOX	b 5.42 a	a 5.97 a	cd 4.88 a	bcd 5.09 a	a 6.36 a	bc 5.28 a	bcd 5.10 a	d 4.80 a
1	TPA		b 5.07 b			a 6.35 a			
	MOX		b 4.77 b			a 6.23 a			
3	TPA	b 4.71 b	c 3.83 c	c 4.12 b	c 4.11 b	a 5.54 b	b 5.00 b	ab 5.15 ab	b 4.79 b
	MOX	d 2.98 b	cd 3.34 c	c 3.45 b	c 3.43 b	a 5.50 d	b 4.45 ab	b 4.57 ab	b 4.22 b
5	TPA		b 3.11 d			a 5.04 c			
	MOX		b 2.72 d			a 4.93 bc			
7	TPA	c 3.62 c	e 2.29 e	cd 3.46 bc	d 3.12 bc	a 5.22 bc	b 4.45 c	b 4.80 ab	b 4.48 bc
	MOX	d 2.28 b	e 1.60 e	c 2.94 bc	c 2.85 bc	a 5.18 bc	b 4.05 bc	b 4.29 abc	b 4.09 b
10	TPA		b <1.60 f 3			a 5.22 bc			
	MOX		b 1.60 e			a 5.11 bc			
14	TPA	d 3.19 d	f <1.60 f 2	de 2.87 c	e 2.63 cd	a 5.09 c	c 3.75 d	b 4.33 bc	b 4.43 bc
	MOX	e 0.73 c	e <1.60 f	cd 2.47 c	d 2.22 c	a 4.89 c	bc 3.33 cd	b 3.78 bcd	b 3.93 bc
21	TPA	bc 2.04 e 3	d <1.60 f 0	c 1.23 d 3	c 1.55 de 3	a 4.55 d	b 2.82 e	a 4.07 bcd	a 4.04 cd
	MOX	bc 0.53 c	c <1.60 f	bc 1.07 d	bc 1.23 d	a 4.23 d	b 1.69 ef	a 3.73 bcd	a 3.58 c
28	TPA	cd 2.00 e 3	f <1.60 f 0	ef 0.53 de 1	de 1.33 def 2	bc 3.00 e	bc 2.84 e	ab 3.66 cd	a 4.07 c
	MOX	d <1.60 c	d <1.60 f	c 1.07 d	c 1.07 d	c 1.17 e	b 2.62 de	ab 3.44 cd	a 3.60 c

Table 2.11., continued

Storage time (days)	Recovery medium[c]	P	e[d]	A	e	B	e	C	e	D	e	E	e	F	e	G	e
		Product codes[a] and population (log_{10} CFU/sample)[b]															
42	TPA	d <1.60 f	2	d <1.60 f	0	d <1.60 e	1	d <1.60 f	1	d <1.60 f	2	c 1.80 f	3	b 3.16 d		a 3.53 d	
	MOX	c <1.60 c		c <1.60 f		c <1.60 e		c <1.60 e		c <1.60 f		b 1.07 f		a 3.03 d		a 2.89 d	
63	TPA	c <1.60 f	2			c <1.60 e	0	bc 0.77 ef	1	c <1.60 f	0	c <1.60 g	3	ab 1.72 e	3	a 2.69 e	
	MOX	b <1.60 c				b <1.60 e		b <1.60 e		b <1.60 f		b <1.60 g		a 1.64 e		a 2.00 e	
78	TPA	c <1.60 f	0			c <1.60 e	0	bc 0.53 ef	0	c <1.60 f	0	c <1.60 g	3	ab 1.23 e	3	a 2.06 f	3
	MOX	b <1.60 c				b <1.60 e		b <1.60 e		b <1.60 f		b <1.60 g		a 1.90 e		a 1.93 e	
94	TPA	b <1.60 f	0			b <1.60 e	0	b <1.60 f	0	b <1.60 f	0	b <1.60 g	3	b <1.60 f	3	a 2.23 ef	3
	MOX	b <1.60 c				b <1.60 e		b <1.60 e		b <1.60 f		b <1.60 g		b <1.60 f		a 1.90 e	

[a] P = Plug used to inoculate Products B, C, E, F, and G; A = I Can't Believe It's Not Butter Spray; B = Country Crock Shedd Spread; C = Blue Bonnet Light; D = Parkay Squeeze; E = Promise;F = Fleischmann's stick; G = Food service butter and margarine blend

[b]Samples consisted of 3.5 ml of Product A, 4 g of Product D, 4 g of products B, C, E, F, or G plus the plug, or the plug (P). Within recovery medium, mean values in the same column that are not followed by the same letter are significantly different ($P \leq 0.05$). Mean values in the same row that are not preceded by the same letter are significantly different ($P \leq 0.05$).

[c] Tryptose phosphate agar (TPA) and Modified Oxford medium (MOX) were used to recover *L. monocytogenes* .

[d] Number out of three replicate samples that were positive for *L. monocytogenes* .

FIGURE 2.7. Populations of *L. monocytogenes* recovered on TPA from products stored at 4.4°C for up to 94 days.

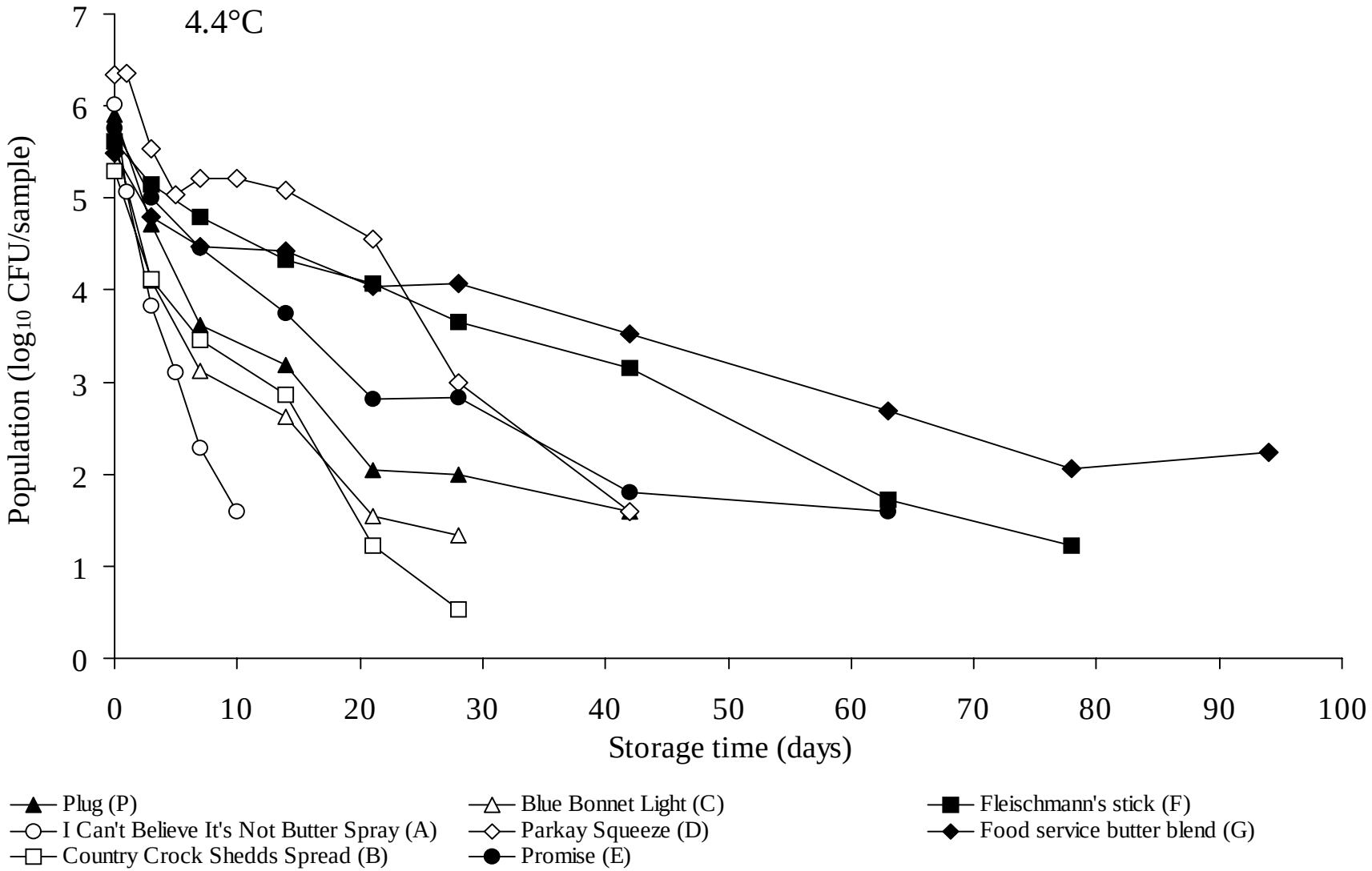

4.4°C
Population (log10 CFU/sample)
Storage time (days)
0
1
2
3
4
5
6
7
0
10
20
30
40
50
60
70
80
90
100
Plug (P)
I Can't Believe It's Not Butter Spray (A)
Country Crock Shedds Spread (B)
Blue Bonnet Light (C)
Parkay Squeeze (D)
Promise (E)
Fleischmann's stick (F)
Food service butter blend (G)

Table 2.12. Populations of *Listeria monocytogenes* recovered from products stored at 10°C

Storage time (days)	Recovery medium[c]	Product codes[a] and population (log_{10} CFU/sample)[b]							
		P	A	B	C	D	E	F	G
0	TPA	b 5.91 a e[d]	ab 6.02 a e	d 5.29 a e	bcd 5.62 a e	a 6.34 a e	bc 5.76 a e	bcd 5.62 a e	cd 5.48 a e
	MOX	b 5.42 a e	a 5.97 a e	cd 4.88 a e	bcd 5.07 a e	a 6.36 a e	bc 5.28 a e	bcd 5.10 a e	d 4.80 a e
1	TPA		a 5.53 b			a 6.02 b			
	MOX		a 5.48 b			a 5.84 a			
3	TPA	bc 4.81 ab	bc 4.79 c	d 3.64 b	d 3.44 b	ab 5.06 c	c 4.49 b	a 5.49 a	bc 4.73 b
	MOX	b 3.78 ab	a 4.83 c	c 3.11 b	c 2.85 b	a 5.01 b	b 4.04 b	a 5.01 a	b 4.21 abc
5	TPA		a 4.37 d			a 4.60 d			
	MOX		a 4.3 d			a 4.50 bc			
7	TPA	a 4.94 ab	c 3.28 e	c 2.79 c	d 1.65 c	a 4.87 cd	bc 3.78 c	ab 4.72 b	ab 4.74 b
	MOX	ab 4.22 ab	c 3.22 e	d 2.34 c	e 1.23 cd	a 4.65 bc	bc 3.44 c	a 4.32 ab	a 4.36 abc
10	TPA		b 2.46 f			a 4.68 d			
	MOX		b 2.52 f			a 4.28 c			
14	TPA	a 4.39 bc	c 2.06 g	d 1.17 d 3	cd 1.80 c	a 3.96 e	b 3.04 d	ab 3.76 c	a 4.41 bcd
	MOX	ab 3.34 ab	c 2.02 g	d 0.53 d	c 1.76 c	ab 3.63 d	b 3.00 c	ab 3.56 bc	a 4.00 bcd
21	TPA	a 4.26 bc	d <1.60 h 3	d <1.60 e 2	d <1.60 d 2	c 2.47 f	c 2.35 e	b 3.24 cd	a 3.92 de
	MOX	a 3.69 ab	c <1.60 h	c <1.60 d	c 0.53 de	c 0.53 e	b 2.24 d	ab 2.99 cd	a 3.36 def
28	TPA	a 4.95 ab	d <1.60 h 0	d 0.53 de 0	d <1.60 d 0	d <1.60 g 0	c 2.24 e	c 2.68 de	b 3.74 e
	MOX	a 3.16 ab	b <1.60 h	b <1.60 d	b <1.60 e	b <1.60 f	a 1.96 d	a 2.50 d	a 3.26 ef

Table 2.12., continued

Storage time (days)	Recovery medium[c]	Product codes[a] and population (log_{10} CFU/sample)[b]															
		P		A		B		C		D		E		F		G	
			e[d]		e		e		e		e		e		e		e
42	TPA	a 4.11 bc		c <1.60 h	0	c <1.60 e	0	c <1.60 d	0	c <1.60 g	0	c 0.53 f	3	b 2.42 e		b 3.05 f	
	MOX	a 2.83 b		c <1.60 h		c <1.60 d		c <1.60 e		c <1.60 f		b 1.07 e		a 2.30 d		a 2.89 f	
63	TPA	a 4.04 bc		b <1.60 h	0	b <1.60 e	0	b <1.60 d	0	b <1.60 g	0	b <1.60 f	1	b <1.60 f	0	a 3.78 e	
	MOX	b 2.24 b		c <1.60 h		c <1.60 d		c <1.60 e		c <1.60 f		c <1.60 f		c <1.60 e		a 3.77 cde	
78	TPA	a 4.18 bc								b <1.60 g	1	b <1.60 f	1	b <1.60 f	3	a 4.15 cde	
	MOX	b 2.90 b								c <1.60 f		c <1.60 f		c <1.60 e		a 4.11 bc	
94	TPA	b 3.41 c								c <1.60 g	0	c <1.60 f	0	c <1.60 f	0	a 4.58 bc	
	MOX	b 1.99 b								c <1.60 f		c <1.60 f		c <1.60 e		a 4.54 ab	

[a] P = Plug used to inoculate Products B, C, E, F, and G; A = I Can't Believe It's Not Butter Spray; B = Country Crock Shedd Spread; C = Blue Bonnet Light; D = Parkay Squeeze; E = Promise;F = Fleischmann's stick; G = Food service butter and margarine blend

[b] Samples consisted of 3.5 ml of Product A, 4 g of Product D, 4 g of Products B, C, E, F, or G plus the plug, or the plug (P). Within recovery medium, mean values in the same column that are not followed by the same letter are significantly different ($P \leq 0.05$). Mean values in the same row that are not preceded by the same letter are significantly different ($P \leq 0.05$).

[c] Tryptose phosphate agar (TPA) and Modified Oxford medium (MOX) were used to recover *L. monocytogenes* .

[d] Number out of three replicate samples that were positive for *L. monocytogenes* .

FIGURE 2.8. Populations of *L. monocytogenes* recovered on TPA from products stored at 10°C for up to 94 days.

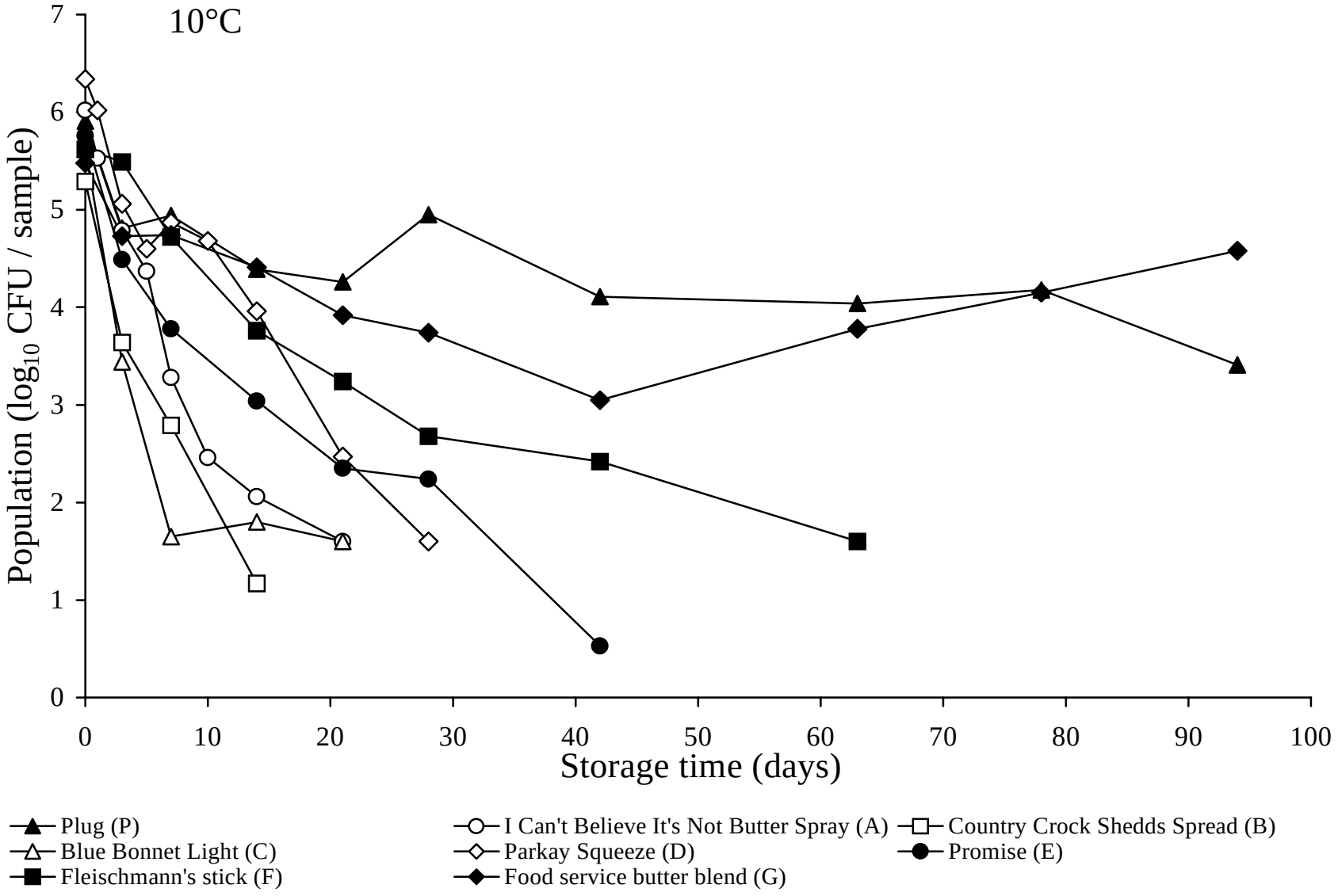

10°C
Population (log10 CFU / sample)
Storage time (days)
0
1
2
3
4
5
6
7
0
10
20
30
40
50
60
70
80
90
100
Plug (P)
I Can't Believe It's Not Butter Spray (A)
Country Crock Shedds Spread (B)
Blue Bonnet Light (C)
Parkay Squeeze (D)
Promise (E)
Fleischmann's stick (F)
Food service butter blend (G)

Table 2.13. Populations of *Listeria monocytogenes* recovered from products stored at 21°C

Storage time	Recovery	Product codes[a] and population (log_{10} CFU/sample)[b]							
		P	A	B	C	D	E	F	G
(days)	medium[c]	e[d]	e	e	e	e	e	e	e
0	TPA	b 5.91 a	ab 6.02 a	d 5.29 a	bcd 5.62 a	a 6.34 a	bc 5.76 a	bcd 5.62 a	cd 5.48 c
	MOX	b 5.42 a	a 5.97 a	cd 4.88 a	bcd 5.09 a	a 6.36 a	bc 5.28 a	bcd 5.10 a	d 4.81 c
1	TPA		a 5.37 b			a 5.32 ab			
	MOX		a 5.24 b			a 5.21 ab			
3	TPA	a 5.26 ab	c 3.77 c	d 2.51 b	d 2.74 b	b 4.65 b	c 3.75 b	b 4.75 b	b 4.73 d
	MOX	a 4.48 b	b 3.6 c	c 2.19 b	c 2.27 b	a 4.11 bc	b 3.56 b	a 4.54 a	a 4.31 d
5	TPA		a 3.21 d			a 4.24 b			
	MOX		a 3.11 cd			a 3.82 bcd			
7	TPA	a 4.85 b	cd 2.62 e	e 0.77 c 3	de 1.70 c	b 3.78 bc	bc 2.92 bc	bc 3.39 c	a 5.38 c
	MOX	b 4.05 b	cd 2.78 d	f <1.60 c	e 1.70 b	cd 2.94 cde	d 2.58 c	c 3.21 b	a 5.30 b
10	TPA		a 1.8 f 3			a 2.48 cd 3			
	MOX		a 1.17 e			a 1.36 efg			
14	TPA	b 4.56 b	e <1.60 g 0	e <1.60 c 0	de 0.53 d 1	cd 2.10 d 2	cd 2.06 c 3	c 2.35 d	a 6.35 ab
	MOX	b 3.83 b	e <1.60 f	e <1.60 c	de 0.53 c	cd 2.09 def	cd 1.96 d	c 2.18 c	a 6.33 a
21	TPA	b 3.56 c	c <1.60 g 0	c <1.60 c 0	c <1.60 d 0	c 1.05 de 1	c 0.73 d 3	c <1.60 e 3	a 6.50 a
	MOX	b 2.75 c	c <1.60 f	c <1.60 c	c <1.60 c	c 0.90 fg	c 0.53 e	c 0.53 d	a 6.41 a
28	TPA	b 3.27 c	c <1.60 g 0	c <1.60 c 0	c <1.60 d 0	c 1.24 de 1	c 0.53 d 1	c <1.60 e 2	a 6.32 ab
	MOX	b 2.82 c	c <1.60 f	c <1.60 c	c <1.60 c	c 1.15 efg	c <1.60 f	c 0.53 d	a 6.38 a

Table 2.13., continued

Storage time (days)	Recovery medium[c]	Product codes[a] and population (log_{10} CFU/sample)[b] P	e[d]	A	e	B	e	C	e	D	e	E	e	F	e	G	e
42	TPA	b <1.60 d	1	b <1.60 g	0	b <1.60 c	0	b <1.60 d	0	b <1.60 e	3	b <1.60 d	1	b <1.60 e	0	a 6.31	ab
	MOX	b <1.60 d		b <1.60 f		b <1.60 c		b <1.60 c		b <1.60 g		b <1.60 f		b 0.53 d		a 6.28	a
63	TPA	b <1.60 d	0					b <1.60 d		b <1.60 e	1	b <1.60 d	0	b <1.60 e	0	a 6.04	b
	MOX	b <1.60 d						b <1.60 c		b <1.60 g		b <1.60 f		b <1.60 d		a 6.06	a
78	TPA	b <1.60 d	0							b <1.60 e	0	b <1.60 d	0	b <1.60 e	0	a 5.64	c
	MOX	b <1.60 d								b <1.60 g		b <1.60 f		b <1.60 d		a 5.52	b
94	TPA	b <1.60 d	0							b <1.60 e	0	b <1.60 d	0	b <1.60 e	0	a 5.50	c
	MOX	b <1.60 d								b <1.60 g		b <1.60 f		b <1.60 d		a 5.43	b

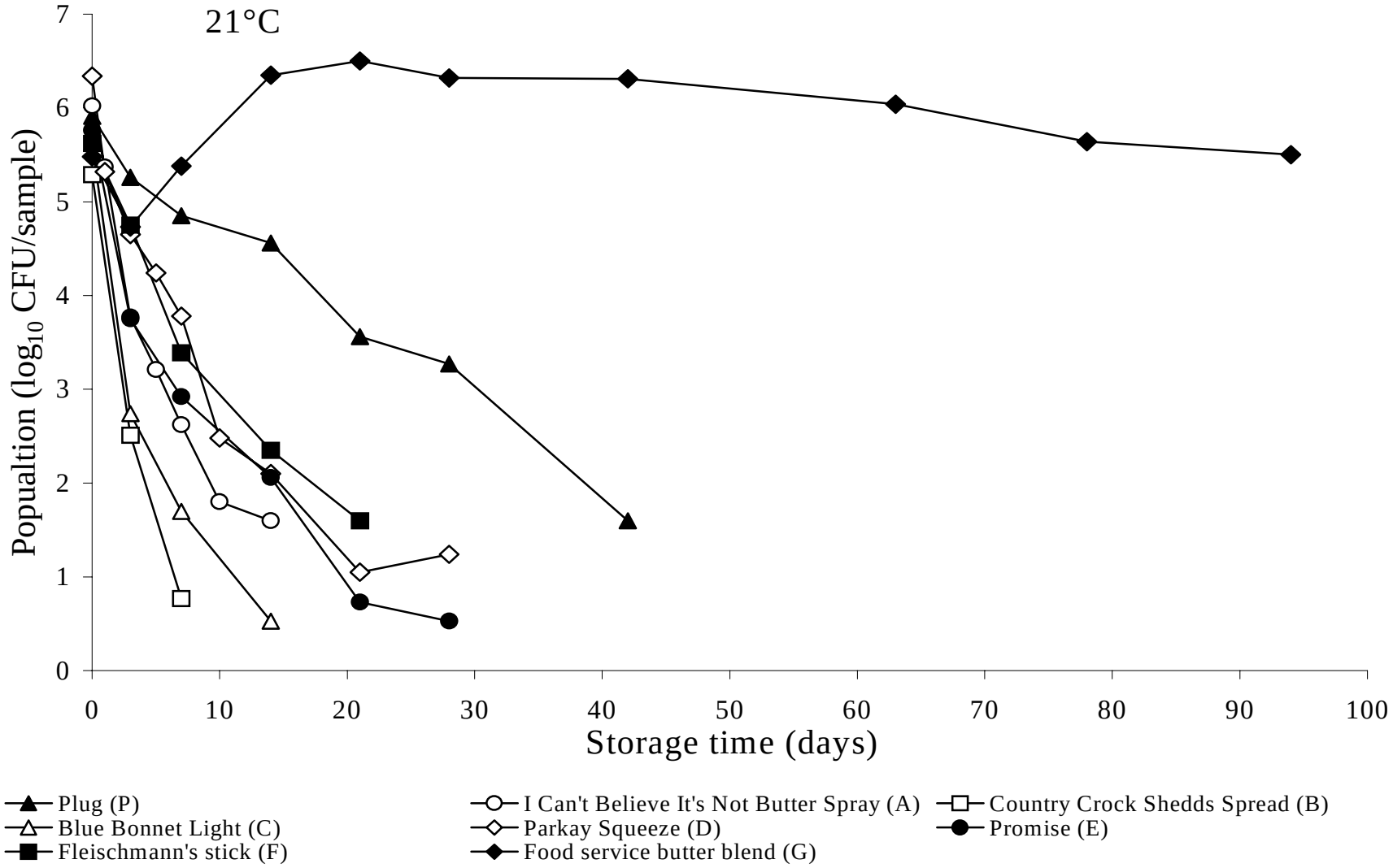
[a] P = Plug used to inoculate Products B, C, E, F, and G; A = I Can't Believe It's Not Butter Spray; B = Country Crock Shedd Spread; C = Blue Bonnet Light; D = Parkay Squeeze; E = Promise;F = Fleischmann's stick; G = Food service butter and margarine blend

[b] Samples consisted of 3.5 ml of Product A, 4 g of Product D, 4 g of Products B, C, E, F, or G plus the plug, or the plug (P). Within recovery medium, mean values in the same column that are not followed by the same letter are significantly different ($P \leq 0.05$). Mean values in the same row that are not preceded by the same letter are significantly different ($P \leq 0.05$).

[c] Tryptose phosphate agar (TPA) and Modified Oxford medium (MOX) were used to recover *L. monocytogenes*.

[d] Number out of three replicate samples that were positive for *L. monocytogenes*.

FIGURE 2.9. Populations of *L. monocytogenes* recovered on TPA from products stored at 21°C for up to 94 days.


21°C
Popualtion (log10 CFU/sample)
Storage time (days)
0
1
2
3
4
5
6
7
0
10
20
30
40
50
60
70
80
90
100
Plug (P)
I Can't Believe It's Not Butter Spray (A)
Country Crock Shedds Spread (B)
Blue Bonnet Light (C)
Parkay Squeeze (D)
Promise (E)
Fleischmann's stick (F)
Food service butter blend (G)

viability of *L. monocytogenes* on plugs was higher at 10°C than at 4.4 or 21°C. An initial population of 5.91 $\log_{10}$ CFU/plug was reduced to <1.60 $\log_{10}$ CFU/plug when storage was at 4.4 or 21°C for 42 days. A population of 3.41 $\log_{10}$ CFU/plug was recovered from plugs stored at 10°C for 94 days.

L. monocytogenes died in Products A, B, C, and D within 94 days, regardless of the storage temperature. Populations in Products A, B, and C decreased most rapidly at all storage temperatures. The pathogen was not detected in Product A after storage for 21, 28, and 14 days at 4.4, 10, and 21°C, respectively, or in Product B after storage for 63, 28, and 14 days at 4.4, 10, and 21°C, respectively. A similar trend was observed in Product C, in which cells survived longest at 4.4°C and died most rapidly at 21°C. In Products E and F, *L. monocytogenes* survived longest at 4.4 and 10°C, as was observed with *Salmonella.*

Unlike inactivation of *E. coli* O157:H7 and *Salmonella* in the food service butter and margarine blend (Product G), *L. monocytogenes* began to grow between 42 and 63 days of storage at 10°C and continued to grow through day 94. An initial population of 5.48 $\log_{10}$ CFU/4-g sample decreased to 3.05 $\log_{10}$ CFU/4-g sample of Product G stored at 10°C for 42 days, then increased to 4.58 $\log_{10}$ CFU/sample by day 94. Our findings on the behavior of *L. monocytogenes* at 4.4 and 10°C are inconsistent with those of Olsen et al. (1988), in which populations of *L. monocytogenes* increased by two orders of magnitude in refrigerated (4 - 6°C and 13°C) butter made from contaminated cream (Champagne, 1994). In Product G stored at 21°C, growth of *L. monocytogenes* was observed between 3 and 7 days. Populations increased from 4.73 $\log_{10}$ CFU/sample on day 3 to 6.50 $\log_{10}$ CFU/sample on day 21. Other studies have shown that refrigerated storage (0 - 10°C) retards but does not prevent growth of *L. monocytogenes* in UHT milk (Walker et al., 1990). Donnelly and Briggs (1986) observed a tremendous increase in growth of *Listeria* in milk when the incubation temperature was increased from 4°C to

10°C. The pathogen is known to grow at pH 4.4 when incubated at 30 or 20°C for less than 7 or 14 days, respectively (George et al., 1988). At 7 and 10°C, growth occurred in TPB at a pH <5.0; growth at 4°C occurred in TPB at pH 5.23. Sorrells et al. (1989) reported that *L. monocytogenes* grew in TSB at pH 4.4 - 5.0, depending on the type of acidulant used.

The yellow fat products evaluated in this study varied in composition. The concentration of salt ranged from 1.30 to 4.12%, while fat ranged from 29.6 to 81.6%. Products contained potassium sorbate at concentrations of up to 1255 ppm. Sorbates are known to inhibit *Salmonella* and *S. aureus* in cooked and uncured sausage, and *Salmonella*, *S. aureus*, and *E. coli* in poultry (Davidson, 1997). Conflicting reports of the efficacy of sorbate in controlling the growth of *L. monocytogenes* may be due to variations in composition of food and sorbate concentration. In a study reported by Thomas (1999), 500 ppm sorbate was required for complete inhibition of *E. coli* O157:H7 at pH > 4 - 5. Products C, D, and F contained sodium benzoate at concentrations of 470 to 590 ppm. Benzoate, as well as sorbate, is used primarily as an antifungal agent. Benzoic acid at a concentration of 1000 ppm has been shown to be effective in reducing *E. coli* O157:H7 populations in apple cider (pH 3.6 – 4.0) by 3 to 5 $\log_{10}$ CFU/ml when the cider was stored at 8°C for 7 days (Davidson, 1997).

CONCLUSIONS

Neither *Salmonella* nor *E. coli* O157:H7 grew in the seven yellow fat products and butter and margarine blend. Populations of both pathogens in all inoculated test products were reduced more rapidly at 21°C than at 4.4°C or 10°C. Growth of *L. monocytogenes* occurred in a butter and margarine blend (Product G) after 42 days at 10°C or 7 days at 21°C. The presence of butter in Product G, as well as emulsion characteristics, apparently provided a more favorable source and availability of nutrients for growth of *L. monocytogenes*. Failure of pathogens to grow in other products was likely due to a combination of unfavorable conditions, including acidic pH, lack of

nutrients, the presence of sorbate, benzoate, sodium chloride, and emulsion characteristics. *Salmonella* survived longer than *E. coli* O157:H7 or *L. monocytogenes* in higher fat products with the exception of Product G. Overall, *E. coli* O157:H7 died more rapidly than *Salmonella* and *L. monocytogenes* in test products.

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CHAPTER 3

VIABILITY OF *SALMONELLA, ESCHERICHIA COLI* O157:H7, AND *LISTERIA MONOCYTOGENES* IN YELLOW FAT PRODUCTS AS AFFECTED BY TEMPERATURE AND PHYSICAL ABUSE

ABSTRACT

Sweet cream whipped salted butter, sweet cream whipped unsalted butter, salted light butter, and three non-butter yellow fat products containing 30-78% fat were surface inoculated with *Salmonella*, *Escherichia coli* O157:H7, or *Listeria monocytogenes*. Products (pH 4.60-5.39, at 4.4°C) were kept at 37°C under high relative humidity (85%) for 1 h to induce condensation of water on the surface before storing at 4.4°C or 21°C for up to 21 days. All three pathogens grew in sweet cream salted whipped butter within 2 days at 21°C. *Listeria monocytogenes* also grew between 7 and 21 days in the same product stored at 4.4°C. None of the pathogens grew in sweet cream unsalted whipped butter or in non-butter products stored at 4.4°C or 21°C. Rates of inactivation of the three pathogens increased in these products stored at 21°C, compared to 4.4°C. Death of all pathogens was retarded in products containing ≥ 57% fat. *Salmonella, E. coli* O157:H7, and *L. monocytogenes* died most rapidly in two non-dairy products containing salt, preservatives, and acidulants. Death of the pathogens is attributed to differences in emulsion characteristics of test products, presence of preservatives, acidic pH, and nutrient deprivation. The adverse affects of these factors are amplified at 21°C compared to 4.4°C.

INTRODUCTION

With the increasing popularity of large multiple-use containers of yellow fat products in food service establishments, restaurants, and the home, the possibility of cross contamination increases as products are repeatedly taken from containers for use over long periods of time. Moisture may condense on the surface of refrigerated or frozen yellow fat products when placed in a warm humid environment such as a commercial or home kitchen (Zijl and Klapwijk, 2000). This introduces more water onto the surface of the product and may aid in the leaching of nutrients, allowing microorganisms to grow. The physical structure of yellow fat product also depends on refrigerated storage (Zijl and Klapwijk, 2000). Storing a product at $\geq$23 °C may destabilize the emulsion, compromising the preservative effects of its compartmentalized structure.

The abuse of products and continuous removal of portions of products in large containers over time may increase the opportunity for cross contamination by utensils or by food service employees and consumers with poor hygiene. Sneezing, coughing, spitting, or handling yellow fat products with unclean hands may result in contamination of the product. Lack of temperature control, disturbance of the emulsion, and condensation of water as a result of exposure of the surface of chilled yellow fat products to high relative humidity may enhance the ability of pathogens to survive and, perhaps, grow.

Outbreaks of human infections involving dairy products, e.g., raw milk and cheese, have been attributed to contamination with *Salmonella*, *Escherichia coli* O157:H7, or *Listeria monocytogenes.* Foods such as ice cream, pasteurized and non-pasteurized milk, and cheese have been vehicles for numerous outbreaks involving *Salmonella* (El-Gazzar and Marth, 1992). Butter supports the growth of *Salmonella* at room temperature (24°C) and cannot be eliminated using refrigeration or by freezing (Sims et al., 1970). The pathogen is widespread in nature and is capable of

contaminating many foods and food ingredients (El-Gazzar and Marth, 1992). *E. coli* O157:H7 has been responsible for outbreaks of infections associated with milk and acid foods such as mayonnaise and apple cider (Meng and Doyle, 1997). *E. coli* is able to survive in butter and butter production facilities (Abbar and Mohamed, 1987). Outbreaks of *Salmonella* or *E. coli* O157:H7 infections associated with yellow fat products, however, have not been reported.

Listeriosis has also been associated with consumption of dairy products. An outbreak in 1983 implicated pasteurized milk and another in 1985 was attributed to Mexican style cheese (NACMCF, 1991). Raw milk may also contain the pathogen but proper pasteurization time and temperature will eliminate it. Many products implicated in foodborne illness are contaminated post pasteurization (NACMCF, 1991). An outbreak of listeriosis in Finland (1999) was reported in which several people became ill after consuming butter containing *L. monocytogenes* serotype 3a (Lyytikinen et al., 2000). Olsen et al. (1988) reported that *L. monocytogenes* is capable of growing in butter containing 1.2% salt at 4 and 13°C. Lanciotti et al. (1992) reported that *L. monocytogenes* and *Yersinia entercolitica* grew in light butter at 4.4°C but rapidly decreased in populations during storage. ter Steeg et al. (1995) reported that *Listeria innocua* survived in a water-in-oil emulsion for 120 days at 25°C with <1 log CFU/g reduction. Survival at pH 4.25 was usually longer in course emulsions than in fine emulsions. A reduction of 4 $\log_{10}$ CFU/g occurred when the water-in-oil emulsion was stored at 25°C, while a reduction of less than 2 $\log_{10}$ was observed at 7°C.

This study was undertaken to determine the survival and growth characteristics of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* on the surface of yellow fat products subjected to temperature abuse and high relative humidity followed by storage at 4.4 or 21°C for up to 21 days.

MATERIALS AND METHODS

Products evaluated. Six commercial brands of margarine and butter products were studied (Table 3.1). Products were selected for evaluation based on the presence or absence of dairy ingredients, fat content, and the presence or absence of salt.

Pathogens. Five serotypes of *Salmonella* were used: Anatum (isolated from a patient with salmonellosis associated with consuming alfalfa sprouts), Enteritidis E190-88 (human isolate), Montevideo (isolated from a patient in a tomato-associated outbreak), Muenchen 372 (orange juice isolate), and Typhimurium DT104 (multi-antibiotic resistant strain).

Five strains of *E. coli* O157:H7 were used: F500 (human isolate from an outbreak associated with mayonnaise), E0018 (calf fecal isolate), SEA 13B88 (outbreak associated with unpasteurized apple cider), 994 (salami isolate), and 932 (human isolate from a meat-associated outbreak).

Six strains of *L. monocytogenes* were used: Scott A (serotype 4b) (isolate from a patient in an outbreak associated with milk), 302 (serotype 1) (isolate from Cheddar cheese), 310 (serotype 4) (from goat cheese), G1091 (serotype 4b) (patient isolate from a coleslaw-associated outbreak), F8369 (serotype 1/2a) (isolate from corn), and F8255 (serotype 1/2b) (isolate from peach/plum).

Preparation of inocula. Microorganisms were adapted to grow in broth and agar containing 50 μg/ml nalidixic acid to minimize the potential growth of background microorganisms on enumeration media and in enrichment broth. Salmonellae and *E. coli* O157:H7 were grown in tryptic soy broth (TSB) (BD Diagnostic Systems, Sparks, Md.) and *L. monocytogenes* was grown in tryptose phosphate broth (TPB) (BD Diagnostic Systems), both supplemented with 1% glucose and 50 μg/ml nalidixic acid, for 28 h at 37°C. All serotypes or strains were transferred (1 loopful to 10 ml) two times at 28-h intervals immediately preceding use as inocula. Cells for inoculation of products were harvested by centrifugation (2000 x *g*, 10 min, 4°C) and washed in sterile Butterfield's

Table 3.1. Products evaluated in studies designed to determine the survival of pathogens in yellow fat products subjected to high relative humidity (85%) at 37°C for 1 h followed by storage at 4.4°C and 21°C for up to 21 days

Product code	Product name	Net weight (g)	Ingredients	pH	Fat (%)	Manufacturer
A	Land O'Lakes Sweet Cream Whipped Butter, salted	227	Cream, salt, and annatto	5.39	78	Land O' Lakes Inc., Arden Hills, Mn.
B	Land O' Lakes Sweet Cream Whipped Butter, unsalted	227	Cream and natural flavoring	4.60	78	Land O' Lakes Inc., Arden Hills, Mn.
C	Land O'Lakes Salted Light Butter	453	Butter (cream and salt), water, tapioca maltodextrin, modified food starch, beta carotene, vitamin A palmitate, natural flavor, lactic acid, salt, vegetable mono and diglycerides, potassium sorbate and sodium benzoate	5.06	43	Land O' Lakes Inc., Arden Hills, Mn.
D	Shedd's Spread Country Crock®	453	Liquid soybean oil and partially hydrogenated soybean oil, water, whey, salt, vegetable mono and diglycerides, soy lecithin, potassium sorbate, citric acid, and calcium disodium EDTA as preservatives, artificial flavor, colored with beta carotene, vitamin A (palmitate) added	4.56	50	Van Den Bergh Foods Co., Lisle, Ill.
E	Blue Bonnet® Light Margarine	453	Water, liquid soybean oil and partially hydrogenated soybean oil, maltodextrin, salt, vegetable monoglycerides, potassium sorbate and sodium benzoate and calcium disodium EDTA, citric acid, artifical flavor, vitamin A palmitate, colored with beta carotene	5.18	32	Beatrice Foods, Indianapolis, Ind.
F	Promise®	453	Liquid sunflower oil, sweet dairy whey, water, hydrogenated soybean oil, partially hydrogenated soybean oil, liquid canola oil, salt, vegetable mono- and diglycerides, soy lecithin, potassium sorbate and citric acid, artificial flavor, colored with beta carotene, vitamin A (palmitate)	4.79	57	Lipton, Englewood Cliffs, N.J.

[a]Products A, B, D, E, and F were packaged in 227-g or 453-g plastic tubs; Product C consisted of four 113-g sticks wrapped in foil and packaged in a waxed paper box

phosphate buffer (pH 7.2) (BPB) (FDA Bacteriological Analytical Manual, 1984). Pellets of *Salmonella* and *E. coli* O157:H7 were resuspended in 200 ml BPB, whereas pellets of *L. monocytogenes* were resuspended in 40 ml of BPB. Ten milliliters of each serotype or strain of each pathogen were combined to give five-serotype or five-strain mixtures of *Salmonella* and *E. coli* O157:H7, respectively, and a six-strain mixture of *L. monocytogenes* containing 10^7 CFU/ml. Each mixture contained approximately equal numbers of all of the serotypes or strains. Populations in single-serotype and single-strain cultures, and in each mixture of multi-serotype or multi-strain suspensions, were determined by serially diluting in sterile 0.1% peptone and surface plating (0.1 ml) in duplicate on tryptic soy agar (TSA, BD Diagnostic Systems) supplemented with 50 μg/ml nalidixic acid (TSAN) (*Salmonella* and *E. coli* O157:H7) or tryptose phosphate agar (TPA, BD Diagnostic Systems) supplemented with 50 μg/ml nalidixic acid (TPAN) (*L. monocytogenes*).

Procedure for inoculation. Five days before inoculation, indentations 5 mm deep were made in the surface of each yellow fat product by pressing the bottom of a sterile 16 x 125 mm glass test tube into the products and twisting one full rotation. Products at 4.4°C were held at 21°C for 1 h before indentations were made. Four indentations were made in the surface of products in each tub of Products A, B, D, E and F, and three indentations were created in each 113 g stick of Product C. Products were sealed and stored at 4.4°C for 5 days before inoculating with pathogens.

Products at 4.4°C were inoculated with 20 μl of mixed-serotype or mixed-strain suspensions of pathogens. Inocula were deposited in the center of the indentations. All tubs of Products A, B, D, E and F and 113-g sticks of Product C were inoculated within 30 min of removal from 4.4°C. After inoculation, products were incubated at 37°C in a high relative humidity (85%) for 1 h. The high temperature and high humidity caused water to condense on the surface of products. After 1 h, lids were applied to containers of Products A, B, D, E, and F; sticks of Product C were placed in a tray (28.5 x 15 x 10

cm) and sealed. Inoculated products were stored at 4.4°C or 21°C for up to 21 days before analyzing for populations and presence of pathogens.

Microbiological analysis. Inoculated products were analyzed for numbers (CFU/ 4-g sample) and presence (by enrichment) of each pathogen after storing for 0, 1, 2, 3, 7, 14, and 21 days at 4.4 or 21°C. Samples consisted of 4 ± 0.1g of product plus inoculum and water that collected in the indentation. Inoculum and water were removed from the indentation using a micropipette before removal of 4 g of product surrounding and beneath the indentation with a sterile spatula. Inoculum, water, and product were placed in a stomacher 80 bag (Seward Medical Ltd, London, U.K.). Care was taken to remove from the margarine or butter all of the product immediately surrounding the area that was surface inoculated.

Bags containing samples were placed in a water bath at 40°C for 5 min or until products were fluid. Separation of the fat and serum fractions was minimal. Forty milliliters of warmed (40°C) lactose broth (BD Diagnostic Systems), modified TSB (mTSB) (Padhye and Doyle, 1992), or *Listeria* enrichment broth (BD Diagnostic Systems) was combined with samples that had been inoculated with *Salmonella*, *E. coli* O157:H7, or *L. monocytogenes*, respectively, and pummeled in a stomacher (Seward Medical, Ltd., London, U.K.) at high speed for 1 min. Pipettes were warmed by drawing in warm sterile water (40°C) and discharging before drawing in the samples. Samples (avoiding the fat layer) of the stomachate were surface plated (0.25 ml in quadruplicate and 0.1 ml in duplicate) on TSAN to enumerate *E. coli* O157:H7 and *Salmonella* or TPAN to enumerate *L. monocytogenes*; samples serially diluted in sterile 0.1% peptone were also surface plated on TSAN or TPAN. Plates were incubated at 37°C for 24 h (*Salmonella* and *E. coli* O157:H7) or 48 h (*L. monocytogenes*) before counting presumptive colonies. Selected colonies were confirmed using *Salmonella* latex agglutination (Oxoid, Basingstroke, U.K.), *E. coli* O157 latex agglutination test (Oxoid), and API *Listeria* assay (bioMerieux Sa, Marcy-l'Etoile, France).

Stomachates containing lactose broth and products inoculated with *Salmonella* were incubated at 37°C for 24 h, then inoculated (1 ml) into 10 ml of selenite cystine broth (BD Diagnostic Systems). The selenite cystine broth was incubated for 24 h at 37°C, then streaked on Hektoen Enteric (HE) agar (BD Diagnostic Systems) agar. Presumptive selected colonies of pathogens were counted and confirmed using agglutination and biochemical tests. The remainder of stomachates containing mTSB and products inoculated with *E. coli* O157:H7 was incubated at 37°C for 24 h, then streaked on sorbitol MacConkey (SMAC) agar (Oxoid) supplemented with 50 μg/ml nalidixic acid (SMACN). Stomachates containing *Listeria* enrichment broth and products inoculated with *L. monocytogenes* were incubated at 37°C for 48 h before streaking on modified Oxford medium (MOX) supplemented with 50 μg/ml nalidixic acid (MOXN). Selected presumptive colonies were subjected to confirmation tests described above.

Statistical analysis. All experiments were replicated three times. Trials for each pathogen were conducted in a randomized complete block using a full-factorial design. Data were subjected to statistical analysis using the SAS System (SAS Institute Inc., Cary, NC). Analysis of variance of mean values was done using general linear model (PROC GLM) with mean separation using Fisher's LSD. The full model was analyzed, but due to main effect interactions, the model was reduced.

RESULTS AND DISCUSSION

Product composition. Products ranged in pH from 4.56 to 5.39 and contained 43-78% fat (Table 3.1). The minimum pH at which the test pathogens can grow depends on temperature, acidulant used to decrease pH, nutrient availability, and the serotype or strain used (El-Gazzar and Marth, 1992). The whipped butter products (Products A and B) did not contain preservatives or added ingredients to maintain the stability of the emulsion, other than salt in Product A. Products A and B, the salted and unsalted whipped butter products, respectively, contained added colorant or natural flavoring.Product E did not contain dairy ingredients, which can serve as sources of

nutrients needed for the survival and potential growth of microorganisms in yellow fat products. Products C, D, E, and F contained ingredients such as preservatives (potassium sorbate and/or sodium benzoate), acidulants (citric acid or lactic acid), and structuring or emulsifying agents. The combination of preservatives, salt, and a low pH may have prevented the growth and survival of pathogens in these products.

Populations of pathogens in inocula. Populations ($\log_{10}$ CFU/ml) of serotypes or strains of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* and their respective mixed-serotype or mixed-strain suspensions used to inoculate products are listed in Table 3.2.

Products inoculated with *Salmonella*. Results of microbiological examination of products inoculated with *Salmonella* are presented in Table 3.3 and graphically in Figures 3.1 and 3.2. The lowest populations of pathogens plotted in figures represent the lowest counts ($\log_{10}$ CFU/4-g sample) obtained on TSAN. Populations of approximately 5.57 $\log_{10}$ CFU/20µl were inoculated onto the surface of indentations in all products. On day 0 (within 90 min of inoculation), decreases in initial populations of *Salmonella* of up to 0.75 $\log_{10}$ CFU/sample occurred. Slight decreases, up to 0.27 $\log_{10}$, were observed in Products A and B, while greater decreases occurred in Products C, D, E, and F. Reductions in populations of *Salmonella* were observed in all products stored at 4.4°C. With the exception of Product A, populations decreased more rapidly in products stored at 21°C than at 4.4°C.

Product A (salted whipped butter) retained the highest population of *Salmonella* over the 21-day storage period, regardless of storage temperature. *Salmonella* grew in Product A between 0 and 2 days of storage at 21°C. Growth may have occurred as a result of availability of nutrients leached from the whipped butter into the condensate present on the surface of the product as a result of exposure to the high-humidity

Table 3.2. Populations of microorganisms in inocula

Microorganism	Serotype or strain	Population[a] (log_{10} CFU/ml)
Salmonella	Anatum	7.22
	Montevideo	7.82
	Typhimurium	7.26
	Muenchen	7.32
	Enteritidis	7.11
	Five-serotype mixture	7.27
E. coli O157:H7	932	7.49
	SEA 13B88	7.98
	E0018	7.65
	994	7.04
	F500	6.84
	Five-strain mixture	7.22
L. monocytogenes	G1091	6.18
	F8369	7.55
	310	7.77
	Scott A	7.39
	F8255	6.74
	302	7.72
	Six-strain mixture	7.59

[a]Inocula consisted of 20 ul of mixed-serotype or mixed-strain suspensions depositied in each indentation on the surface of yellow fat products

Table 3.3. Populations of *Salmonella* recovered from yellow fat products.

Storage temperature	Storage time	Product codes[a] and population (log_{10} CFU/sample)[b]											
		A		B		C		D		E		F	
(°C)	(days)	log_{10} CFU	e[c]	log_{10} CFU	e	log_{10} CFU	e	log_{10} CFU	e	log_{10} CFU	e	log_{10} CFU	e
4.4	0	a 5.50 a		b 5.30 a		c 4.92 a		c 4.82 a		c 4.92 a		b 5.17 a	
	1	a 5.42 abc		a 5.16 ab		b 4.49 ab		c 3.82 b		d 3.51 b		b 4.43 b	
	2	a 5.49 ab		b 4.57 c		c 4.12 b		d 3.32 bc		e 2.97 b		c 4.16 bc	
	3	a 5.36 c		b 4.76 bc		d 3.17 c		d 2.82 c		d 2.89 b		c 3.64 c	
	7	a 5.37 bc		b 4.10 d		d 0.53 d	1	d 1.07 d	3	d 1.23 c	3	c 2.50 d	
	14	a 5.31 c		b 3.41 e		d <1.60 d	0	cd 0.53 ed	1	d <1.60 d	0	c 1.17 e	
	21	a 5.00 d		b 2.38 f	3	c <1.60 d	0	c <1.60 e	0	c <1.60 d	0	c 0.53 e	2
21	0	a 5.50 c		b 5.30 a		c 4.92 a		c 4.82 a		c 4.92 a		b 5.17 a	
	1	a 5.40 c		b 4.27 b		cd 3.25 b		d 3.14 b		cd 3.34 b		c 3.54 b	
	2	a 6.44 a		b 3.66 c		d 0.53 c	3	c 2.44 c		c 3.00 c		c 2.88 c	
	3	a 6.34 a		b 3.38 c		e <1.60 c	3	d 1.07 d	3	c 2.32 d		c 2.51 d	
	7	a 6.02 b		b 0.53 d	3	b <1.60 c	0	b <1.60 e	0	b <1.60 e	1	b <1.60 e	0
	14	a 4.51 d		b <1.60 e	0	b <1.60 c	0	b <1.60 e	0	b <1.60 e	0	b <1.60 e	0
	21	a 2.35 e		b <1.60 e	0	b <1.60 c	0	b <1.60 e	0	b <1.60 e	0	b <1.60 e	0

[a] A = Land O'Lakes whipped, salted, sweet cream butter; B = Land O' Lakes whipped, unsalted, sweet cream butter; C = Land O'Lakes Light butter; D = Shedd's Spread Country Crock; E = Blue Bonnet Light; F = Promise

[b] Samples consisted of 4 g of Products A, B, C, D, E, or F. Within storage temperature, mean values in the same column that are not followed by the same letter are significantly different ($P \leq 0.05$). Mean values in the same row that are not preceded by the same letter are significantly different ($P \leq 0.05$).

[c] Number out of three replicates that were positive for *Salmonella* .

FIGURE 3.1. Populations of *Salmonella* recovered from yellow fat products stored at 4.4°C for up to 21 days.

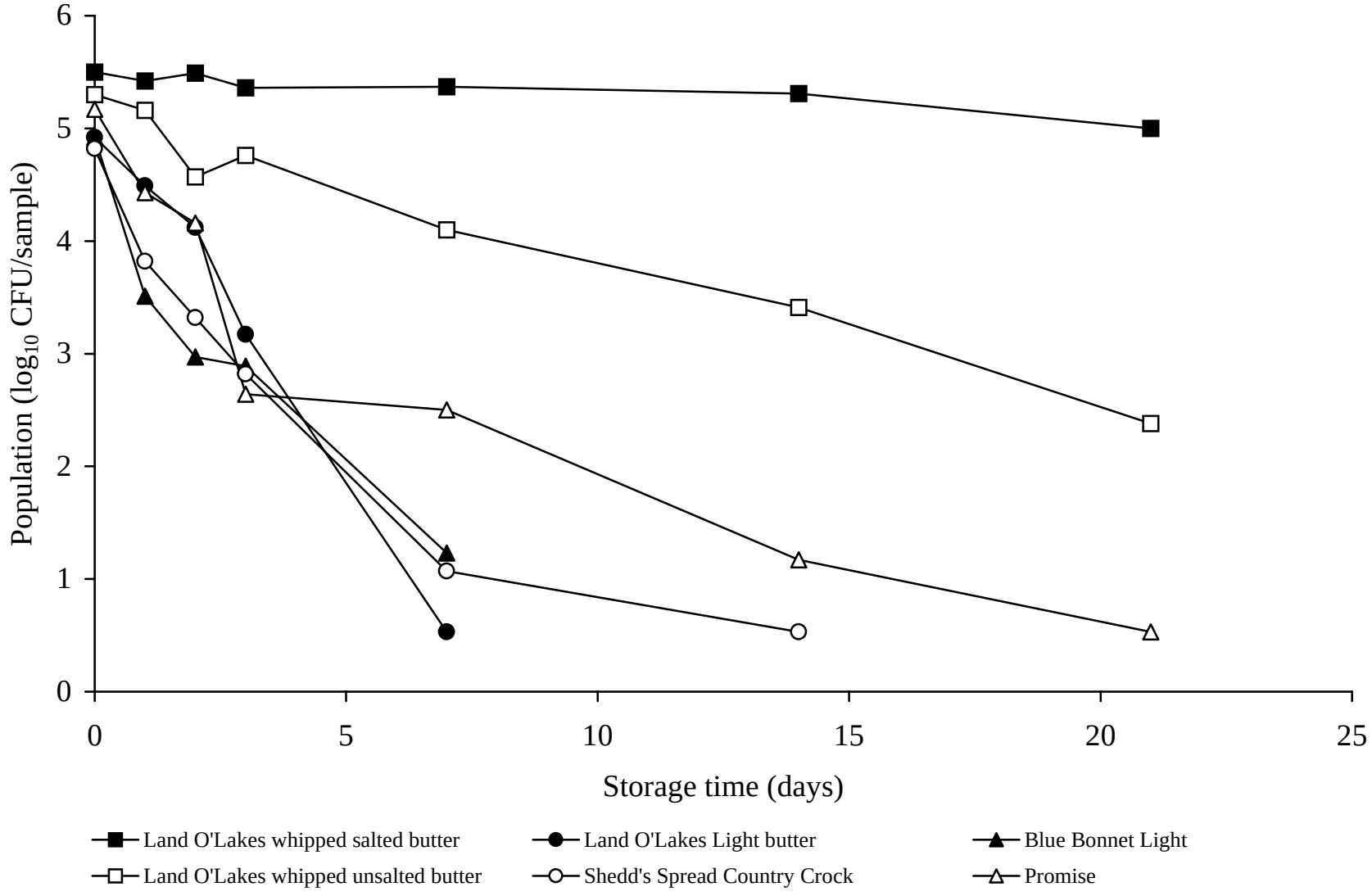
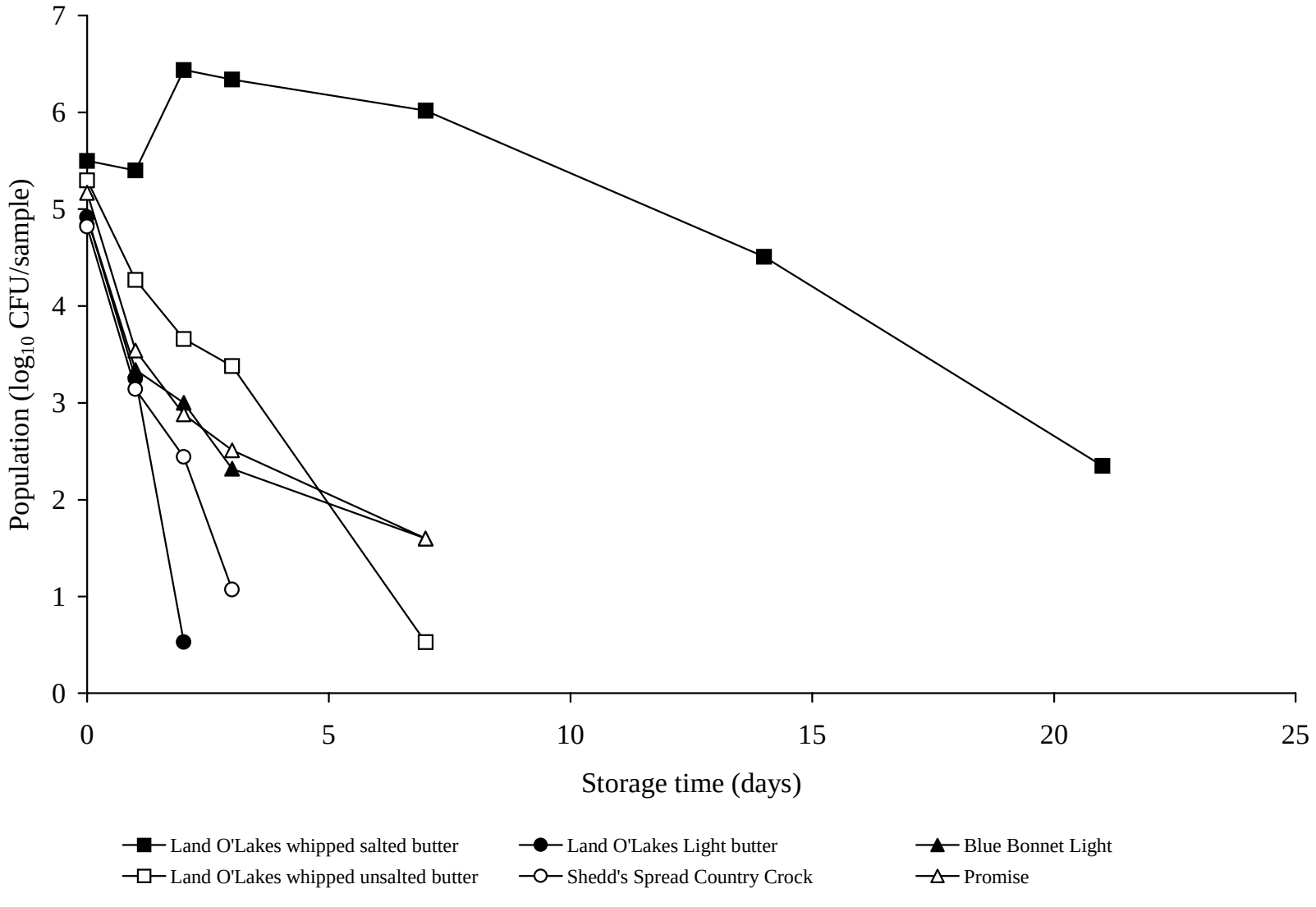

Population (log10 CFU/sample)
0
1
2
3
4
5
6
0
5
10
15
20
25
Storage time (days)
Land O'Lakes whipped salted butter
Land O'Lakes Light butter
Blue Bonnet Light
Land O'Lakes whipped unsalted butter
Shedd's Spread Country Crock
Promise

FIGURE 3.2. Populations of *Salmonella* recovered from yellow fat products stored at 21°C for up to 21 days.


Population (log10 CFU/sample)
0
1
2
3
4
5
6
7
0
5
10
15
20
25
Storage time (days)
Land O'Lakes whipped salted butter
Land O'Lakes Light butter
Blue Bonnet Light
Land O'Lakes whipped unsalted butter
Shedd's Spread Country Crock
Promise

environment at 37°C for 1 h. The condensate that formed on the surface may have provided a favorable environment for growth. The population significantly ($P \leq 0.05$) increased by 1.04 $\log_{10}$ CFU/sample between 1 and 2 days at 21°C but was not maintained and death of the cells followed throughout the 21-day storage period. After storage for 3 days, a more rapid decrease in populations in Product A was observed at 21°C than at 4.4°C. The initial population (5.50 $\log_{10}$ CFU/sample) recovered from Product A was reduced by only 0.50 $\log_{10}$ after storage for 21 days at 4.4°C. Death of cells may be attributable, in part, to the depletion of available nutrients during growth. When nutrient demands are higher than can be met by the aqueous environment surrounding cells in yellow fat products, death occurs (Verrips, 1980).

Product B (unsalted whipped butter) retained the second highest population of *Salmonella* at 4.4 and 21°C. Growth did not occur at 21°C in this product, even though it differs from Product A only in that it does not contain salt. This is inconsistent with observations by Jensen et al. (1983). They reported that unsalted sweet cream butter was the only type of butter tested that supported the growth of bacteria during storage above 0°C. Results of studies to evaluate the survival of *Salmonella* in unsalted and salted batches of butter stored at various temperatures (Sims et al., 1970) were, however, similar to ours. The most significant decline in populations occurred in the unsalted butter followed by lightly salted and moderately salted butter stored at 0 or -10°F.

Higher fat products (A, B, and F) was correlated with higher survival of *Salmonella*, compared to survival in lower-fat products. *Salmonella* was detected by enrichment in two of three samples of Product F stored for 21 days at 4.4°C. The pathogen was not detected by enrichment in Product F held at 21°C for 7-21 days. Whether stored at 4.4 or 21°C, death of *Salmonella* occurred most rapidly in Product C. S*almonella* behaved similarly in Products D and E stored at 4.4 or 21°C. The pathogen was not detected in either product stored for 14 days at 4.4°C or 7 days at 21°C. *Salmonella* was not detected by enrichment of Product C stored at 4.4°C for 14 or 21

days or 21°C for 7-21 days. The addition of citric or lactic acid, potassium sorbate, and/or sodium benzoate may have enhanced the rate of death of *Salmonella*. *Staphylococcus aureus*, *Salmonella,* and *E. coli* are known to be inhibited by sorbates in cooked and uncured sausages (Davidson, 1997). Sorbate (500 ppm) at pH >4-5 has been shown to inhibit the growth of *E. coli* O157:H7 (Thomas, 1999).

Products inoculated with *E. coli* O157:H7. Populations of *E. coli* O157:H7 recovered from inoculated yellow fat products are listed in Table 3.4 and presented graphically in Figures 3.3 and 3.4. A population of approximately 5.52 $\log_{10}$ CFU was inoculated onto the surface of indentations made on each product. Decreased populations were observed in all products on day 0 (within 90 min of inoculation). The highest population was recovered from Product A, followed by Products C, F, B, D, and E, in that order.

As with *Salmonella,* in general, populations of *E. coli* O157:H7 decreased more rapidly in products stored at 21°C, compared to 4.4°C, although growth of *E. coli* O157:H7 did occur in Product A between 1 and 2 days at 21°C. Populations increased from 5.49 $\log_{10}$ CFU/sample on day 0 to 6.50 $\log_{10}$ CFU/sample on day 3. *E. coli* O157:H7 was not detected in any of the products stored for 21 days at 21°C and only in Product A (0.63 $\log_{10}$ CFU/sample) after 14 days at 21°C. Significant ($P \leq 0.05$) reductions in populations of *E. coli* O157:H7 occurred in Product A stored for 7 days at 21°C, but the pathogen was not detected by enrichment after storage for 21 days at 21°C. Jensen et al. (1983) reported a bactericidal effect of salt on cells incubated at temperatures higher than 0°C. Our findings on the survival of *Salmonella* and *E. coli* O157:H7 in water-in-oil emulsions do not support that observation. Both pathogens grew in salted whipped butter (Product A) but not in unsalted whipped butter (Product B) stored at 21°C. Death of *E. coli* O157:H7 was more rapid in Product B than in Product A, regardless of storage temperature.

Table 3.4. Populations of *Escherichia coli* O157:H7 recovered from yellow fat products.

Storage temperature	Storage time	Product codes[a] and population (log_{10} CFU/sample)[b]											
		A		B		C		D		E		F	
(°C)	(days)	log_{10} CFU	e[c]	log_{10} CFU	e	log_{10} CFU	e	log_{10} CFU	e	log_{10} CFU	e	log_{10} CFU	e
4.4	0	a 5.49	a	cd 4.68	a	b 4.98	a	d 4.62	a	e 4.49	a	c 4.81	bc
	1	a 5.39	ab	c 4.59	a	b 5.01	a	d 4.21	b	e 3.14	b	b 5.05	ab
	2	a 5.19	bc	b 4.31	ab	a 5.24	a	b 4.24	b	c 2.88	b	a 5.20	a
	3	a 5.11	c	b 4.23	abc	a 4.85	a	c 3.31	c	d 2.83	b	a 4.95	abc
	7	a 4.81	d	b 3.71	bc	b 3.56	b	c 2.10	d	c 2.20	c	a 4.70	b
	14	a 4.70	d	b 3.39	c	c 1.37	c 3	d <1.60	e 0	d <1.60	d 0	ab 4.09	c
	21	a 4.63	d	b 1.95	d 3	c <1.60	d 0	c <1.60	e 0	c <1.60	d 0	b 3.03	d
21	0	a 5.49	b	cd 4.68	a	b 4.98	a	d 4.62	a	e 4.49	a	c 4.74	a
	1	a 5.87	b	b 4.26	b	b 4.15	bc	b 4.37	a	c 3.15	b	b 4.55	a
	2	a 6.41	a	b 4.54	ab	c 3.84	c	bc 3.99	a	d 2.67	c	bc 4.14	a
	3	a 6.50	a	b 4.39	ab	b 4.47	b	c 3.17	b	d 2.30	d	b 4.13	a
	7	a 5.51	b	c <1.60	c 3	c <1.60	d 1	c <1.60	c 1	c <1.60	e 0	b 1.74	b 2
	14	a 0.63	c 3	a <1.60	c 0	a <1.60	d 0	a <1.60	c 0	a <1.60	e 0	a <1.60	c 0
	21	a <1.60	d 0	a <1.60	c 0	a <1.60	d 0	a <1.60	c 0	a <1.60	e 0	a <1.60	c 0

[a] A = Land O'Lakes whipped, salted, sweet cream butter; B = Land O' Lakes whipped, unsalted, sweet cream butter;C = Land O'Lakes Light butter; D = Shedd's Spread Country Crock; E = Blue Bonnet Light; F = Promise

[b] Samples consisted of 4 g of Products A, B, C, D, E, or F. Within storage temperature, mean values in the same column that are not followed by the same letter are significantly different ($P \leq 0.05$). Mean values in the same row that are not preceded by the same letter are significantly different ($P \leq 0.05$).

[c] Number out of three replicates that were positive for *E. coli* O157:H7.

FIGURE 3.3. Populations of *E. coli* O157:H7 recovered from yellow fat products stored at 4.4°C for up to 21 days.

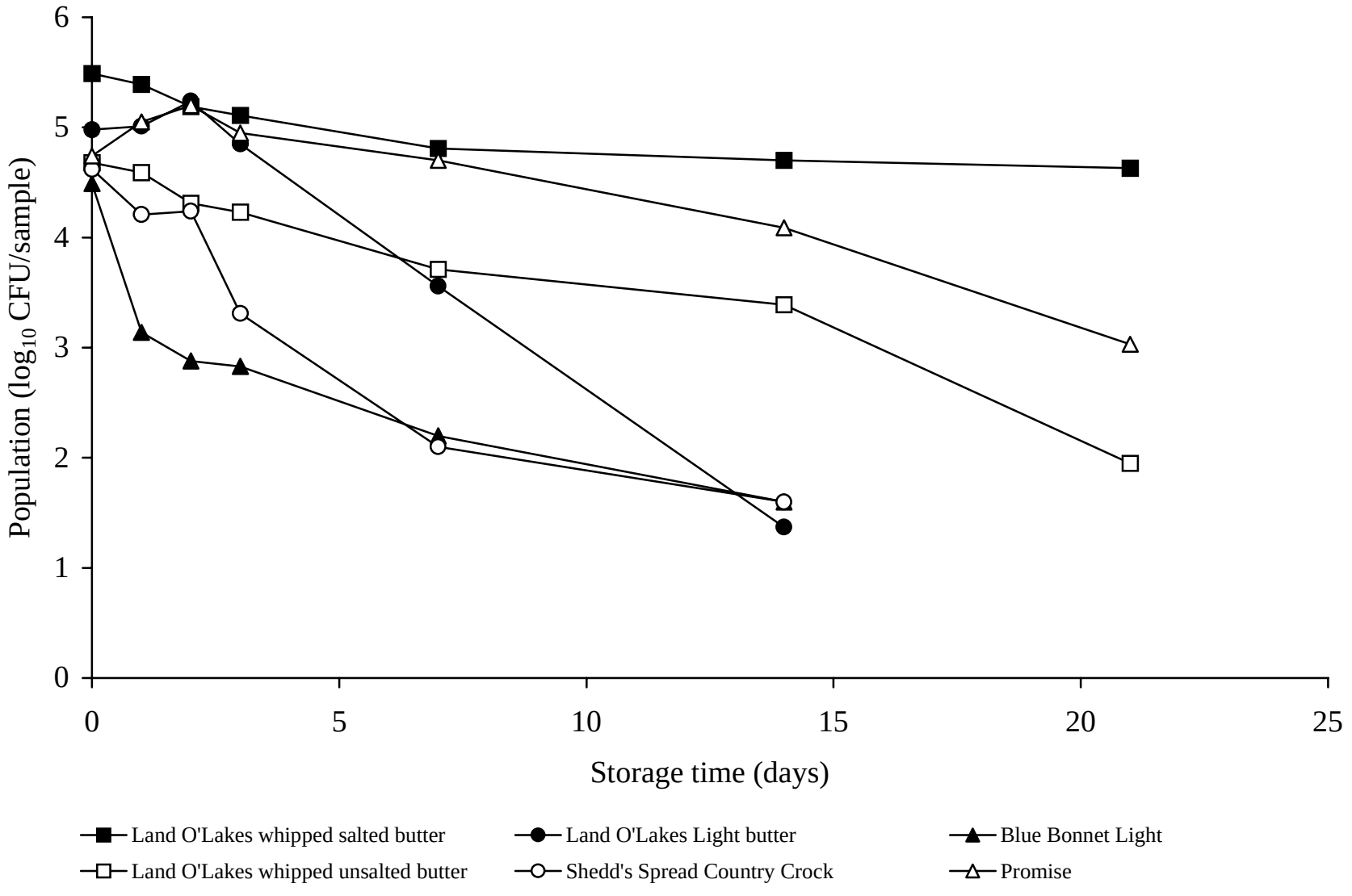
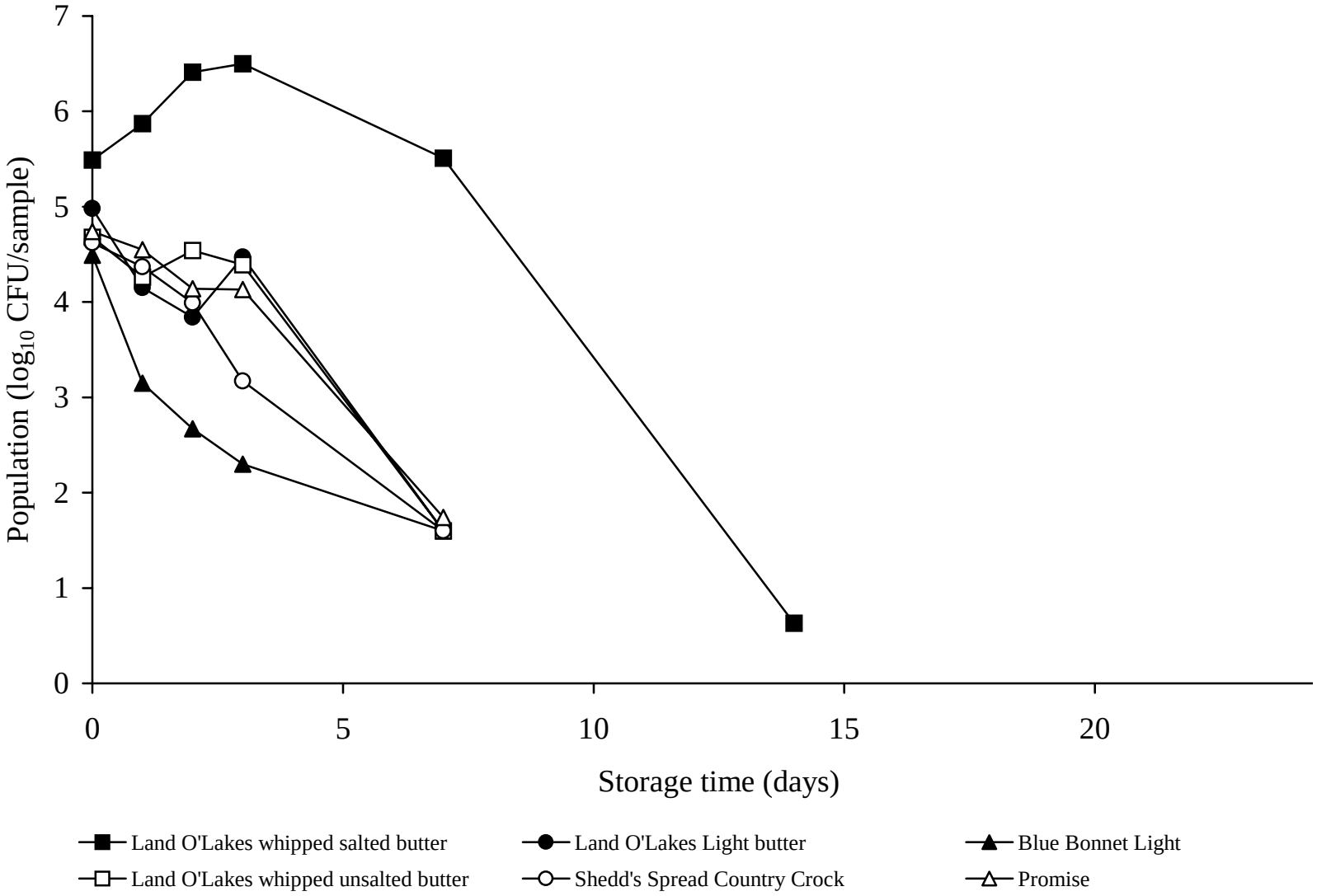

Population (log10 CFU/sample)
6
5
4
3
2
1
0
0
5
10
15
20
25
Storage time (days)
Land O'Lakes whipped salted butter
Land O'Lakes Light butter
Blue Bonnet Light
Land O'Lakes whipped unsalted butter
Shedd's Spread Country Crock
Promise

FIGURE 3.4. Populations of *E. coli* O157:H7 recovered from yellow fat products stored at 21°C for up to 21 days.


Population (log10 CFU/sample)
0
1
2
3
4
5
6
7
0
5
10
15
20
Storage time (days)
Land O'Lakes whipped salted butter
Land O'Lakes Light butter
Blue Bonnet Light
Land O'Lakes whipped unsalted butter
Shedd's Spread Country Crock
Promise

A similar trend was observed with *E.coli* O157:H7 as was observed with *Salmonella* in that both pathogens survived longer in higher fat products (A, B, and F). A slight increase in population was also observed with Product F stored for 1 day at 4.4°C. An increase from 4.81 $\log_{10}$ CFU/sample on day 0 to 5.20 $\log_{10}$ CFU/sample on day 2 was followed by a reduction to 3.03 $\log_{10}$ CFU/sample on day 21. *Escherichia coli* O157:H7 was present at populations of 4.63, 3.03, and 1.95 $\log_{10}$ CFU/sample in Products A, F, and B, respectively, while the pathogen was not detected in Products C, D, and E after storage for 21 days at 4.4°C. The most rapid rates of inactivation of *E. coli* O157:H7 occurred in Products D and E. *Escherichia coli* O157:H7 behaved similarly in both of these products in that it was undetectable by enrichment of 4-g samples after 14 days of storage at 4.4 and 21°C. The rate of reduction in Product E was most rapid.

Products inoculated with *L. monocytogenes*. Populations of *L. monocytogenes* recovered from inoculated products are listed in Table 3.5 and presented graphically in Figures 3.5 and 3.6. A population of approximately 5.89 $\log_{10}$ CFU was surface inoculated (20 µl) onto indentations made in each product. The greatest decrease in population of *L. monocytogenes* on day 0 was observed in Product E (0.93 $\log_{10}$ CFU/sample) while the smallest decrease was observed in Product A (0.35 $\log_{10}$ CFU/sample). As observed with *Salmonella* and *E. coli* O157:H7, with the exception of Product A, the population of *L. monocytogenes* were reduced more quickly in products stored at 21°C compared to 4.4°C. Retention of viability of *L. monocytogenes* was longest in high-fat products (A, B, and F) stored at 4.4°C and 21°C, as was observed with *Salmonella* and *E.coli* O157:H7.

The ability of L. monocytogenes to grow at refrigeration temperatures was demonstrated, as the pathogen grew at 4.4°C in Product A. Growth in Product A also occurred at 21°C. At 4.4°C, populations in Product A decreased from 5.64 $\log_{10}$ CFU/sample on day 0 to 4.69 $\log_{10}$ CFU/sample on day 7, then significantly ($P \leq 0.05$) increased to 5.75 $\log_{10}$ CFU/sample on day 14 and 6.28 $\log_{10}$ CFU/sample on day 21Significant increases in

Table 3.5. Populations of *Listeria monocytogenes* recovered from yellow fat products.

Storage temperature	Storage time	A		B		C		D		E		F	
(°C)	(days)	$\log_{10}$ CFU	e[c]	$\log_{10}$ CFU	e	$\log_{10}$ CFU	e	$\log_{10}$ CFU	e	$\log_{10}$ CFU	e	$\log_{10}$ CFU	e
4.4	0	a 5.64	b	c 5.08	a	c 5.04	a	b 5.24	a	c 4.96	a	b 5.22	a
	1	a 5.48	c	b 4.96	a	c 4.62	ab	b 4.91	a	d 4.29	a	b 4.99	a
	2	a 5.23	d	a 4.82	ab	b 3.87	bc	b 4.30	b	c 3.23	b	b 4.30	b
	3	a 4.74	e	a 4.49	b	b 3.34	c	b 3.48	c	c 1.65	c	b 3.70	c
	7	a 4.69	e	ab 4.10	c	c 1.46	d	c 0.63	d 3	c 0.53	d 3	b 3.00	d
	14	a 5.75	b	b 4.08	c	d 2.16	d	e <1.60	e 0	e <1.60	d 0	c 2.68	de
	21	a 6.28	a	b 3.37	d	d 0.53	e 3	d <1.60	e 0	d <1.60	d 0	c 2.33	e
21	0	a 5.64	c	c 5.08	a	c 5.04	a	b 5.24	a	c 4.96	a	b 5.22	a
	1	a 5.69	c	b 4.97	a	c 3.71	b	d 3.14	b	e 2.54	b	c 3.86	b
	2	a 6.31	b	b 4.76	b	c 2.70	c	d <1.60	c 3	d 0.53	c 3	c 1.67	c 3
	3	a 6.64	a	b 4.41	c	c 1.90	d 3	e <1.60	c 1	e <1.60	c 0	d 0.94	cd 2
	7	a 5.90	c	b <1.60	d 0	b <1.60	e 1	b <1.60	c 0	b <1.60	c 0	b <1.60	d 3
	14	a 4.57	d	b <1.60	d 0	b <1.60	e 0	b <1.60	c 0	b <1.60	c 0	b 0.53	cd 1
	21	a 4.15	e	b <1.60	d 0	b <1.60	e 0	b <1.60	c 0	b <1.60	c 0	b <1.60	d 0

[a] A = Land O'Lakes whipped, salted, sweet cream butter; B = Land O' Lakes whipped, unsalted, sweet cream butter; C = Land O'Lakes Light butter; D = Shedd's Spread Country Crock; E = Blue Bonnet Light; F = Promise

[b] Samples consisted of 4 g of Products A, B, C, D, E, or F. Within storage temperature, mean values in the same column that are not followed by the same letter are significantly different ($P \leq 0.05$). Mean values in the same row that are not preceded by the same letter are significantly different ($P \leq 0.05$).

[c] Number out of three replicates that were positive for *Listeria monocytogenes*.

FIGURE 3.5. Populations of *L. monocytogenes* recovered from yellow fat products stored at 4.4°C for up to 21 days.

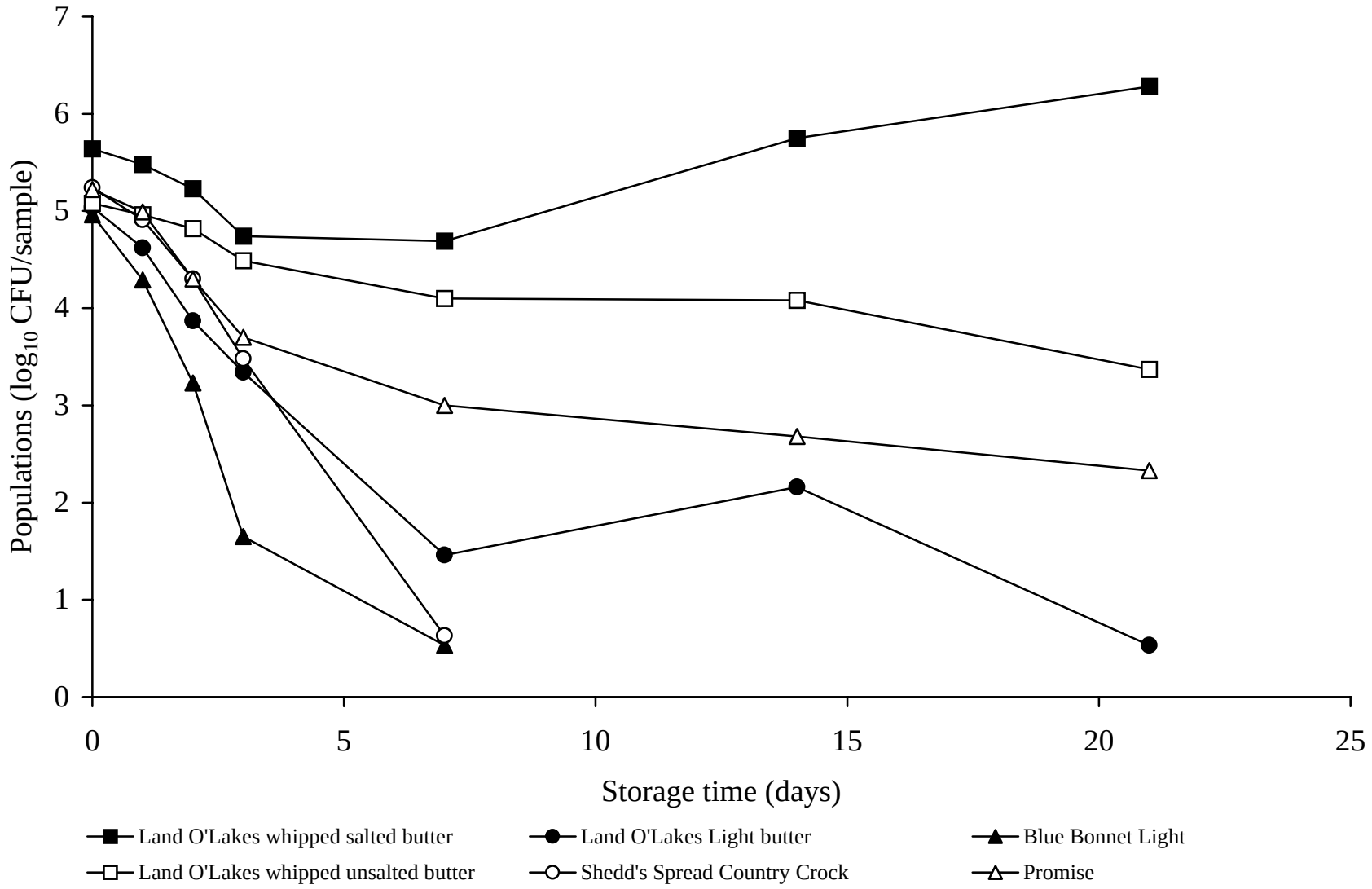
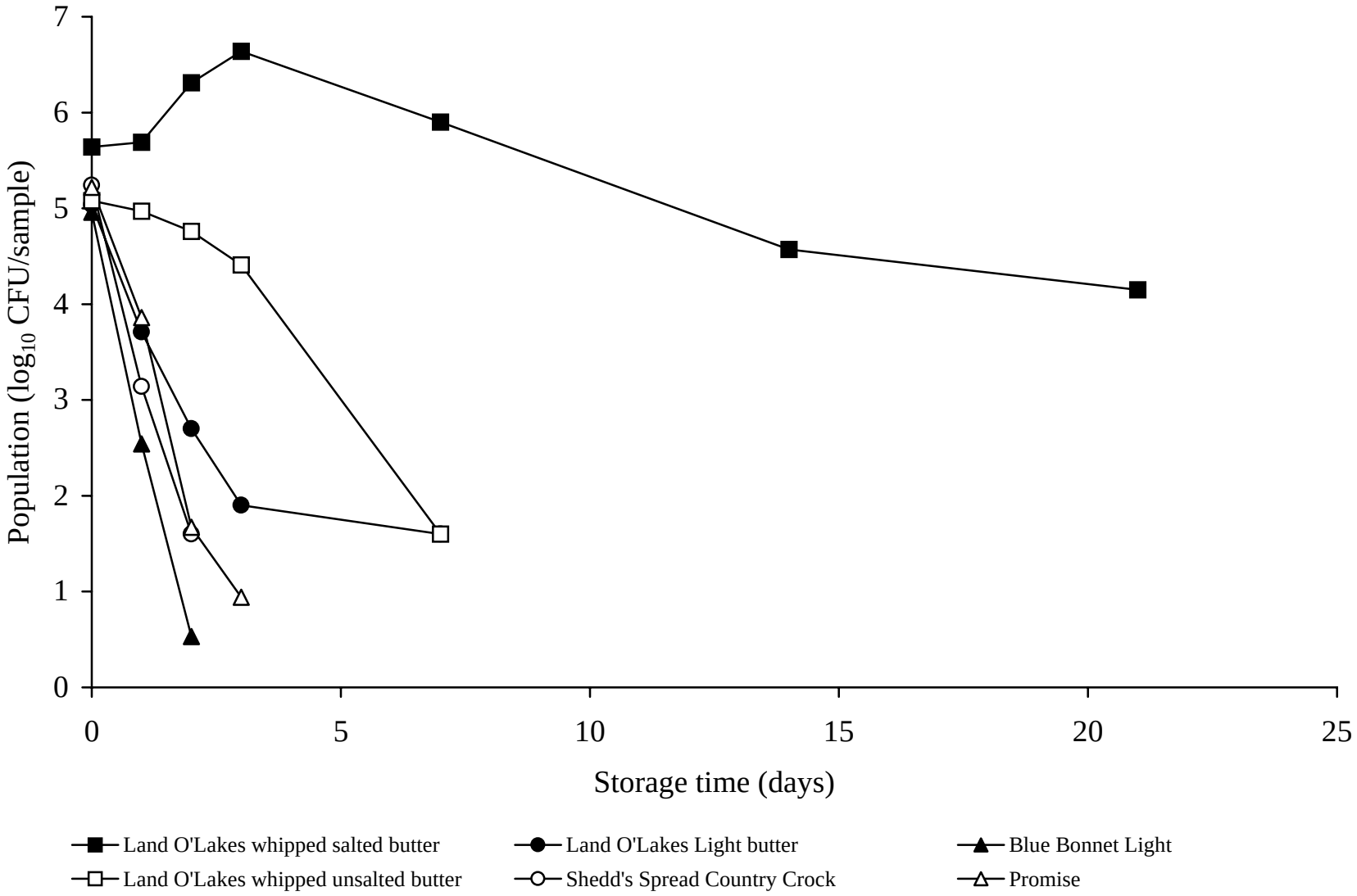

Populations (log10 CFU/sample)
0
1
2
3
4
5
6
7
0
5
10
15
20
25
Storage time (days)
Land O'Lakes whipped salted butter
Land O'Lakes Light butter
Blue Bonnet Light
Land O'Lakes whipped unsalted butter
Shedd's Spread Country Crock
Promise

FIGURE 3.6. Populations of *L. monocytogenes* recovered from yellow fat products stored at 21°C for up to 21 days.


Population (log10 CFU/sample)
0
1
2
3
4
5
6
7
0
5
10
15
20
25
Storage time (days)
Land O'Lakes whipped salted butter
Land O'Lakes Light butter
Blue Bonnet Light
Land O'Lakes whipped unsalted butter
Shedd's Spread Country Crock
Promise

populations were observed within 2 days in Product A stored at 21°C, and continued to significantly increase to 6.64 $\log_{10}$ CFU/sample on day 3. These results are in agreement with observations by Walker et al. (1990), who observed that a reduction in storage temperature resulted in a longer lag time and a slower rate of growth of *L. monocytogenes* in UHT milk.

Populations of *L. monocytogenes* decreased most rapidly in Products D and E at both storage temperatures. This trend was observed with the same products inoculated with *E. coli* O157:H7. *Listeria monocytogenes* survived longer in Product C stored at 4.4°C (0.53 $\log_{10}$ CFU/sample after 21 days compared to an undetectable level after 7 days at 21°C). Lanciotti et al. (1992) reported that the death of *L. monocytogenes* in light butter products was not attributable to a low pH. In their study, light butter products had a mean pH value of 6.5, yet death of *L. monocytogenes* occurred. The lower pH of products evaluated in our study, in combination with the adverse effects of salt and preservatives, may have contributed to increased rates of reduction in populations of all test pathogens.

In summary, *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* were not able to grow in sweet cream unsalted whipped butter or in the of the three margarine type products. Growth of the three pathogens was observed in the sweet cream whipped butter containing salt (Product A) at 21°C but not in the same base product without salt (Product B). Growth of *L. monocytogenes* was also observed in sweet cream salted whipped butter between 7 and 14 days of storage at 4.4°C, and continued through 21 days. Reductions in populations in products stored at 4.4 or 21°C are attributed in part to an . Reductions in populations of *Salmonella* and *E. coli* o157:H7 in Product A at 21°C occurred in later stages of the 21-day storage period, suggesting that nutrient depletion may also have occurred in this product.

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