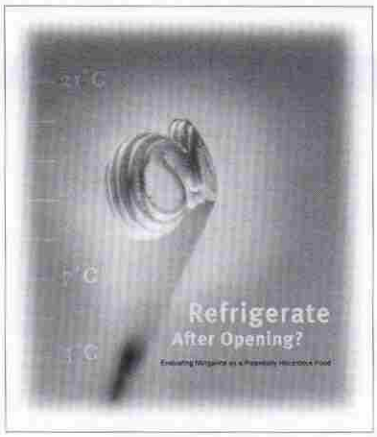


Growth and Survival of Selected Pathogens in Margarine-Style Table Spreads

Ann M. Guentert
Richard H. Linton, Ph.D.

Abstract

Although margarine-style table spreads can have a pH above 4.6 and a water activity greater than 0.85, there is some question if such products can support the growth of pathogenic bacteria. The objective of this study was to evaluate the growth and survival of *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella typhi* in 60-percent- and 70-percent-vegetable-oil, margarine-style, water-in-oil-emulsion table spreads stored at different temperatures. Samples of 25 grams of each table spread were inoculated with 1×10^3 cells of each bacterial mixture. The samples were stored at 5°C, 7°C, and 21°C, and the microbial population in colony-forming units per gram (CFU/gram) was enumerated over time. In almost all storage conditions, bacterial levels were shown to decrease over time. Inactivation was observed in (listed from fastest to slowest, respectively) *S. aureus*, *L. monocytogenes*, *E. coli* O157:H7, and *S. typhi*. Growth was observed only for *S. typhi* in table spreads stored at 21°C, but the rate of growth was extremely slow. Based on these findings, the table spreads evaluated in this study are not potentially hazardous foods, and cold temperature storage is not necessary from a food safety perspective.

Introduction

The Food Code (Food and Drug Administration [FDA], 1999) defines a potentially hazardous food as "a food that is natural or synthetic and that requires temperature control because it is in a form capable of supporting the rapid and progressive growth of infectious or toxigenic microorganisms." A potentially hazardous food has a pH greater than 4.6 and a water activity (a_w) greater than 0.85. The Food Code recommends storage of potentially hazardous foods at or below 5°C (41°F), and most states require storage of potentially hazardous foods at or below 7°C (45°F) to reduce the risk of microbial growth. Some foods that have suitable pH and a_w characteristics may not be considered potentially hazardous because they do not support the "rapid and progressive growth" of harmful microorganisms. There has been debate about whether margarine-style table spreads truly constitute potentially hazardous foods, and there has been further discussion regarding the safe handling of table spreads. More information on microbial growth in table spreads is needed to determine safe recommendations for temperature storage conditions.

Margarine-style edible table spreads are formulated as 5 to 80 percent water-in-oil emulsions with added milk solids, salt, preservatives such as sodium benzoate and potassium sorbate, and additional ingredients such as acidulants and colorants (Lund, Baird-Parker, & Gould, 2000). Their physical characteristics suggest the ability to support growth or survival of harmful microorganisms under appropriate temperature conditions and oxygen availability. The pH of these spreads generally ranges from 3.6 to 6.0, and the water activity is usually at 0.87 or higher (Troller, 1983). Microbial growth is limited to the aqueous phase of the table spreads (Lund et al.). The manufacture of table spreads is not very different from the manufacture of butter or margarine, but, according to Lund and coauthors, dairy and nondairy table spreads are "microbiologically more vulnerable than 80 percent fat spreads and may require additional measures to ensure a safe and wholesome product." Such measures include decontamination of raw materials, pasteurization, and proper hygiene during packing.

The primary safety concerns associated with edible table spreads are incidental contamination by food handlers, the environment, or other sources, and temperature abuse that would permit bacterial growth to occur. While no confirmed outbreaks of foodborne illness have been associated with margarine specifically (Delamarre & Batt, 1999), similar products have been found to be subject to

contamination. An outbreak of more than 265 cases resulted in a recall of blended-margarine-and-butter products after microbial testing revealed contamination with staphylococcal enterotoxin-A and *Staphylococcus intermedius* (FDA, 1992). A whipped-butter product contaminated with *Staphylococcus aureus* was implicated in a foodborne outbreak ("Presumed Staphylococcal," 1977). The death of a woman in the United Kingdom was suspected to be a result of margarine contaminated with *Listeria monocytogenes*, but subsequent microbial testing of the recalled product and throughout the manufacturing facility could not confirm the presence of the organism (Barnes, 1989). In 1999, an outbreak of listeriosis in Finland implicated butter contaminated with *L. monocytogenes* serotype 3a. This organism was detected in environmental and product samples taken from the manufacturing facility (Lyytikäinen et al., 1999).

The purpose of this study was to determine if common table spreads fit the definition of a potentially hazardous food. The investigation was accomplished through evaluation of the growth and survival of selected pathogens under different temperature storage conditions likely to be used in the retail food industry and the homes of consumers. Determination of the ability of pathogens to survive and grow in these products under different storage temperatures will aid in the development of recommendations for storage conditions to ensure safety.

Materials and Methods

Experimental Design

The experiment was designed as a challenge study to determine the survival, growth, and inactivation of four pathogens inoculated into two spread formulations over storage time. Three different storage temperatures were evaluated. The temperatures 5°C (41°F) and 7°C (45°F) were chosen in connection with recommended cold-holding temperatures as described in sections 3-501.16, 3-501.17, 3-501.18, and 4-204.111 of the Food Code (FDA, 1999). The temperature 21°C (70°F) was selected to simulate ambient room temperature and potential temperature abuse conditions. Inoculated samples were placed in incubators at each of the storage temperatures. Samples were pulled in triplicate and enumerated by aerobic plate count on days 0, 1, 4, 7, 10, 14, 21, and 28, and then biweekly for up to 70 days. Enumeration continued three weeks beyond the observation of a nondetectable level of inoculum.

TABLE 1

Temperature, pH, and a_w Conditions Required to Support Growth of Bacterial Species, Compared with Conditions in Which Water-in-Oil Emulsions Were Held

Organism or Table Spread	Temperature	pH	Aw
*Staphylococcus aureus**	7–45°C	4.2–9.3	0.86 or above
*Listeria monocytogenes**	2–45°C	4.8–9.6	0.95 or above
*Escherichia coli**	2.5–45°C	4.6–9.5	0.935 or above
*Salmonella spp.**	6.5–45°C	4.5–NA[a]	0.95 or above
60% emulsion table spread	5°C, 7°C, 21°C	4.9–5.3	0.90 ± 0.15
70% emulsion table spread	5°C, 7°C, 21°C	4.9–5.3	0.90 ± 0.10

[a] NA = not available.
Source: FDA (2001).

FIGURE 1

Growth and Survival of *Staphylococcus aureus* at 5°C, 7°C, and 21°C on a 60% Emulsion Stick Spread (a) and a 70% Emulsion Spread (b)

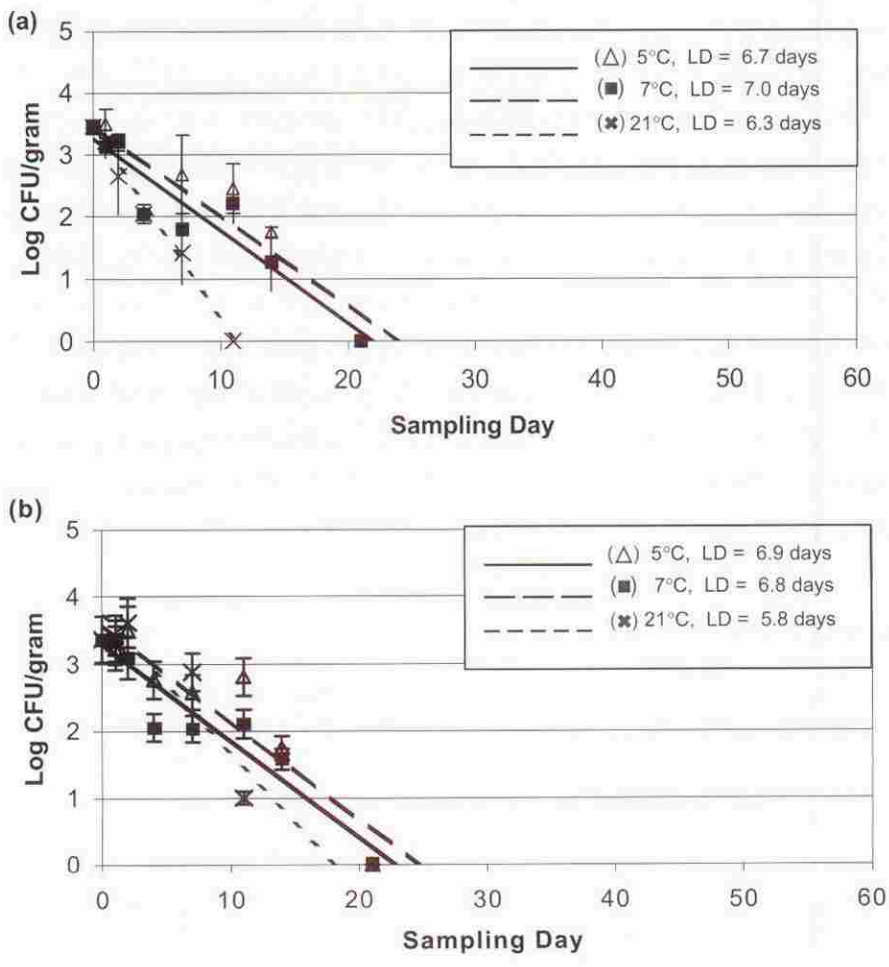
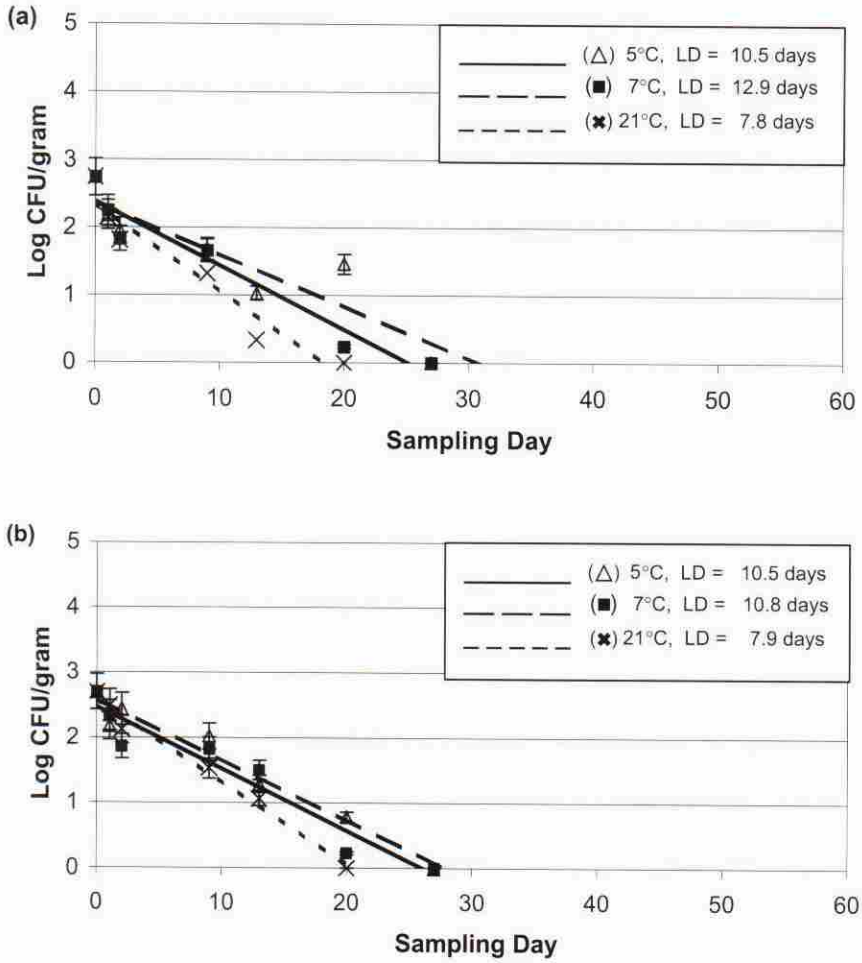


FIGURE 2

Growth and Survival of *Listeria monocytogenes* at 5°C, 7°C, and 21°C on a 60% Emulsion Stick Spread (a) and a 70% Emulsion Spread (b)



Pathogen Selection

Four pathogens were selected for evaluation based on several characteristics. *S. aureus* was selected because it is commonly carried on human skin and can tolerate relatively high levels of salt. *L. monocytogenes* was selected because of the ubiquitous nature and potentially low infective dose of the organism. *S. aureus* and *L. monocytogenes* also were selected because of outbreaks suspected to be associated with similar food products. *E. coli* O157:H7, also characterized by a potentially low infective dose, represents a particularly severe risk because of the potentially fatal nature of its pathological effects. *S. typhi* was selected because it may be present in small numbers in some of the ingredients typically included in spread formulations such as whey, spray-dried milk powder, and buttermilk powder (Charteris, 1996). The concern about low levels of *E. coli* O157:H7 and *S. typhi* is further emphasized in Section 2-201.12 of the Food Code, which calls for exclusion of any food employee from a food establishment if the employee "has a stool that yields a specimen culture that is positive for *Salmonella typhi, Shigella* spp., or *Escherichia coli* O157:H7" (FDA, 1999). Specific strains used in this study included:

- *L. monocytogenes* Scott A, LCDC 81-861 and ATCC 19115;
- *S. aureus* ATCC 5341, 6538 and 8095;
- *E. coli* O157:H7 C7927, MF6707A and A9124-1; and
- *S. typhi*, ATCC 4931, 6962, and 6994.

Table Spread Formulations

Two formulations of commercially available spreads, one spread-style and one stick, were evaluated in the study. The spread-style product was formulated as 60 percent vegetable oil in a water-in-oil emulsion, 1.8 ± 0.12 percent sodium chloride (NaCl), with an aqueous-phase pH of 4.9 to 5.3 and a water activity of 0.90 ± 0.15 (Aqualab Model CX-2 water activity meter, Pullman, Washington). Sodium benzoate and potassium sorbate were present as preservatives and phosphoric acid as an acidulant. The stick product was formulated as 70 percent vegetable oil in a water-in-oil emulsion, 1.8 ± 0.12 percent NaCl, with an aqueous-phase pH of 4.9 to 5.3 and a water activity of 0.90 ± 0.10. Sodium benzoate was present as a preservative and phosphoric acid as an acidulant. Both products remained in a solid state at 5°C (41°F) and 7°C (45°F), and the 60 percent product softened and had a tendency to separate into oil and aqueous phases at 21°C (70°F).

Preparation of the Inoculums

Four separate inoculum mixtures (one for each organism) were prepared by grouping of the three strains of each genus together. Preparation of the inoculum consisted of growing individual cultures in trypticase soy broth (TSB, DIFCO, Detroit, Michigan) at 37°C for 18 hours. Equal volumes of the individual cultures were mixed in a sterile 50-mL centrifuge tube. The mixture was centrifuged, washed, and re-suspended in a volume of phosphate-buffered saline (PBS, Sigma, St. Louis, Missouri) and serially diluted in PBS to a level of approximately 1×10^4 CFU per milliliter (CFU/mL). Before use, cultures were maintained on trypticase soy agar (TSA) slants held at 5°C. An aerobic plate count of the inoculum was used to verify the initial inoculum level and was compared with aerobic plate counts of three samples of each inoculated product at the time of initial inoculation (time zero).

Because of the difficulty of ensuring homogeneous distribution of inoculum throughout a large batch of product and the potential for contamination through sampling of a bulk container (Lund et al., 2000), individual 25-gram samples were dispensed into sterile, covered containers. Each 25-gram sample was inoculated with 100 microliters (µL) of the ~1×10^4 CFU/mL culture mixture. The 100-µL inoculum was distributed in 10 to 12 droplets onto the surface of each 25-gram sample of product. The inoculum was not blended into samples, as blending would have reduced available oxygen and restricted bacterial growth to the confines of the aqueous-phase droplets (Verrips, Smid, & Kerkhof, 1980). Since the surface tension of the aqueous phase would be diminished on the surface of the product, the

aqueous-phase droplets would be larger and provide better access and availability to bacteria added to the surface. Therefore, surface inoculation provided the most conservative conditions for the evaluation of pathogen growth and inactivation. Negative controls were prepared in an identical manner, with sterile PBS substituted for the inoculum. Aerobic plate counts of the two products indicated no detectable background, as there were no visible colonies present after 48 hours of incubation at 37°C.

Samples were prepared in triplicate and in sufficient number to accommodate sampling over 70 days. On each sampling day, three samples and one negative control (un-inoculated) of each spread from each storage temperature and pathogen inoculum were pulled for enumeration. Samples were positioned in each on a numbered grid, which permitted the use of a random number generator to determine random selection of samples to be pulled. Each 25-gram sample was blended in a Seward 400 Lab Stomacher (Seward Medical, London) for one minute in a stomacher bag containing 225 mL of 0.1 percent peptone that had been tempered to 45°C (113°F), serially diluted, and pour-plated into tryptic soy agar (TSA) in accordance with the procedure for the evaluation of butter, margarine, and related products (Marshall, 1993). Plates were enumerated after 48 hours incubation at 37°C. The average log CFU per gram for the three samples was plotted against sampling time in Microsoft Excel to determine the rates of growth, survival, and inactivation for each pathogen under the conditions studied.

Results and Discussion

Information is presented in Table 1 about the conditions required for potential growth and survival of each pathogen and the pH, water activity, and storage conditions of the two table spreads. Given the microbial-growth characteristics, the potential for growth in the table spreads should be limited to *S. aureus*, but the potential for survival is possible for all organisms tested. It is important to determine survival rates of *L. monocytogenes*, *E. coli* O157:H7, and *S. typhi* because they have been reported to cause illness at very low infective-dose levels.

The behavior of each microorganism in each table spread was described by the extent of growth, survival, and inactivation over storage time at each temperature. Results were illustrated graphically with a plotting of log CFU/gram versus sampling time. The slope of a best-fit trend line was generated for each set of treatment conditions. Positive slopes represented growth and negative slopes represented inactivation. The term "LI," for log increase, was used to indicate the time for a 1-log (90 percent) increase, or growth, in cell numbers. The term "LD," for log decrease, was used to indicate the time required for a 1-log (90 percent) decrease, or inactivation, in cell numbers.

In almost all cases, bacterial cell numbers were shown to decrease over sampling time (Figures 1, 2, 3, and 4). *S. aureus* had the fastest rate of inactivation, with LD values ranging from 6.3 to 7.0 days in the 60 percent emulsion and 5.8 to 6.9 days in the 70 percent emulsion (Figure 1a, Figure 1b). Cells could not be detected in any table spread samples after three weeks of storage. Storage temperature and oil content of the emulsion appeared to have little effect. *S. aureus* is a common pathogen found on human skin and nasal passages, and it is often associated with contamination of ready-to-eat ingredients. It is a public-health concern when it grows in foods, and it produces an enterotoxin that, when ingested, causes an intoxication. The table spreads used in this study would not support bacterial growth and toxin production by *S. aureus*. In fact, these products would reduce the level of contamination during storage.

The data for *L. monocytogenes* were very similar to those for *S. aureus*. For the 60 percent and 70 percent emulsion spreads, the LD values ranged from 7.8 to 12.9 days and 7.9 to 10.8 days, respectively (Figure 2a, Figure 2b). The rate of death appeared

FIGURE 3

Growth and Survival of *Escherichia coli* O157:H7 at 5°C, 7°C, and 21°C on a 60% Emulsion Stick Spread (a) and a 70% Emulsion Spread (b)

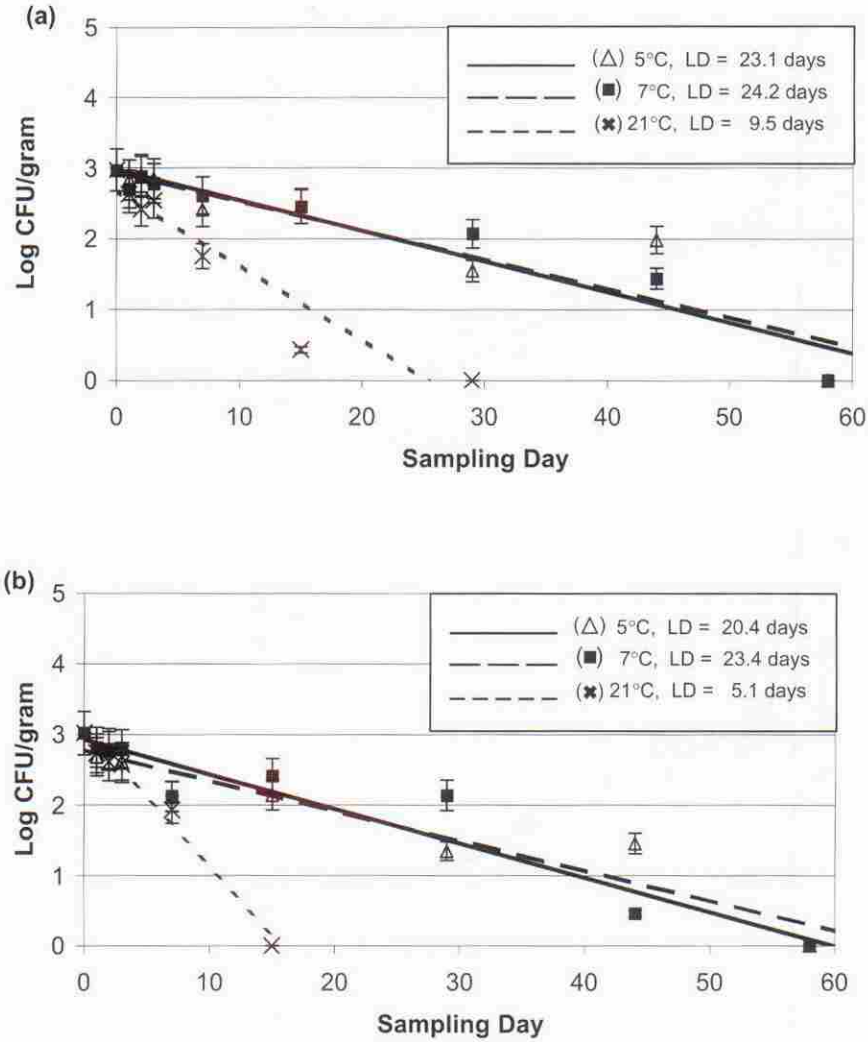
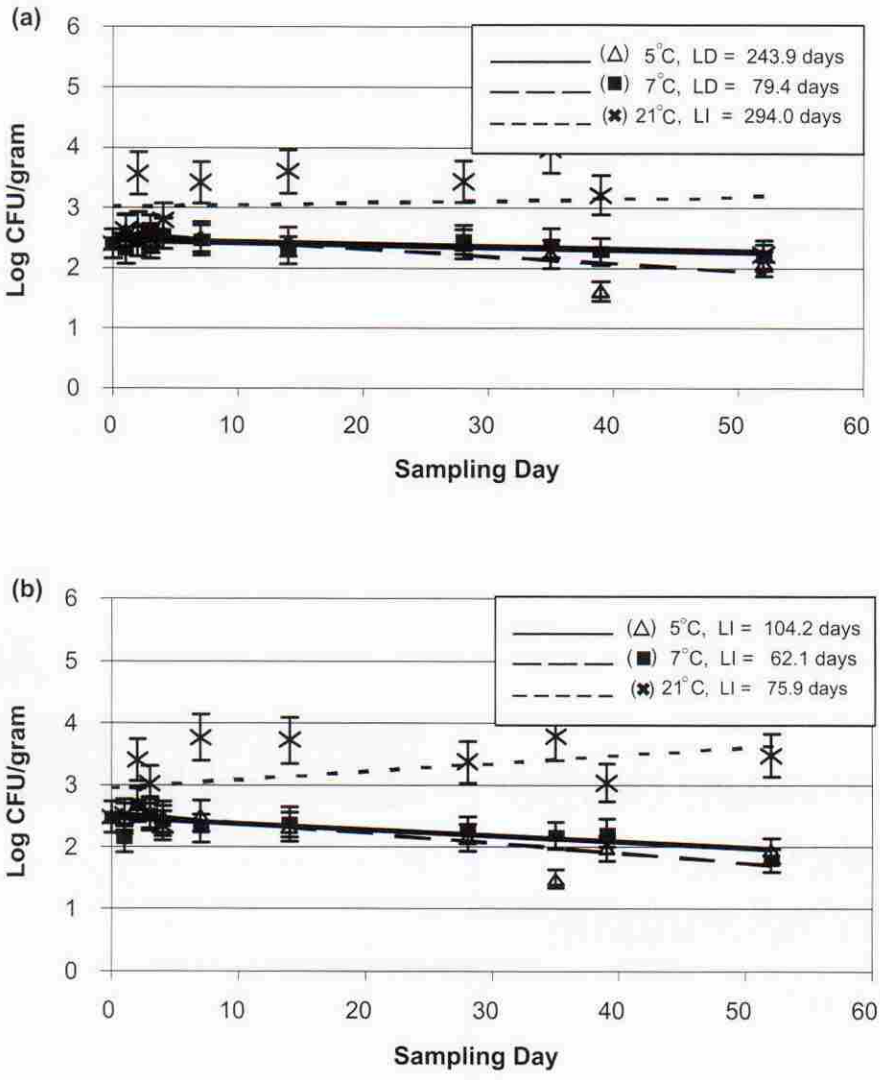


FIGURE 4

Growth and Survival of *Salmonella typhi* at 5°C, 7°C, and 21°C on a 60% Emulsion Stick Spread (a) and a 70% Emulsion Spread (b)



to be slightly faster at 21°C in both table spreads. *L. monocytogenes* is a ubiquitous environmental pathogen that can be found in food-manufacturing plants, retail food establishments, and the homes of consumers. The most important concerns with respect to this pathogen are that the infective dose is thought to be as low as 100 cells and that the organism is capable of growing at temperatures as low as 2°C. The findings of this study demonstrating inactivation in table spreads are reassuring. If table spreads were to be contaminated with *L. monocytogenes*, numbers of the organism would be reduced over storage time.

At colder storage temperatures, *E. coli* O157:H7 was inactivated at a much slower rate than the previously described pathogens (Figure 3a, Figure 3b). Depending on the emulsion oil content, LD values were between 20.4 and 24.2 days. The rate of inactivation was much higher, however, when the table spreads were held at 21°C, at which the LD value was 9.5 days for the 60 percent emulsion and 5.1 days for the 70 percent emulsion. For both *E. coli* and *L. monocytogenes*, the rate of inactivation was faster when the spreads were stored at room temperature (21°C) conditions than when stored at the recommended refrigerated storage temperature (5°C or 7°C).

The behavior of *S. typhi* was quite different. The organisms persisted much longer during storage and even showed signs of growth. In the 60 percent emulsion, the projected LD values were 243.9 days at 5°C and 79.4 days at 7°C. At 5°C and 7°C, LD values were projected out to 104.2 and 62.1 days, respectively, in the 70 percent emulsion. Growth was observed at 21°C in both emulsion mixtures. The projected LI value of the 60 percent emulsion was 294 days and 75.9 days for the 70 percent emulsion. Some data points from the sampling time period, however, suggested a 1-log increase early in storage time.

Conclusion

Storage of both table spreads at the recommended cold-holding temperatures demonstrated a bactericidal effect on *L. monocytogenes*, *S. aureus*, and *E. coli* O157:H7, and a bacteriostatic effect on *S. typhi*. Rates of inactivation in the two formulations of table spreads showed little difference. Whether from human hands, contaminated equipment, or the environment, the level of bacterial contamination in table spreads is likely to be very low. The inability of these table spreads to support growth of low levels of each pathogenic organism indicates a low risk of foodborne illness from incidental contamination.

The goal of this research was to determine if these table spreads were potentially hazardous foods. It was evident that the two spread formulations, when held at 5°C, 7°C, or 21°C, could not support the "rapid and progressive growth" of any of the four pathogens tested. It can be concluded that the edible spread formulations tested in this study should not be classified as potentially hazardous foods as defined by the Food Code (FDA, 1999).

Very little difference was noted between storage at 5°C and 7°C. When the table spreads were stored at 21°C, however, the kinetics of bacterial growth changed. *L. monocytogenes* and *E. coli* died more rapidly at 21°C than at 5°C, and 7°C. *S. typhi* was able to grow when held at 21°C, albeit very slowly. This observation was interesting, since most *Salmonella* species have been reported to require an a_w of 0.95 or higher for growth.

Water-in-oil emulsions present a complex food system whose implications for microbial growth and survival are not fully understood. The inability of *S. aureus* to grow and the inability of *L. monocytogenes* and *E. coli* O157:H7 to survive for longer periods of time are challenging observations to explain. The addition of acidulants and preservatives, although primarily used to inhibit yeast and mold, may play an important role. The volume of the water or oil droplet surrounding the microbial cell may also be an important factor. The dispersion of water droplets in oil

produces an effective microbiological barrier. A contaminating microorganism present in a water droplet would be restricted in its growth either by space limitations or by lack of nutrients in the droplet (Lund et al., 2000). Further study should be dedicated to understanding the mechanisms for microbial growth, survival, and inactivation in water-in-oil emulsion systems.

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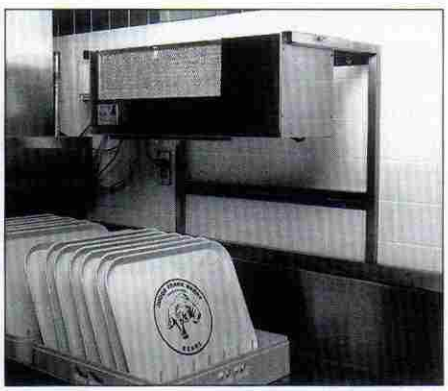

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