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Research Paper

Long-Term Survival of *Listeria monocytogenes* in Nut, Seed, and Legume Butters

Megan L. Fay [1], Joelle K. Salazar [1,*], Xinyuan Zhang [2], Xinyi Zhou [2], Diana S. Stewart [1]

[1] *Division of Food Processing Science and Technology, U. S. Food and Drug Administration, Bedford Park, IL, USA*
[2] *Illinois Institute of Technology, Institute for Food Safety and Health, Bedford Park, IL, USA*

A R T I C L E I N F O

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A B S T R A C T

Nut, seed, and legume butters have become increasingly popular with consumers. *Listeria monocytogenes* contamination of a variety of butters has resulted in several recalls, although no known outbreaks have been identified. *L. monocytogenes* has been shown to survive on a variety of seeds for up to 6 months, legumes and nuts for over 12 months, and in peanut butter and peanut-chocolate spreads for 21 to 60 weeks depending on formulation; however, long-term survival in other butters has not yet been characterized. In this study, the survival of *L. monocytogenes* in various nut, seed, legume, and chocolate-containing butters (n = 10) based on inoculation level, storage temperature, and the pH, a_w, and nutrient contents of the butters was examined. First, butters were inoculated with *L. monocytogenes* at 4 log CFU/g and stored at either 5 or 25°C with enumeration and/or enrichment at intervals over 12 months. *L. monocytogenes* survived in all butters examined with no significant change in population after storage at 5°C, whereas the population was reduced to < 1.70 log CFU/g in as little as 3 months at 25°C; the only exception was for sunflower butter, where *L. monocytogenes* decreased approximately 1 log CFU/g. Subsequently, all butters were inoculated at 1 log CFU/g and stored at 25°C for 6 months with enrichment during storage. *L. monocytogenes* was detected in all butters, except pecan butter, after 6-month storage. Butters containing chocolate did not inhibit *L. monocytogenes* survival, regardless of the inoculation level. Results indicate there may be an association between high-fat and carbohydrate level and survivability of *L. monocytogenes* in various types of butters. This work highlights the need to mitigate the potential for cross-contamination of *L. monocytogenes* into nut, seed, and legume butters due to the potential for long-term survival.

Foodborne illness outbreaks in the U.S. due to contamination of nut, seed, or legume butters have historically been associated with *Salmonella enterica* (CDC, 2007, 2009, 2013, 2022; Harris & Yada, 2019; Sheth et al., 2011) and pathogenic *Escherichia coli* (CDC, 2017). Two of the largest salmonellosis outbreaks in the published literature were linked to peanut butter: one in 2006 causing 715 illnesses in 48 states (CDC, 2007; Sheth et al., 2011) and another in 2008 resulting in 714 illnesses in 46 states (CDC, 2009). More recently, in 2022, a salmonellosis outbreak associated with peanut butter sickened 21 people from 17 states, resulting in four hospitalizations (CDC, 2022). Although no outbreak has been associated with nut, seed, or legume butters due to *Listeria monocytogenes* thus far, recent recalls of peanut, almond, cashew, sesame seed, sunflower seed, and coconut butters have resulted due to possible contamination of this pathogen (FDA, 2018a, 2018b, 2019a, 2019b, 2020) and have highlighted the need for further research.

Manufacturing of these butters includes the same general steps: harvesting/shelling of the nuts, seeds, or legumes, roasting, cooling, blanching/removal of skins, sorting, grinding/milling and mixing, and filling (McHugh, 2016). *L. monocytogenes* is an environmental pathogen capable of contaminating these food products postprocessing. Although roasting of the nuts, seeds, or legumes is conducted at a temperature and length of time sufficient to inactivate *L. monocytogenes*, contamination with *L. monocytogenes* could occur postroasting. Contamination of the mixed ingredients (including the nuts, seeds, or legumes postroasting) could also occur during or after the grinding/milling, mixing, or filling steps.

Nut, seed, and legume butters are low water activity (a_w) foods (a_w < 0.45 (Burnett et al., 2000; He et al., 2013)) and do not support the growth of foodborne bacterial pathogens. However, it is known that *L. monocytogenes*, like *S. enterica*, is capable of surviving on/in low a_w food matrices for extended periods of time. Studies examining

* Corresponding author.
E-mail address: Joelle.Salazar@fda.hhs.gov (J.K. Salazar).

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L. monocytogenes survival on nuts (raw pecans, almonds, and pistachios) have determined that the pathogen could survive 12 months at temperatures ranging from −24 to 24°C (Brar et al., 2015; Kimber et al., 2012). Studies examining the pathogen's survival on legumes (peanuts and chickpeas) have also observed survival over 12 months at −24, 4, and 22°C (for peanuts) (Brar et al., 2015) and 6 months at 25°C (for chickpeas) (Salazar et al., 2019). For seeds (sesame seeds and pine nuts), the pathogen has been observed to survive 6 months at 25°C (Salazar et al., 2019).

Less information, however, is available on the survival of *L. monocytogenes* in nut, seed, and legume butters. Only two studies have assessed *L. monocytogenes* survival in peanut butter (Cunningham et al., 2018; Kenney & Beuchat, 2004). In one study, *L. monocytogenes* declined in population but survived through 24 weeks in peanut butter and chocolate-peanut spread (a_w 0.33 or 0.65) stored at 20°C (Kenney & Beuchat, 2004). In another, the pathogen survived up to 150 to 420 days at 22°C in different formulations of peanut butter (Cunningham et al., 2018). Unfortunately, no information is available on *L. monocytogenes* survival in nut or seed butters; although *L. innocua*, a surrogate for *L. monocytogenes*, has been documented to survive in tahini (sesame seed paste) at temperatures ranging from 10 to 37°C during 28-day storage (Al-Nabulsi et al., 2013). Due to a dearth of information, the aim of this study was to examine the survival of *L. monocytogenes* in different nut, seed, and legume butters based on extrinsic parameters such as inoculation level and storage temperature, as well as intrinsic parameters, including pH, a_w, and nutrient contents of the butters.

Materials and Methods

***L. monocytogenes* strains and inoculum preparation**. Four rifampicin resistant-*L. monocytogenes* strains were utilized in this study: 806 (isolated from hummus), 3132 (isolated from avocado), 0352 (isolated from cream cheese), and ScottA (clinical isolate (Briers et al., 2011)). The four strains were cultured individually in Brain Heart Infusion broth (BHI; Thermofisher Scientific) and incubated at 37°C for 16–18 h. Cultures were washed twice with Butterfield's Phosphate Buffer (BPB, pH 7.2) and combined to form a four-strain cocktail of approximately 9 log CFU/mL. To verify initial levels, the cocktail was serially diluted in BPB and plated onto Brain Heart Infusion agar (BHIA; Thermofisher Scientific) supplemented with 200 µg/mL rifampicin ($BHIA^{rif}$).

Preparation, inoculation, and storage of butters. A selection of nut, seed, legume, and chocolate-containing butters (n = 10) were used in this study (Table 1). Butters were purchased from local retail grocers and online distributors and stored unopened at 25°C prior to use.

For high-level inoculation (4 log CFU/g) trials, butters (2200 g) were placed individually into sterile stainless-steel bowls and inoculated with 2.2 mL of a diluted *L. monocytogenes* cocktail at 4 log CFU/g. Butters were hand-mixed for 15 min using sterile stainless-steel spoons to evenly distribute the inoculum. To verify homogeneity, triplicate 25-g samples from different locations in the mixing bowls were placed into 24 oz. stomacher bags for enumeration of *L. monocytogenes* (see Enumeration and enrichment of *L. monocytogenes*). After mixing, 100-g portions of each butter were transferred to 8 oz. deli containers with lids. Deli containers were stored at 5 or 25°C at 30–33% relative humidity (RH) for up to 12 mo. Three independent trials were conducted with different lots of each butter.

For low-level inoculation (1 log CFU/g) trials, butters (25-g portions) were placed into stomacher bags and individually inoculated with 25 uL of a diluted *L. monocytogenes* cocktail at 1 log CFU/g. Bags were stored at 25°C at 30–33% RH for up to 6 mo. Two independent trials were conducted with different lots of each butter.

Enumeration and enrichment of *L. monocytogenes*. For high-level inoculation experiments, a deli container was removed from 5 and 25°C storage after 0, 1, 3, 6, and 12 mo. Triplicate 25-g samples were homogenized with 225 mL BPB in a stomacher (Stomacher 400 Circulator) for 1 min. Serial dilutions of the homogenate were plated onto $BHIA^{rif}$. Agar plates were incubated at 37°C for 24–48 h. When the population was below the limit of enumeration of the plate count assay (1.7 log CFU/g), the samples were enriched using Buffered *Listeria* Enrichment Broth (BLEB; Thermofisher Scientific) according to the FDA BAM (FDA, 2017).

For low-level inoculation experiments, triplicate 25-g samples were removed from 25°C storage after 0, 1, 2, 4, and 6 mo. The samples were enriched using BLEB according to the FDA BAM (FDA, 2017).

Measurement of pH and a_w of butters. For the high-level inoculation trials, the pH and a_w of each butter were measured immediately after opening (time 0) and after 1, 3, 6, and 12 mo storage at 5 and 25°C. For pH, triplicate 5-g samples were homogenized with 10 mL water and the pH of the homogenate was measured using a pH meter (MP220 pH meter, Mettler Toledo). For a_w, triplicate 1-g samples were measured using a water activity meter (Aqualab 4TE, Meter Group).

Statistical analysis. Differences in pH, aw, or *L. monocytogenes* populations between different butters or times were assessed using Student's t test or ANOVA with Tukey's posttest. When enrichments were used, the differences in the detection of *L. monocytogenes* between butters or times were determined using Fisher's Exact test. A p value less than 0.05 was considered significant.

Table 1
Nutrient contents of the nut, legume, seed, and chocolate-containing butters used in this study

Butter category	Butter type	Nutrient content[a]			
		Fat (%)	Carbohydrate (%)	Protein (%)	Salt (%)
Nut	Almond	56.25	18.75	21.88	0.02
	Hazelnut	60.71	17.86	14.29	0.00
	Pecan	75.00	12.50	9.38	0.00
Legume	Peanut	50.00	18.75	21.88	0.47
	Soy	43.75	28.13	21.88	0.44
Seed	Pumpkin	64.29	5.36	28.57	0.32
	Sesame	53.13	25.00	18.75	0.14
	Sunflower	56.25	15.63	21.88	0.41
Chocolate-containing	Hazelnut	29.73	59.46	5.41	0.04
	Peanut	40.63	34.38	18.75	0.14

[a] As specified on nutrient label.

Results

Characteristics of the butters used in this study

The fat, carbohydrate, protein, and salt contents of the 10 butters used in this study are listed in Table 1, while the initial pH and a_w values are presented in Tables 2, 3, 4, and 5 for nut, seed, legume, and chocolate-containing butters, respectively. The fat contents of the butters ranged from 29.73% (hazelnut) to 75.00% (pecan). The carbohydrate contents ranged from 5.36% (pumpkin) to 59.46% (hazelnut with chocolate). For protein, the lowest value was 5.41% (hazelnut with chocolate) and the highest was 28.57% (sunflower). While two of the butters did not have salt (hazelnut and pecan), the highest salt content was 0.47% (peanut).

The initial (time 0) pH values of the 10 butters ranged from 5.84 ± 0.13 (almond) to 6.57 ± 0.01 (hazelnut with chocolate). The pH values of the almond, sesame, and sunflower butters (5.84 ± 0.13, 5.97 ± 0.02, and 5.95 ± 0.01, respectively) were significantly lower than all the other butters. Alternatively, the pH values of the soy, hazelnut with chocolate, and the peanut with chocolate butters (6.47 ± 0.04, 6.57 ± 0.01, and 6.54 ± 0.04, respectively) were significantly higher than all the other butters. The initial a_w values of the butters ranged from 0.221 ± 0.008 (sunflower) to 0.436 ± 0.015 (hazelnut). The a_w of two of the seed butters (sesame and sunflower at 0.232 ± 0.005 and 0.221 ± 0.008, respectively) were significantly lower than all the other butters. In addition, the a_w of the nut butters (almond (0.412 ± 0.021), hazelnut (0.436 ± 0.015), and pecan (0. 390 ± 0.027)) were significantly higher than all the other butters.

pH and a_w dynamics of the butters during storage

The pH and a_w of the butters during 12 mo storage at both 5 and 25°C are shown in Tables 2, 3, 4, and 5 for nut, seed, legume, and chocolate-containing butters, respectively. The pH values of all butters were fairly consistent during 12 mo storage at both temperatures. At 5°C storage, the pH values of four butters (hazelnut, sunflower, peanut, soy, hazelnut with chocolate, and peanut with chocolate) significantly decreased after 12 mo. The greatest decrease was observed for peanut with chocolate butter (6.54 ± 0.04 to 6.26 ± 0.08 after 12 mo). At 25°C, the pH of sesame, sunflower, and soy butter significantly decreased. The greatest decrease was observed for sunflower butter (5.95 ± 0.01 to 5.57 ± 0.19 after 12 mo).

During 12 mo storage, a downward trend in a_w was observed for all butters at both temperatures. In general, a_w values were lowest after 6 mo storage, at times returning to initial levels after 12 mo. For nut butters, all three butters significantly decreased in a_w at both temperatures after 6 mo storage. The greatest decrease in a_w was observed for hazelnut butter at 25°C (0.446 ± 0.021 to 0.300 ± 0.004) after 12 mo. For seed butters, the a_w of sesame butter was significantly lower at 6 mo storage at 25°C after 6 mo compared to the initial value. The a_w of both pumpkin and sunflower butter did not significantly change during storage at both temperatures. For legume butters, the a_w of both butters decreased after 6 mo storage, however, the initial a_w values did not significantly differ from the values observed after 12 mo. Finally, for the two butters with chocolate, the a_w decreased significantly after 6 mo storage at both temperatures. After 12 mo, the a_w of the hazelnut with chocolate butter was significantly lower than the initial value when stored at 25°C.

Survival of *L. monocytogenes* in high-level inoculated butters

The average initial inoculation level of *L. monocytogenes* in the butters was 3.47 ± 0.22 and 3.59 ± 0.36 prior to storage at 5 and 25°C, respectively, with no significant difference between individual butters. For nut butters (Fig. 1), no significant change in *L. monocytogenes* population was observed during storage at 5°C, with reductions of 0.85, 1.05, and 0.93 log CFU/g after 12 months for almond, hazelnut, and pecan butter, respectively. At 25°C, the *L. monocytogenes* population dropped below 1.70 log CFU/g in all nut butters after 3 mo storage. After 12 mo, *L. monocytogenes* was still detected in both almond (4/9) and pecan (1/9) butter. While the pathogen was detected in hazelnut butter after 6 mo (9/9), it was not detected after 12 mo storage.

For seed butters (Fig. 2), no significant change in *L. monocytogenes* population was observed during storage at 5°C, with reductions of only 0.40, 0.43, and 0.37 log CFU/g after 12 months for pumpkin, sesame, and sunflower seed butter, respectively. At 25°C, the *L. monocytogenes* population dropped below 1.70 log CFU/g in pumpkin and sesame seed butter after 6 and 4 mo storage, respectively. After 12 mo, *L. monocytogenes* was still detected in both pumpkin (4/9) and sesame seed (3/9) butter. For sunflower butter, the *L. monocytogenes* population was still enumerable after 12 mo storage, reducing by only 0.91 log CFU/g after 12 mo.

For legume butters (Fig. 3), no significant change in *L. monocytogenes* population was observed during storage at 5°C, with reductions of 0.84 and 0.05 log CFU/g after 12 months for peanut and soy butter, respectively. At 25°C, the *L. monocytogenes* population dropped below 1.70 log CFU/g in both butters after 4 mo storage. After 12 mo, *L.*

Table 2
pH and water activity (a_w) of the nut butters during storage at 5 and 25°C for 1 year

Butter type	Time (months)	5°C		25°C	
		pH ± SD[b]	a_w[a] ± SD	pH ± SD	a_w ± SD
Almond	0	5.84 ± 0.13 [aA]	0.412 ± 0.021 [aA]	5.84 ± 0.13 [aA]	0.412 ± 0.021 [aA]
	1	6.19 ± 0.27 [bA]	0.446 ± 0.110 [aA]	6.09 ± 0.21 [bA]	0.467 ± 0.053 [bA]
	3	5.92 ± 0.09 [acA]	0.325 ± 0.093 [acA]	5.90 ± 0.16 [acA]	0.217 ± 0.046 [cB]
	6	5.73 ± 0.18 [acA]	0.216 ± 0.040 [bA]	5.86 ± 0.07 [acA]	0.189 ± 0.014 [cA]
	12	5.77 ± 0.20 [acA]	0.324 ± 0.095 [acA]	5.77 ± 0.08 [acA]	0.308 ± 0.022 [dA]
Hazelnut	0	6.18 ± 0.04 [aA]	0.436 ± 0.015 [aA]	6.18 ± 0.04 [abA]	0.446 ± 0.021 [aA]
	1	6.16 ± 0.10 [aA]	0.454 ± 0.090 [aA]	6.21 ± 0.12 [aA]	0.438 ± 0.090 [aA]
	3	6.21 ± 0.17 [aA]	0.301 ± 0.101 [bA]	6.14 ± 0.13 [abA]	0.255 ± 0.087 [bcA]
	6	6.11 ± 0.08 [aA]	0.254 ± 0.092 [bcA]	6.08 ± 0.07 [bA]	0.202 ± 0.038 [bA]
	12	5.97 ± 0.05 [bA]	0.321 ± 0.084 [bcA]	6.15 ± 0.09 [abB]	0.300 ± 0.004 [cA]
Pecan	0	6.09 ± 0.02 [abA]	0.390 ± 0.027 [acA]	6.09 ± 0.02 [aA]	0.390 ± 0.027 [aA]
	1	6.19 ± 0.16 [aA]	0.429 ± 0.088 [aA]	6.11 ± 0.22 [aA]	0.451 ± 0.032 [aA]
	3	6.06 ± 0.30 [abA]	0.290 ± 0.075 [bA]	5.88 ± 0.11 [bA]	0.241 ± 0.029 [bcA]
	6	5.91 ± 0.10 [bA]	0.227 ± 0.071 [bA]	5.88 ± 0.05 [bA]	0.192 ± 0.016 [bA]
	12	5.98 ± 0.10 [abA]	0.300 ± 0.088 [bcA]	6.01 ± 0.08 [abA]	0.302 ± 0.100 [cA]

Different lowercase letters indicate significant difference between pH or a_w values for the same butter at different times (columns). Different uppercase letters indicate significant difference between pH or a_w values for the same butter at the same time (rows).

[a] Water activity.

[b] Standard deviation (n = 9).

Table 3
pH and water activity (a_w) of the seed butters during storage at 5 and 25°C for 1 year

Butter type	Time (months)	5°C		25°C	
		pH ± SD[b]	a_w[a] ± SD[b]	pH ± SD[b]	a_w[a] ± SD[b]
Pumpkin	0	6.19 ± 0.09 aA	0.282 ± 0.018 aA	6.19 ± 0.09 aA	0.282 ± 0.018 aA
	1	6.17 ± 0.04 aA	0.321 ± 0.018 aA	6.20 ± 0.04 aA	0.363 ± 0.083 aA
	3	6.14 ± 0.09 aA	0.266 ± 0.135 aA	6.10 ± 0.13 aA	0.191 ± 0.045 aA
	6	6.03 ± 0.13 aA	0.213 ± 0.057 aA	5.96 ± 0.22 aA	0.192 ± 0.019 aA
	12	5.80 ± 0.58 aA	0.289 ± 0.093 aA	5.80 ± 0.07 aA	0.291 ± 0.002 aA
Sesame	0	5.97 ± 0.02 aA	0.232 ± 0.005 abA	5.97 ± 0.02 aA	0.232 ± 0.005 aA
	1	6.12 ± 0.09 bA	0.291 ± 0.045 aA	5.96 ± 0.02 aB	0.350 ± 0.041 bB
	3	6.08 ± 0.09 bA	0.216 ± 0.092 abA	5.98 ± 0.10 aB	0.215 ± 0.008 aA
	6	5.91 ± 0.04 aA	0.176 ± 0.034 bA	5.94 ± 0.08 aA	0.162 ± 0.020 cA
	12	5.84 ± 0.08 aA	0.231 ± 0.068 abA	5.82 ± 0.06 bA	0.246 ± 0.055 aA
Sunflower	0	5.95 ± 0.01 aA	0.221 ± 0.008 abcA	5.95 ± 0.01 aA	0.221 ± 0.008 aA
	1	5.97 ± 0.03 aA	0.287 ± 0.059 aA	6.03 ± 0.03 aB	0.303 ± 0.063 bA
	3	6.09 ± 0.07 bA	0.244 ± 0.050 aA	5.98 ± 0.06 aB	0.224 ± 0.093 abA
	6	5.86 ± 0.06 cA	0.210 ± 0.070 bA	5.71 ± 0.21 bA	0.183 ± 0.013 aA
	12	5.83 ± 0.09 cA	0.195 ± 0.038 cA	5.57 ± 0.19 bB	0.256 ± 0.068 aB

Different lowercase letters indicate significant difference between pH or a_w values for the same butter at different times (columns). Different uppercase letters indicate significant difference between pH or a_w values for the same butter at the same time (rows).
[a] Water activity.
[b] Standard deviation (n = 9).

Table 4
pH and water activity (a_w) of the legume butters during storage at 5 and 25°C for 1 year

Butter type	Time (months)	5°C		25°C	
		pH ± SD[b]	a_w[a] ± SD[b]	pH ± SD[b]	a_w[a] ± SD[b]
Peanut	0	6.20 ± 0.04 abA	0.272 ± 0.048 aA	6.20 ± 0.04 abA	0.272 ± 0.048 acA
	1	6.17 ± 0.18 abA	0.312 ± 0.035 aA	6.21 ± 0.05 abA	0.325 ± 0.023 aA
	3	6.25 ± 0.05 aA	0.279 ± 0.113 aA	6.40 ± 0.38 aA	0.230 ± 0.038 bcA
	6	6.09 ± 0.08 bcA	0.178 ± 0.039 bA	6.01 ± 0.14 bA	0.173 ± 0.024 bA
	12	6.02 ± 0.05 cA	0.248 ± 0.077 abA	6.07 ± 0.34 bA	0.258 ± 0.070 cA
Soy	0	6.47 ± 0.04 aA	0.308 ± 0.008 aA	6.47 ± 0.04 aA	0.308 ± 0.008 acA
	1	6.45 ± 0.14 aA	0.384 ± 0.087 aA	6.46 ± 0.05 acA	0.369 ± 0.084 aA
	3	6.44 ± 0.02 aA	0.326 ± 0.067 aA	6.46 ± 0.10 acA	0.197 ± 0.040 bdB
	6	6.31 ± 0.09 bA	0.206 ± 0.045 bA	6.30 ± 0.03 bA	0.193 ± 0.033 bA
	12	6.29 ± 0.06 bA	0.305 ± 0.084 aA	6.38 ± 0.06 bcB	0.265 ± 0.066 cdA

Different lowercase letters indicate significant difference between pH or a_w values for the same butter at different times (columns). Different uppercase letters indicate significant difference between pH or a_w values for the same butter at the same time (rows).
[a] Water activity.
[b] Standard deviation (n = 9).

Table 5
pH and water activity (a_w) of the chocolate-containing butters during storage at 5 and 25°C for 1 year

Butter type	Time (months)	5°C		25°C	
		pH ± SD[b]	a_w[a] ± SD[b]	pH ± SD[b]	a_w[a] ± SD[b]
Hazelnut with chocolate	0	6.57 ± 0.01 aA	0.359 ± 0.011 aA	6.57 ± 0.01 aA	0.359 ± 0.011 aA
	1	6.45 ± 0.21 abA	0.374 ± 0.018 aA	6.58 ± 0.22 aA	0.433 ± 0.059 bB
	3	6.26 ± 0.16 bA	0.312 ± 0.137 aA	6.54 ± 0.08 abB	0.224 ± 0.002 cA
	6	6.42 ± 0.18 abA	0.189 ± 0.029 bA	6.34 ± 0.26 bA	0.195 ± 0.041 cA
	12	6.33 ± 0.16 bA	0.276 ± 0.088 abA	6.49 ± 0.07 abB	0.288 ± 0.020 dA
Peanut with chocolate	0	6.54 ± 0.04 aA	0.370 ± 0.014 acA	6.54 ± 0.04 aA	0.370 ± 0.014 acA
	1	6.50 ± 0.04 aA	0.430 ± 0.109 aA	6.55 ± 0.05 aB	0.416 ± 0.141 aA
	3	6.52 ± 0.07 aA	0.279 ± 0.059 bcA	6.43 ± 0.32 aA	0.233 ± 0.083 bA
	6	6.40 ± 0.08 bA	0.220 ± 0.061 bA	6.37 ± 0.09 aA	0.189 ± 0.018 bA
	12	6.26 ± 0.08 cA	0.288 ± 0.075 bcA	6.38 ± 0.08 aB	0.282 ± 0.088 bcA

Different lowercase letters indicate significant difference between pH or a_w values for the same butter at different times (columns). Different uppercase letters indicate significant difference between pH or a_w values for the same butter at the same time (rows).
[a] Water activity.
[b] Standard deviation (n = 9).

monocytogenes was still detected in both peanut (2/9) and soy (3/9) butter.

For the two chocolate-containing butters (Fig. 4), there was also no significant change in *L. monocytogenes* population during storage at 5°C, with reductions of 0.83 log CFU/g for both hazelnut with chocolate and peanut with chocolate butter after 12 months. At 25°C, the *L. monocytogenes* population dropped below 1.70 log CFU/g in hazelnut with chocolate and peanut with chocolate butter after 5 and 4 mo stor-

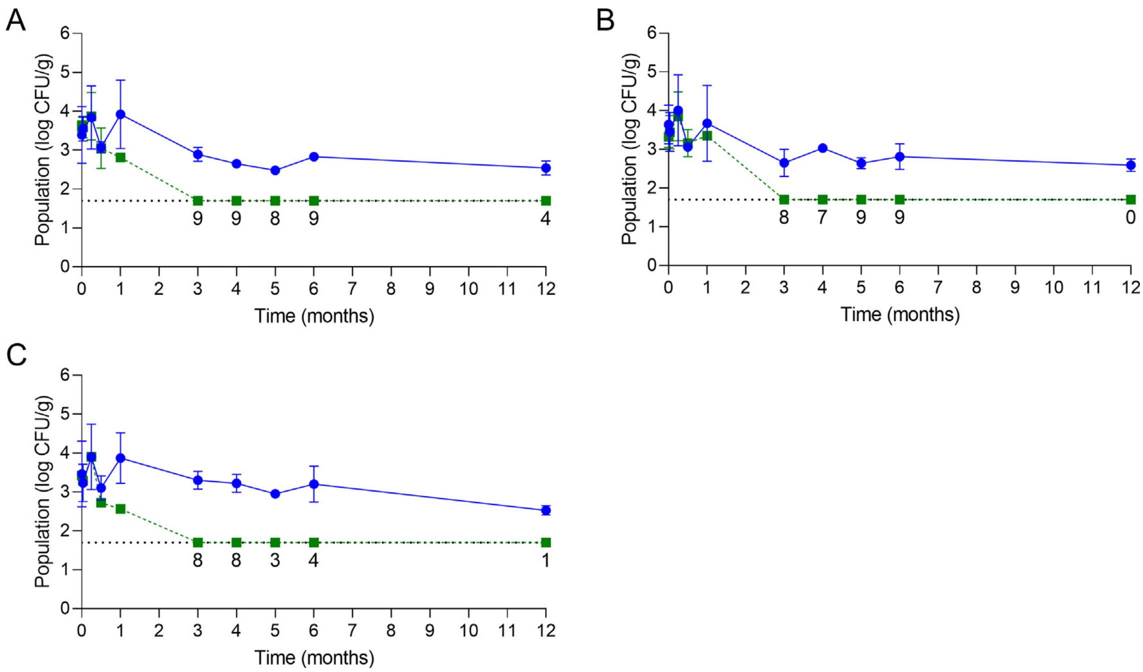

Figure 1. Survival of *L. monocytogenes* in nut butters during storage at 5°C (blue) and 25°C (green). A) Almond; B) Hazelnut; C) Pecan. Data points and error bars represent mean and standard deviation (n = 9). Horizontal dotted line represents the limit of enumeration via plate count assay (1.70 log CFU/g). Data points at the limit of enumeration represent that the samples were enriched and the number below indicates the number of samples where *L. monocytogenes* was detected out of 9 total.

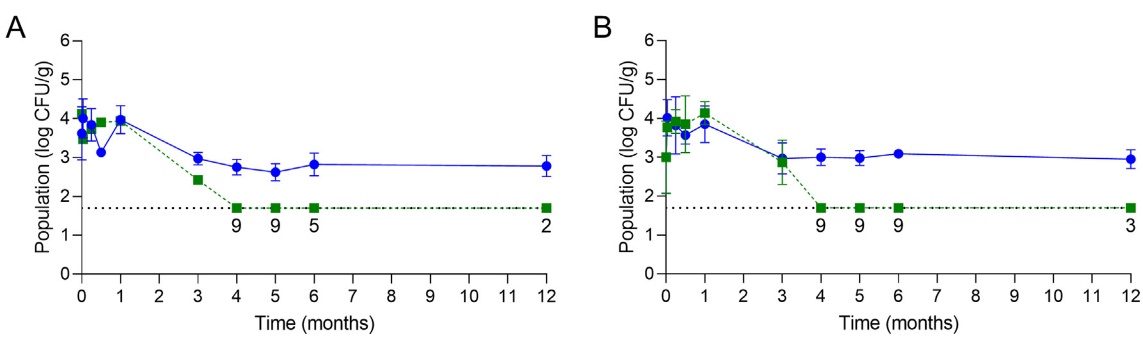

Figure 2. Survival of *L. monocytogenes* in seed butters during storage at 5°C (blue) and 25°C (green). A) Pumpkin; B) Sesame; C) Sunflower. Data points and error bars represent mean and standard deviation (n = 9). Horizontal dotted line represents the limit of enumeration via plate count assay (1.70 log CFU/g). Data points at the limit of enumeration represent that the samples were enriched and the number below indicates the number of samples where *L. monocytogenes* was detected out of 9 total.

age, respectively. After 12 mo, *L. monocytogenes* was still detected in both hazelnut with chocolate (6/9) and peanut with chocolate (1/9) butter.

Survival of *L. monocytogenes* in low-level inoculated butters

Since the greatest decrease in *L. monocytogenes* population was observed at 25°C for high-level inoculation trials, butters were inoculated at a low level (1 log CFU/g) and stored at 25°C for 6 mo to determine if this time/temperature combination could eliminate the pathogen. Prior to storage at 25°C for 6 mo, *L. monocytogenes* was detected in all butter samples (6/6) (Table 6). After 1 mo storage, no significant difference in the detection of *L. monocytogenes* was observed for any of the butters with the exception of hazelnut, where the pathogen was detected in only 2/6 samples. After 4 mo, there was also a significant decrease in the detection of *L. monocytogenes* in sesame seed butter (1/6). After 6 mo, *L. monocytogenes* was still detected in 6/6 samples for soy and peanut with chocolate butter. *L. monocytogenes* was not detected in pecan butter after 6 mo.

Discussion

Due to recent recalls of nut, seed, and legume butters associated with *L. monocytogenes* contamination (FDA, 2018a, 2018b, 2019a, 2019b, 2020), this study aimed to examine the pathogen's ability to survive long-term in select butters stored at 5 or 25°C. Since the typical shelf-life of these butters ranges from 6-9 months unopened and 2–9 months once opened (NPB; USDA, 2019), this study initially examined *L. monocytogenes* survival at both 5 and 25°C during 12-month storage when contamination occurred at a high level (4 log CFU/g). Results of this study suggest that storage of nut, seed, and legume butters at 5°C, compared to 25°C, provides a more conducive environment for the survival of *L. monocytogenes*. While *L. monocytogenes* survived in all butters examined with no significant change in population after 12-month storage at 5°C, the pathogen population was reduced to <1.70 log CFU/g in as little as 3 months at 25°C; the only exception

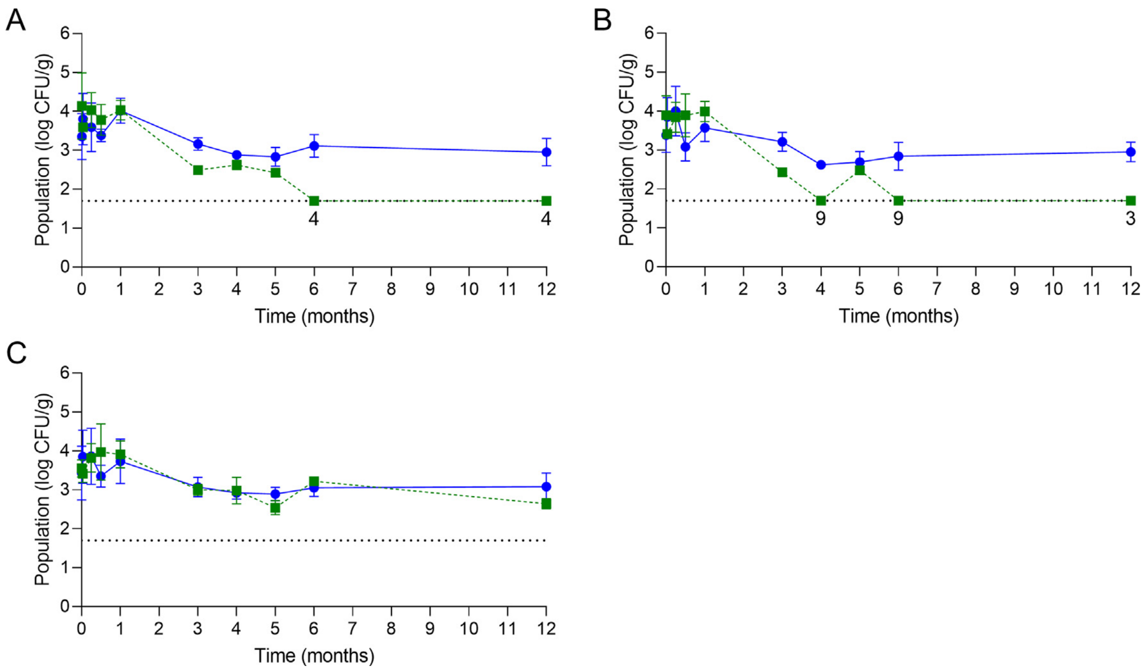

Figure 3. Survival of *L. monocytogenes* in legume butters during storage at 5°C (blue) and 25°C (green). A) Peanut; B) Soy. Data points and error bars represent mean and standard deviation (n = 9). Horizontal dotted line represents the limit of enumeration via plate count assay (1.70 log CFU/g). Data points at the limit of enumeration represent that the samples were enriched and the number below indicates the number of samples where *L. monocytogenes* was detected out of 9 total.

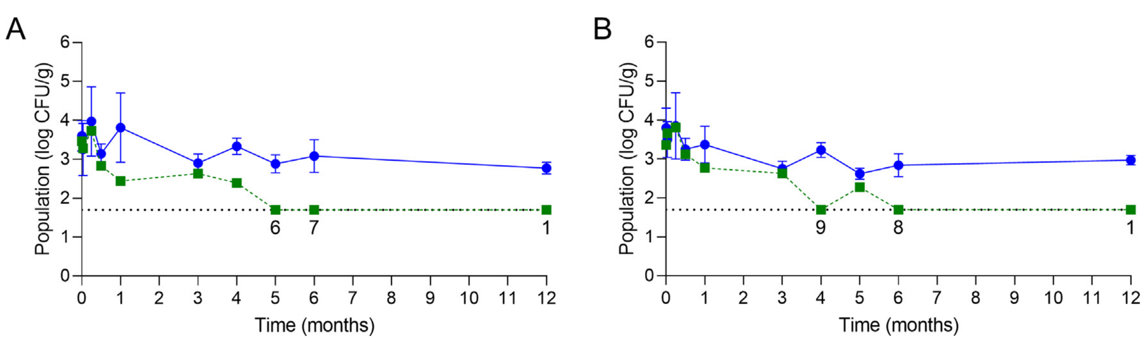

Figure 4. Survival of *L. monocytogenes* in butters containing chocolate during storage at 5°C (blue) and 25°C (green). A) Hazelnut; B) Peanut. Data points and error bars represent mean and standard deviation (n = 9). Horizontal dotted line represents the limit of enumeration via plate count assay (1.70 log CFU/g). Data points at the limit of enumeration represent that the samples were enriched and the number below indicates the number of samples where *L. monocytogenes* was detected out of 9 total.

Table 6
Detection of *L. monocytogenes* in nut, legume, seed, and chocolate-containing butters inoculated at 1 log CFU/g during 25°C storage for 6 months

Time (months)	No. of samples where *L. monocytogenes* was detected (n = 6)									
	Nut			Legume		Seed			Chocolate-containing	
	Almond	Hazelnut	Pecan	Peanut	Soy	Pumpkin	Sesame	Sunflower	Hazelnut	Peanut
0	6^{aA}	6^{aA}	6^{aA}	6^{aA}	6^{aA}	6^{aA}	6^{aA}	6^{aA}	6^{aA}	6^{aA}
1	6^{aA}	6^{aA}	4^{abA}	4^{aA}	6^{aA}	5^{aA}	4^{abA}	4^{aA}	2^{aA}	3^{aA}
2	6^{aA}	5^{aA}	6^{aA}	6^{aA}	4^{aA}	6^{aA}	4^{abA}	5^{aA}	3^{aA}	6^{aA}
4	5^{aA}	5^{aA}	3^{abA}	5^{aA}	6^{aA}	3^{aA}	1^{bA}	4^{aA}	4^{aA}	6^{aA}
6	3^{aAB}	5^{aA}	0^{bB}	5^{aA}	6^{aA}	5^{aA}	5^{abA}	2^{aA}	5^{aA}	6^{aA}

Different lowercase letters indicate significant difference between detection in the same butter at different times (columns). Different uppercase letters indicate significant difference between detection for different butter categories (nut, legume, seed, or chocolate-containing) at the same time (rows).

was for sunflower butter, where *L. monocytogenes* decreased approximately 1 log CFU/g after 12 months.

Since *L. monocytogenes* decreased in population in all butters tested at 25°C, and in the instance of hazelnut butter was not detected after 12-month storage, the subsequent phase of this study assessed the pathogen's survival in the butters at 25°C when inoculated at a low level (1 log CFU/g). After 6 months of storage, *L. monocytogenes* was still detected in all samples (6/6) of both soy and peanut-chocolate butter, whereas it was not detected in pecan butter. Interestingly, in the case of hazelnut butter, *L. monocytogenes* remained detectable in 5/6 samples even though it was not detected after 12 months at 25°C when inoculated at the higher level (4 log CFU/g).

While the pH of the butters remained fairly constant during 12-month storage at either temperature, the a_w values after 12 months were either the same or lower than the initial values. This decrease, however, may not have influenced *L. monocytogenes* survival as the pathogen has been documented to survive for 24 weeks at 20°C in peanut butter (a_w 0.33) (Kenney & Beuchat, 2004), 12 months at 22°C in powdered infant formula (a_w 0.28) (Koseki et al., 2015), and 6 months at 22°C in wheat flour (a_w 0.31) (Taylor et al., 2018). The nutrient contents of the butters, however, may have played a role in the survival of *L. monocytogenes*. All butters used in this study had fairly high-fat contents (average 53%) yet very different carbohydrate contents (ranging from 5.36 to 59.46%). It is known that low a_w foods with high-fat/oil and high carbohydrate contents can protect bacterial pathogens, allowing them to persist in these food products for longer periods of time (Olaimat et al., 2020).

In the case of sunflower butter, where the *L. monocytogenes* population remained fairly constant during 12-month storage at 25°C, the fat and carbohydrate contents were 56.25 and 15.63%, respectively. However, three butters had higher fat contents and seven butters had higher carbohydrate contents. Therefore, there may be properties of the sunflower seeds themselves which contribute to *L. monocytogenes* survival. In 2016, there were 196 recalls associated with *L. monocytogenes* contamination of foods in the U.S.; of the 196 recalls, 50 of them were due to contaminated sunflower seeds and products containing sunflower seeds, including trail mixes, granola bars, and sunflower butter (Maberry, 2017). The reason for the association between *L. monocytogenes* and sunflower seeds is not clear, and this association may or may not translate to sunflower seed butter.

This study included two chocolate-containing butters (hazelnut and peanut) to determine if the addition of chocolate plays a role in the survival of *L. monocytogenes*. No significant difference in *L. monocytogenes* survival was determined in the chocolate-containing butters, compared to the other butters examined in this study. Contrary information is available in the literature about the effect of chocolate on pathogen survival. Chocolate is known to contain antimicrobial properties (flavonoids and polyphenols); cocoa added to broth medium has been shown to inhibit the growth of *L. monocytogenes* (Pearson & Marth, 1990). However, the addition of chocolate or cocoa to foods, such as milk and peanut butter, has also been reported to enhance the growth or survival of *L. monocytogenes* (Rosenow & Marth, 1987). For example, the pathogen survived at a higher population in chocolate-peanut spread compared to peanut butter stored at 20°C for 24 weeks: log reductions of 2.42 and 3.47 at a_w 0.65 and 2.42 and 3.80 CFU at a_w 0.33, respectively (Kenney & Beuchat, 2004).

While minimal information in the published literature is available on the survivability of *L. monocytogenes* in nut, seed, or legume butters, the results of this study are similar to those examining the survival of *S. enterica* or *E. coli* O157:H7 in peanut butter or peanut paste (Burnett et al., 2000; He et al., 2011; Kataoka et al., 2014; Park et al., 2008). For example, when inoculated into peanut butter and peanut butter spread at 5.68 log CFU/g, *S. enterica* survived for 24 weeks at 5 and 21°C, decreasing by 2.86–4.28 and 4.14–4.50 log CFU/g, respectively (Burnett et al., 2000). In another study, when inoculated into peanut butter (a_w 0.40) at either 4 or 8 log CFU/g, *S. enterica* and *E. coli* O157:H7 populations decreased by less than 1 log CFU/g after 4 weeks of storage at 4 or 25°C (He et al., 2011).

Since outbreaks of salmonellosis have occurred due to contaminated peanut butter, the U.S. Food and Drug Administration released a guidance document for industry in 2009 outlining measures to mitigate the risk associated with *Salmonella* spp. contamination of peanut butter and peanut-derived product used as ingredients (such as peanuts or peanut paste) (FDA, 2009). The guidance document provides considerations for evaluating the effectiveness of certain *Salmonella* control measures as well as recommendations. Some of the recommendations include obtaining peanut-derived products only from suppliers with validated processes in place and determining processing conditions which appropriately reduce *Salmonella* spp. via challenge testing, taking into consideration aw, pH, and fat content. Although specifically written for *Salmonella* spp. and peanut butter or peanut-derived ingredients, the same recommendations could also apply to other types of nuts, seed, and legume butters, as well as nut-, seed-, and legume-derived ingredients, as well as to the mitigation of *L. monocytogenes* in these products.

This study determined that *L. monocytogenes* was capable of surviving long-term in nut, seed, and legume butters stored at 5 and 25°C, with few exceptions. Temperature was found to be the most influential factor in *L. monocytogenes* survival, with populations dropping below 1.70 log CFU/g in as early as 3 months when butters were stored at 25°C. Results also indicate that even low levels of *L. monocytogenes* (1 log CFU/g) can survive in these butters and remain viable long-term. This study provides information on the survival of *L. monocytogenes* in different types of butters based on temperature, nutrient content, pH, and a_w, and may assist in informing discussions surrounding the safety of these food products.

Declaration of Competing Interest

The authors declare that no competing interests exist.

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