



Salmonella Growth during the Soaking Step of “Sprouted” Grain, Nut, and Seed Production

ABSTRACT

Food companies are marketing products with “sprouted,” “activated,” or “awakened” ingredients. The label implies that germination of grains, nuts, and seeds confers improved health benefits; however, the “sprouting” process consists of a simple overnight soak in water often at ambient temperature, followed immediately by drying and further processing. Our objective was to quantify *Salmonella* growth during soaking of grains, nuts, and seeds commonly included in “sprouted” products. Raw grains (buckwheat, millet, quinoa, rice), nuts (almond, cashew, hazelnut, peanut, pecan, walnut), and seeds (flax, hemp, pumpkin, sunflower) were inoculated with *Salmonella* to achieve ~3 log CFU/g after drying. Inoculated samples (20 g) were soaked in water (20 mL) with incubation at 7 or 25°C for 24 h. *Salmonella* bacteria were enumerated using serial dilution and spread plating on Hektoen enteric agar (37°C, 24 h). *Salmonella* was capable of significant growth (> 3 log CFU/g) in all 15 grains, nuts, and seeds during ambient temperature soaking, reaching an average concentration of 7.05 log CFU/g. High salt concentrations

(> 10%) and refrigeration (< 7°C) were verified to prevent growth of *Salmonella* during soaking. This study demonstrates the risk of ambient temperature “sprouting” practices and presents practical strategies to improve safety of these products.

INTRODUCTION

The healthy snack market was valued at \$21 billion globally in 2017 and is projected to grow 5% annually over the next decade (8). Many established food businesses as well as food entrepreneurs in the Pacific Northwest are marketing new products to target entry into the healthy snack market category. Differentiation in this product category is important to product success in this crowded marketplace. Recent differentiators have included “raw,” “gluten-free,” “non-GMO,” “sustainably produced,” “locally sourced,” and “paleo-friendly.” Grains, nuts, and seeds are significant ingredients in the healthy snack segment due to their high-quality protein and “healthy” fats, along with abundant antioxidant, fiber, and mineral content (5, 17, 18). New products including grains, nuts, and seeds that are “sprouted,” “awakened,” and

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“activated” are perceived to provide added value. Companies producing these “sprouted” products simply soak raw ingredients (nuts, seeds, grains, etc.) in water overnight, often at ambient temperature. Soaking softens the hull of the nuts, seeds, or grains and leads to swelling that initiates the activation of enzymes and reduction of antinutrients (e.g., phytic acid), which are marketed as providing health benefits (16). Following soaking, these ingredients are typically dried under low temperature (often < 55°C) and low humidity (< 50% relative humidity) to their original water activity and are packaged as single-ingredient snacks (e.g., raw, sprouted almonds), incorporated into a complex snack (e.g., granola, bars, trail mix), or pureed into nut or seed butters or as a base for fermented nondairy “cheeses” (personal communication). These “sprouted” products are considered by consumers to be “raw” and “living,” and they serve the needs of the general consumer as well as vegan and raw food dieters (13).

Three outbreaks have been linked to the consumption of “sprouted” products, specifically sprouted chia seed powder, cashew nut cheese, and raw sprouted nut butters (11, 12, 19). In response to the chia powder outbreak, Health Canada and the Canadian Food Inspection Agency conducted an investigation to determine the level of *Salmonella* contamination in the implicated product (sprouted chia powder) and a co-manufactured product (sprouted flax powder). The finished sprouted chia powder was determined to be contaminated with *Salmonella* at levels of 7.9 to 17.5 most probable number per 100 g. Many of the ingredients (grains, nuts, and seeds) used in “sprouted” products have been associated with a number of recalls and outbreaks in recent years (9–11, 20, 25). Further studies on tree nuts have quantified low levels of *Salmonella* contamination (typically < 20 CFU/100 g) (1, 3, 4, 26). Grains, nuts, and seeds do not have sufficient moisture to support the growth of foodborne pathogens; however, the soaking stage of “sprouting” may create an environment that could support the growth of various microorganisms, including *Salmonella*. A single study conducted by Keller et al. (2018) demonstrated significant growth (>3 log CFU/g) of *Salmonella* during the 24-h soaking period of pumpkin, sunflower, and chia seeds at 25°C (14). These results indicated that growth of *Salmonella* during the soaking period may have been a significant factor in the chia seed powder outbreak. Beyond this study, there is limited information on whether the hydration of other grains, nuts, and seeds used in the formulation of “sprouted” products would be sufficient for *Salmonella* replication within the 24-h soaking period.

Significant research has been conducted that demonstrates the growth of *Salmonella*, Shiga toxin-producing *Escherichia coli*, and other foodborne pathogens during the production of traditional sprouts (alfalfa, radish, etc.) that are characterized by obvious plant growth (shoots, leaves, etc.). Sprouts production includes multiple stages characterized as storage, rinsing, pregermination soak, rinse, germination and growth,

packaging, cooling and storage, and distribution (15, 21). This production process spans several days with incubation at high humidity at ideal temperatures 25 to 30°C that leads to high total bacterial cell density (>7 log CFU/g), which could include high numbers of pathogenic species (7, 22, 24). The result is a product intended to be directly consumed that has the potential for hosting high cell densities of bacterial pathogens. Although there has been significant research on traditional sprouts, the process and characteristics of the finished “sprouted” product are substantially different to true sprouts and provide little insight on the relative risk of these production steps.

The objective of this study was to demonstrate the potential for *Salmonella* to grow during the soaking period of various grains, nuts, and seeds commonly used in commercially available “sprouted” products. A secondary objective of this study was to determine the utility of easily implementable and cost-effective controls (i.e., refrigeration or salt addition) to prevent growth of *Salmonella* during soaking. Demonstration of pathogen growth (or lack thereof) during soaking would provide clear evidence to facilitate hazard analysis and preventive control decisions by the food industry and provide regulators with clear guidance on the risks associated with this new category of “sprouted” products.

MATERIALS AND METHODS

Bacterial strains and inoculum preparation

Six *Salmonella enterica* strains previously associated with tree nut and peanut products were selected for use in the inoculation cocktail (Table 1). Individual strains were transferred from frozen stock (–80°C) to tryptic soy broth (TSB; Neogen, Lansing, MI) and incubated at 37°C for 24 h. Individual strains were confirmed to be *Salmonella* by displaying typical morphology on Hektoen enteric agar (Neogen) following incubation at 37°C for 24 to 48 h. Isolated colonies were transferred from Hektoen enteric agar to fresh TSB and cultured as described above. Aliquots (100 µL) of TSB culture were spread plated onto tryptic soy agar (TSA; Neogen) and incubated at 37°C for 24 h to produce a bacterial lawn. Bacterial lawns were harvested with 5 mL of 0.1% peptone water (Neogen) using a sterile cell spreader. Immediately following harvest, individual strains were mixed in equal volume in a 50-mL conical tube to create a *Salmonella* cocktail. The cocktail was diluted in 0.1% peptone water to achieve a cell density of ~5 log CFU/mL.

Inoculation of grains, nuts, and seeds

Minimally processed grains (brown rice, buckwheat, millet, quinoa), nuts (almond, cashew, hazelnut, peanut, pecan, pili nut, walnut), and seeds (flax, hemp, pumpkin, sunflower) were supplied by local food companies or purchased from the bulk food section of local grocery stores. Individual nut types (20 g) were aseptically transferred

TABLE 1. *Salmonella enterica* strains included in the inoculation cocktail for soaking studies of grains, nuts, and seeds

<i>Salmonella</i> Serotype	Isolate Identifier	Description	Isolate Source
Enteritidis PT30	ATCC BAA-1045	Almond isolate	American Type Culture Collection
Enteritidis PT9c	RM4635	Clinical isolate from almond outbreak	Rob Mandrell USDA-ARS
Montevideo	GRC1	Pistachio isolate	Food and Drug Administration
Paratyphi B var. L(+) tartrate(+)	OSPHL 15092808070	Clinical isolate from 2015 outbreak associated with raw sprouted nut butters	Oregon State Public Health Labs
Oranienburg	MDD317	Pecan isolate	Michelle Danyluk University of Florida
Tennessee	MDD319	Clinical isolate from peanut butter outbreak	Larry Beuchat University of Georgia

onto sterile weighing boats and mixed with 2 mL of diluted inoculum with a sterile utensil to coat. For seeds and grains, the inoculum was applied with an atomizer (Specialty Bottle, Seattle, WA) via spraying 1 mL of diluted inoculum directly onto seed and grain products (20 g) spread out in weigh boats. After coating or mixing, inoculated product was dried in the biological safety cabinet at ambient temperature for 24 h to return product to original moisture content. Inoculated product (~3 log CFU/g) was used immediately for experimentation after the drying period.

Soaking process

Inoculated product was mixed and aliquoted (20 g) into sterile 50-mL conical bottom tubes. Sterile distilled water (20 mL; pH 6.44) was added to each tube and incubated at 22°C for 24 h. Additionally, almond samples were soaked in salt solutions at various concentrations (1.5, 10, 20%) for 24 h. Uninoculated controls were prepared in the same manner to evaluate changes in the cell density of natural microbiota, pH, water activity, and moisture content.

Microbial analyses

Upon completion of a 24-h soak, the hydrated product and soaking solution were mixed thoroughly by shaking the conical tubes for 30 s. For products that thickened during soaking (i.e., flax and hemp), the entire sample (product and solution) was homogenized with an equal volume (1:1) of 0.1% peptone water. Soaking solution was aseptically transferred and serially diluted in 0.1% peptone water, and appropriate dilutions were spread plated on Hektoen enteric agar (inoculated samples) or TSA (uninoculated samples).

Inoculated, unsoaked samples were mixed with 20 mL of 0.1% peptone water, mixed, serially diluted, and plated as described for soaked samples. Plates were incubated at 37°C for 24 to 48 h prior to enumeration.

pH, water activity, and moisture content

Samples were tested for water activity with the AquaLab CX-2 or 4TE water activity benchtop meter (Decagon Devices, Inc., Pullman, WA). Moisture content was measured with the HB43-S halogen moisture analyzer using method 1530.08 (Mettler Toledo, Columbus, OH). Product samples were finely chopped in a food processor prior to moisture content and water activity analyses. The pH of water, soaking solution, and product were measured with the SympHony H10P pH meter (VWR, Radnor, PA) or the Apera SX811-SS (Apera Instruments, Columbus, OH). Prior to pH measurement, distilled water was added to product samples (1:1) and blended to homogenize.

Statistical analysis

Biological replication at the cocktail preparation level was performed in triplicate, and *Salmonella* growth experiments for each product were performed in triplicate for each biological replicate ($n = 9$). Natural microbiota growth experiments were performed on uninoculated products on 2 days in triplicate ($n = 6$). Moisture content, water activity, and pH of uninoculated unsoaked and soaked products were measured on one sample per product per day ($n = 2$). Mixed model and one-way analysis of variance with a Tukey's test as the post hoc analysis were performed to determine the impact of water activity, moisture content, pH, and initial

TABLE 2. Moisture content, water activity, and pH of grains, nuts, and seeds before and after soaking in water at 22°C for 24 hrs. Values are expressed as the mean ± standard error (n = 2). Within each column, means with different capital letters are significantly different (P < 0.05). Columns with no capital letters indicate that there was no significant difference in that variable as a function of product type. Within each row, means with different lowercase letters are significantly different (P < 0.05) for a given variable (moisture content, water activity, pH) for initial and soaked product

Product		Moisture Content (%)		Water Activity (a _w)		pH		
		Initial	After 24-hr Soak	Initial	After 24-hr Soak	Initial	After 24-hr Soak	Soaking Solution ^d
Grains	Brown Rice	9.01 ± 0.59 ^{Ba}	22.44 ± 1.07 ^{EFb}	0.501 ± 0.003 ^{Aa}	0.991 ± 0.004 ^b	6.39 ± 0.03 ^{DEa}	6.29 ± 0.01 ^{DEFa}	5.73 ± 0.04 ^{DEFb}
	Buckwheat	8.41 ± 0.02 ^{B^cCa}	36.51 ± 3.24 ^{CDEb}	0.359 ± 0.002 ^{CDa}	0.988 ± 0.002 ^b	6.37 ± 0.03 ^{DEa}	6.35 ± 0.04 ^{CDEa}	6.03 ± 0.02 ^{Bb}
	Millet	7.48 ± 0.02 ^{Ca}	25.26 ± 1.26 ^{CDEFb}	0.300 ± 0.007 ^{DEFGa}	0.986 ± 0.000 ^b	6.56 ± 0.05 ^{BCDa}	6.12 ± 0.03 ^{Fb}	5.71 ± 0.01 ^{EFc}
	Quinoa	11.35 ± 0.06 ^{Aa}	40.16 ± 2.17 ^{BCb}	0.503 ± 0.000 ^{Aa}	0.986 ± 0.004 ^b	6.29 ± 0.09 ^{Ea}	6.40 ± 0.09 ^{BCDEa}	6.30 ± 0.00 ^{Aa}
Nuts	Almond	2.41 ± 0.03 ^{EFGa}	31.47 ± 5.70 ^{CDEFa}	0.343 ± 0.033 ^{CDEFa}	0.977 ± 0.011 ^b	6.37 ± 0.01 ^{DEa}	6.47 ± 0.04 ^{ABCDa}	5.47 ± 0.01 ^{Gb}
	Cashew	3.23 ± 0.38 ^{EFa}	39.56 ± 2.78 ^{BCDb}	0.435 ± 0.007 ^{ABa}	0.980 ± 0.002 ^b	6.46 ± 0.05 ^{CDEa}	6.51 ± 0.01 ^{ABCa}	5.66 ± 0.02 ^{Fb}
	Hazelnut	2.99 ± 0.07 ^{EFa}	28.84 ± 0.39 ^{CDEFb}	0.416 ± 0.009 ^{BCa}	0.984 ± 0.003 ^b	6.42 ± 0.02 ^{DEb}	6.57 ± 0.04 ^{A^{Ba}}	5.83 ± 0.01 ^{CDEc}
	Peanut	1.13 ± 0.13 ^{Ga}	25.33 ± 2.51 ^{CDEFb}	0.256 ± 0.004 ^{Ga}	0.986 ± 0.003 ^b	6.74 ± 0.01 ^{Ba}	6.64 ± 0.03 ^{Aa}	6.30 ± 0.02 ^{Ab}
	Pecan	2.38 ± 0.12 ^{EFGa}	16.66 ± 2.66 ^{Fb}	0.382 ± 0.024 ^{BCa}	0.983 ± 0.004 ^b	6.27 ± 0.03 ^{Eb}	6.49 ± 0.02 ^{ABCa}	5.87 ± 0.01 ^{CDc}
	Pili nut	3.14 ± 0.08 ^{EFa}	25.99 ± 0.41 ^{CDEFb}	0.507 ± 0.027 ^{Aa}	0.985 ± 0.004 ^b	6.41 ± 0.04 ^{DEa}	6.52 ± 0.02 ^{ABCa}	5.71 ± 0.02 ^{EFb}
	Walnut	2.04 ± 0.14 ^{FGa}	23.58 ± 0.18 ^{DEFb}	0.352 ± 0.004 ^{CDEa}	0.985 ± 0.000 ^b	6.41 ± 0.01 ^{DEb}	6.58 ± 0.00 ^{ABa}	5.66 ± 0.03 ^{Fc}
Seeds	Flax	5.35 ± 0.06 ^{Da}	69.48 ± 3.33 ^{Ab}	0.276 ± 0.001 ^{FGa}	0.977 ± 0.000 ^b	5.88 ± 0.05 ^{Fa}	5.85 ± 0.02 ^{Ga}	5.94 ± 0.05 ^{BCa}
	Hemp	5.41 ± 0.04 ^{Da}	54.30 ± 0.68 ^{ABb}	0.291 ± 0.000 ^{DEFGa}	0.999 ± 0.001 ^b	7.20 ± 0.04 ^{Aa}	6.64 ± 0.03 ^{Ab}	ND ^b
	Pumpkin	3.46 ± 0.58 ^{Ea}	36.97 ± 3.77 ^{CDEb}	0.284 ± 0.001 ^{EFGa}	0.979 ± 0.002 ^b	6.70 ± 0.05 ^{BCa}	6.48 ± 0.02 ^{ABCb}	4.68 ± 0.02 ^{Hc}
	Sunflower	3.65 ± 0.11 ^{Ea}	29.42 ± 5.08 ^{CDEFa}	0.264 ± 0.001 ^{Ga}	0.986 ± 0.000 ^b	6.54 ± 0.04 ^{BCDa}	6.24 ± 0.01 ^{EFb}	5.82 ± 0.05 ^{CDEc}

^aSoaking solution pH was measured after the completion of the 24-hr soak. The initial pH of the water used for soaking was 6.44.

^bNot determined.

cell density on the growth of *Salmonella* during soaking of different grains, nuts, and seeds.

RESULTS AND DISCUSSION

Moisture content, water activity, and pH values for the 15 grains, nuts, and seeds evaluated in this study are shown

in *Table 2*. The initial moisture content for each product category ranged from 1.13 to 3.23% for nuts, 3.46 to 5.41% for seeds, and 7.48 to 11.35% for grains. Prior to soaking, moisture content was correlated with water activity for nuts (P -value = 0.0158; R^2 = 0.769) and grains (P -value < 0.0001; R^2 = 0.649), but not for seeds (P -value = 0.3176;

$R^2 = 0.165$). Initial water activities had a similar range across all product categories, from a low of 0.256 (peanut) to a high of 0.507 (pili nut). As expected, the 24-h soak led to significant increases in moisture content and water activity of all products. Flax seed absorbed the most water, from an initial moisture content of 5.35% to a final moisture content of 69.48%. Hemp seed absorbed the next highest amount of water, increasing from 5.41 to 54.30%. Quinoa and cashew achieved final moisture contents of approximately 40%. Brown rice and pecans absorbed the least amount of water, increasing by 13.43 and 14.28%, respectively. All products reached a water activity of at least 0.977 (almond and flax seed) after the 24-h soaking period, with no significant difference among the soaked products.

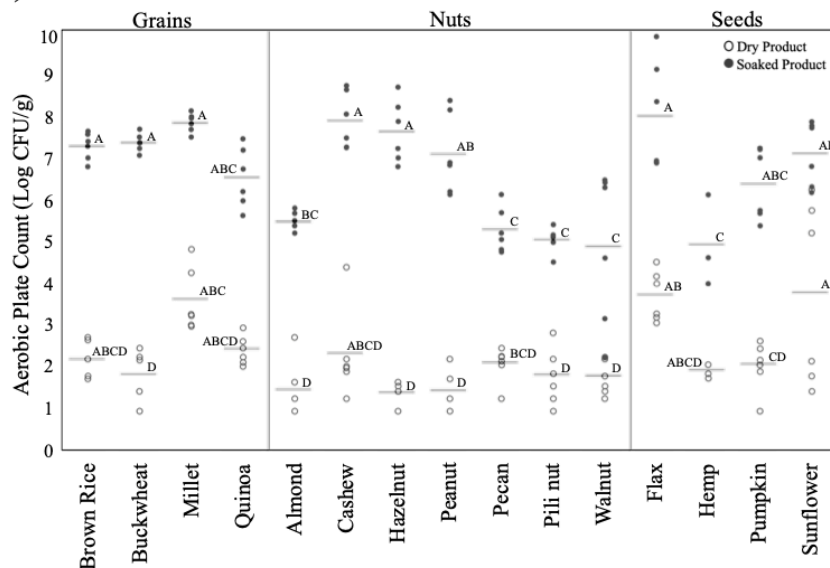
All of the grains, nuts, and seeds used in this study are considered to be low-moisture and low-acid products prior to soaking. The initial pH of each product is shown in [Table 2](#). With the exception of flax seed (pH 5.88), all of the unsoaked products fell within the narrow pH range of 6.27 to 6.74. Soaking did not have a significant impact on the pH of most grains, nuts, and seeds. However, soaking did lead to a statistically significant increase in the pH of walnuts (6.41 to 6.58) and quinoa (6.29 to 6.40) and a significant decrease in the pH of hemp seeds (7.20 to 6.64), pumpkin seeds (6.70 to 6.48), and sunflower seeds (6.54 to 6.24). Soaking solutions of peanut and quinoa maintained pH (6.30) throughout the 24-h soaking periods, whereas the pH of the solution of all other products was significantly decreased at the end of the 24-h soak (the pH of the hemp seed solution was not measured). Soaking of buckwheat and flax seed led to modest pH decreases, to achieve final solution pH values of 6.03 and 5.94, respectively. The pH of soaking solution for the

majority of products ranged from 5.47 to 5.87. The soaking solution of pumpkin seeds was drastically reduced to a final value of 4.68 after the 24-h period.

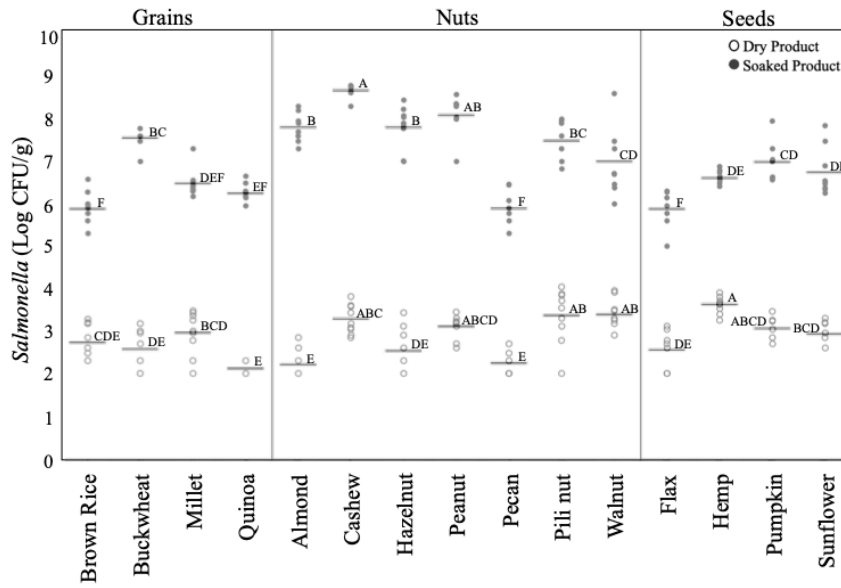
The pH of the soaking solution is influenced by the hydration of components from the grains, nuts, and seeds along with their intrinsic enzymatic processes as they are rehydrated. The metabolism and reproduction of the native microbiota of each product type is likely the major contributor to changes in the pH of the soaking solution. Changes in the natural microbiota of the grains, nuts, and seeds before and after soaking are shown in [Fig. 1A](#). The initial concentration of mesophilic bacteria on these low-moisture products was fairly low, with average aerobic plate counts ranging from 1.40 (hazelnut) to 3.83 (sunflower seed) log CFU/g; however, the cell density varied substantially between replicates, particularly for sunflower seed (1.48 to 6.34 log CFU/g) and cashew (1.30 to 4.48 log CFU/g) samples. Minimally processed, low-moisture products typically have lower aerobic plate counts; however, the variability can be significant, even within a product lot. Aerobic plate counts and their variability for similar products have been reported in previous reports (1, 4, 6, 23). Ambient soaking in water for 24 h led to large and significant increases in microbial populations on all products. All products supported the growth of >3.00 log CFU/g of bacteria, with brown rice, buckwheat, cashew, hazelnut, and peanut supporting increases of >5 log CFU/g within 24 h ([Fig. 1C](#)). Final cell densities were comparable to those previously reported for soaked pumpkin, sunflower, and chia seeds (14).

All of the products were inoculated and dried in a similar manner; however, there were small, but statistically significant, differences in the survival of the *Salmonella*

A) Natural Microbiota



B) *Salmonella*



C) Growth

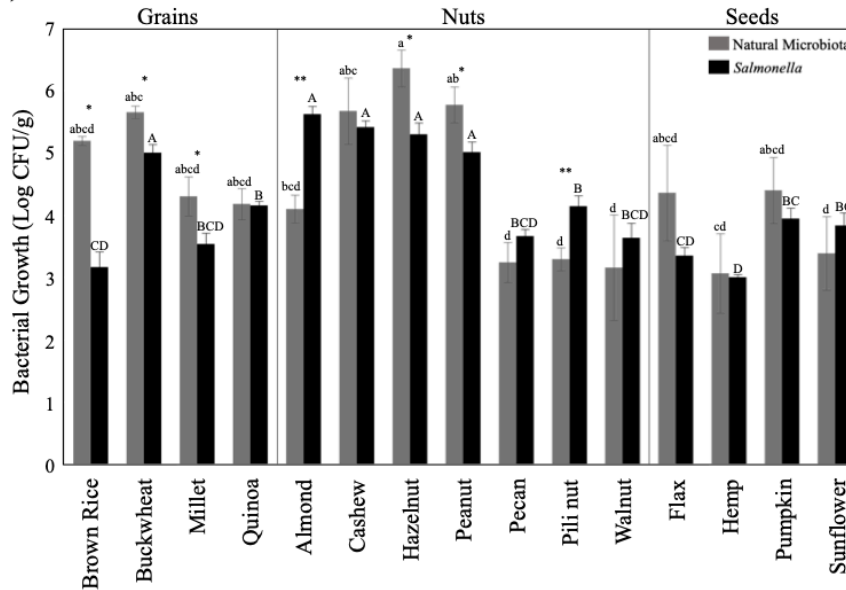


FIGURE 1. Natural microbiota (A) and *Salmonella* (B) cell density on grains, nuts, and seeds before (open circle) and after (closed circle) soaking. Growth (Log CFU/g) of natural microbiota and *Salmonella* after soaking (C). The soaking process consisted of a 24-hrs hold in water at ambient temperature ($22 \pm 3^\circ\text{C}$). Natural microbiota cell densities for individual samples are represented as a circle with the mean indicated by a horizontal line ($n = 6$). *Salmonella* cell densities presented in an identical manner ($n = 9$). Bar height represents the mean \pm standard error ($n = 9$). At each timepoint, means with different letters are significantly different ($P < 0.05$). * indicates significantly more growth of the natural microbiota at the end of the soaking period when compared to *Salmonella* ($P < 0.05$). ** indicates significantly more growth of *Salmonella* at the end of the soaking period when compared to the natural microbiota ($P < 0.05$).

inoculum on these dried products (Fig. 1B). *Salmonella* levels averaged between 2.10 (quinoa) and 3.61 (hemp) log CFU/g on the raw products prior to soaking. *Salmonella* inoculum levels were significantly higher on hemp, walnuts, and pili nuts and significantly lower on almonds, pecans, and quinoa. Initial pH (positively correlated; P -value < 0.0001) and moisture content (negatively correlated; P -value = 0.0022) were statistically significant parameters associated with the stability of the *Salmonella* inoculum on the grains, nuts, and seeds. The small reduction of the *Salmonella* inoculum during the drying phase was expected and has been reported in numerous previous studies of low-moisture foods (e.g., 2, 13, 15, 22).

The primary purpose of this study was to quantify the potential for *Salmonella* to grow on grains, nuts, and seeds during the soaking process (ambient temperature, 24 h) used for creating “sprouted” products. The growth of *Salmonella* on various commodities commonly “sprouted” is shown in Fig. 1B and 1C. All 15 of these commodities supported significant growth of *Salmonella* (>3.00 log CFU/g) within the 24-h soaking period. *Salmonella* populations increased by >5.00 log CFU/g during the soaking of almonds, cashews, hazelnuts, peanut, and buckwheat. Significantly less growth (3- to 4-log CFU/g increase) was measured in hemp, brown rice, and flax. The growth of *Salmonella* during soaking of pumpkin seeds (3.95 log CFU/g) and sunflower seeds (3.84 log CFU/g) is very similar to that seen in the previous study by Keller et al. (14). For the seeds, population increases in the natural microbiota and *Salmonella* were similar. Interestingly, *Salmonella* had significantly more growth during the soaking period than did the natural microbiota in two nuts (almond and pili) and significantly less growth in three of the grains (brown rice, buckwheat, millet) and two nuts (hazelnut and peanut). *Salmonella* cell density after the 24-h soaking is likely influenced by the rate of hydration and the release of nutrients into solution. These, along with many other factors (temperature, pH, macronutrient profile, mineral content, competing natural microbiota, etc.), will influence the lag time and the growth rate of *Salmonella*. Keller et al. (14) reported average lag times of 3.45 h (sunflower) to 6.68 h (chia) during soaking at 25°C; however, these times were highly variable between replicates (standard deviations averaging 2 h). Shortening the soaking time to < 3 h to mitigate the potential growth of *Salmonella* is not a viable option because many of these commodities would not be fully hydrated in this time frame.

The overall goal of the soaking step is to hydrate the grains, nuts, and seeds; however, this hydration also stimulates previously dormant bacterial cells into rapid proliferation. Due to the market demand for “sprouted” ingredients, it is important to identify and verify practical measures that would prevent the growth of pathogens during the soaking step. The potential for grains, nuts, and seeds to be contaminated with low levels of *Salmonella* or other

foodborne pathogens is a significant risk; however, the risk for foodborne illness increases significantly if processing steps would support the proliferation of these pathogens. Almonds were selected as the target matrix due to their ability to support the most growth of *Salmonella* during soaking as well as for their high utilization in commercially available “sprouted” products. Through conversations with industry partners, the inclusion of salt in the soaking process and refrigeration were determined to be the most cost-effective and easily implementable options for modifying current procedures. High salt concentrations (>10%) prevented the growth of *Salmonella* throughout the 24-h soaking of almonds under ambient conditions (Fig. 2). At higher salt concentrations (20%), there was a significant inactivation (1.44-log CFU/g reduction) of *Salmonella* during soaking. Low-salt concentration (1.5%) had no impact on the growth of *Salmonella* during soaking. Refrigeration (< 7°C) was also effective at preventing the growth of *Salmonella* on almonds within the 24-h soaking period. Whereas *Salmonella* is capable of growth at 7°C, the growth rate is substantially slower and did not result in a measurable increase in cell density within the 24-h soaking period. Processors relying on refrigeration must take care to confirm that refrigeration conditions are < 4°C and/or limit the soaking time at lower temperatures to prevent *Salmonella* growth. Investigations surrounding the 2015 *Salmonella* Paratyphi B outbreak linked to “sprouted” nut butters reported that the company conducted their soaking process under refrigeration but they did not apply a validated kill step (12). This situation demonstrates that refrigerated soaking may be an important control to prevent the growth of *Salmonella*; however, this approach was insufficient to eliminate risk associated with contaminated starting ingredients. Had their “sprouting” process occurred at ambient temperature without a subsequent kill step, the outbreak would likely have led to significantly more illnesses.

CONCLUSIONS/RECOMMENDATIONS

The use of typical soaking processes in the production of “sprouted” grains, nuts, and seeds at ambient temperature poses a significant risk due to the ability of *Salmonella* to grow rapidly in all 15 of the commodities tested. Although many companies further process “sprouted” ingredients, most use low-temperature dehydration to maintain a “raw” label. Dehydration, or other processing steps, could be optimized and validated to achieve a significant pathogen reduction; however, the current study demonstrates that the soaking stage of the “sprouting” process could lead to an increase in *Salmonella* of > 5 log CFU/g. Therefore, the standard 5-log reduction typically targeted in process validation studies would be inadequate to achieve food safety goals in these processing systems. Potential growth of pathogens in a processing facility also creates a number of challenges for sanitation and other prerequisite programs that provide

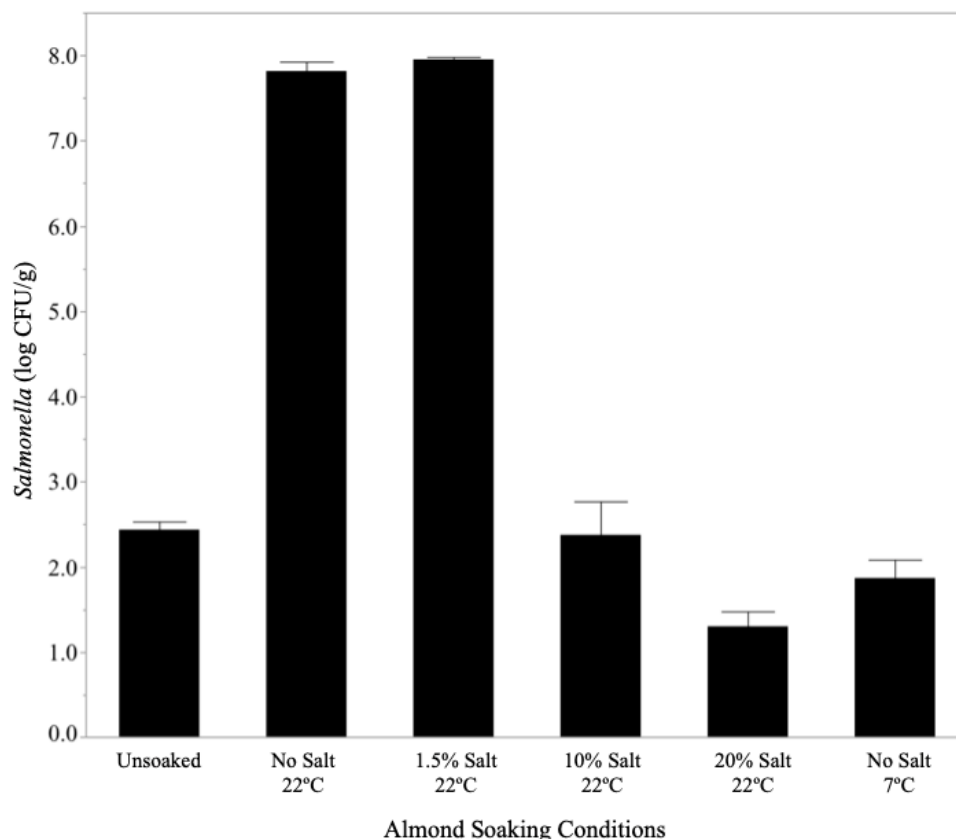


FIGURE 2. Impact of salt concentration and temperature on the cell density of *Salmonella* following a 24-hr soak of almonds in water. Bar height represents the mean \pm standard error ($n = 18$ unsoaked (dry product); $n = 9$ no salt/22°C, $n = 3$ remaining treatments).

foundational food safety management within the processing environment. A mitigation strategy during the soaking stage must be implemented to prevent pathogen growth. This study demonstrates that high salt concentration (>10%) or refrigeration (< 7°C) could be used as cost-effective strategies to reduce or prevent the growth of *Salmonella* during soaking.

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