

## Research Paper

# Fate of *Salmonella* throughout Production and Refrigerated Storage of Tahini

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## ABSTRACT

Tahini, a low-moisture food that is made from sesame seeds, has been implicated in outbreaks of salmonellosis. In this study, the fate of *Salmonella* was determined through an entire process for the manufacture of tahini, including a 24-h seed soaking period before roasting, subsequent grinding, and storage at refrigeration temperature. *Salmonella* populations increased by more than 3 log CFU/g during a 24-h soaking period, reaching more than 7 log CFU/g. Survival of *Salmonella* during roasting at three temperatures, 95, 110, and 130°C, was assessed using seeds on which *Salmonella* was grown. *Salmonella* survival was impacted both by temperature and the water activity ( $a_w$ ) at the beginning of the roasting period. When roasted at 130°C with a high initial  $a_w$  ( $\geq 0.90$ ) and starting *Salmonella* populations of  $\sim 8.5$  log CFU/g, populations quickly decreased below detection limits within the first 10 min. However, when the seeds were reduced to an  $a_w$  of 0.45 before roasting at the same temperature, 3.5 log CFU/g remained on the seeds after 60 min. In subsequent storage studies, seeds were roasted at 130°C for 15 min before processing into tahini. For the storage studies, tahini was inoculated using two methods. The first method used seeds on which *Salmonella* was first grown before roasting. In the second method, *Salmonella* was inoculated into the tahini after manufacture. All tahini was stored for 119 days at 4°C. No change in *Salmonella* populations was recorded for tahini throughout the entire 119 days regardless of the inoculation method used. These combined results indicate the critical importance of  $a_w$  during a roasting step during tahini manufacture. *Salmonella* that survive roasting will likely remain viable throughout the normal shelf life of tahini.

Key words: *Salmonella*; Sesame seeds; Tahini; Thermal processing

*Salmonella* is a major cause of foodborne disease worldwide, resulting in approximately 93.8 million salmonellosis cases and 155,000 deaths every year (6). In general, *Salmonella* outbreaks are frequently associated with the consumption of contaminated fresh fruits and vegetables, live poultry, eggs, raw meat, and meat products (25). However, low-moisture foods (i.e., sesame seeds, tahini, peanuts, peanut butter, chocolate, spices, cereal products, and powdered infant formula) have been implicated in multiple outbreaks of salmonellosis. According to the Centers for Disease Control and Prevention, from 2006 to 2016 there were at least 12 *Salmonella* outbreaks linked to low-moisture foods, 9 of which involved seeds and nuts or products produced from seeds and nuts (4). Although a low-water activity ( $a_w$ ) environment does not support the growth of *Salmonella*, these microorganisms can persist in low-moisture foods for long periods (9, 23). Therefore, prevention of *Salmonella* contamination in low-moisture foods is particularly important for food manufacturers.

One low-moisture product that has caused particular concern is tahini, also called sesame paste. It is typically

produced both in-home and in commercial operations from dehulled and roasted sesame seeds. Sesame seeds and tahini are typically not cooked before they are consumed (16). From 2002 to 2003, three *Salmonella* Montevideo outbreaks reported in Australia and New Zealand were related to tahini imported from Egypt (28). A 2013 outbreak of salmonellosis in the United States was associated with tahini, resulting in 16 cases, including one death (29).

The manufacturing process of tahini can be complicated: raw, unhulled sesame seeds frequently go through a multistep process including an initial 24-h soak step, a dehulling step, and draining and drying before thermal treatment. The effect of roasting has been examined to some extent by Torlak et al. (26). In that study, typical roasting temperatures of 110, 130, and 150°C were shown to destroy *Salmonella*. However, Torlak et al. (26) did not examine the survival of *Salmonella* during the entire manufacturing process; they focused solely on the survival of *Salmonella* during seed roasting and storage of tahini. Of particular concern may be the 24-h soaking period before dehulling and roasting of sesame seeds, which was not examined previously. In addition, the mode of inoculation and contamination can play a significant role in subsequent survival of *Salmonella* (3). Finally, formation of biofilms

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could significantly impact the thermal resistance of *Salmonella* during subsequent roasting. In this study, the effect of the entire process beginning with a 24-h soaking of sesame seeds was examined with respect to the survival of *Salmonella* in tahini manufacture.

## MATERIALS AND METHODS

**Salmonella strains.** A cocktail of four *Salmonella* serotypes was used for inoculation of sesame seeds and tahini: *Salmonella* Anatum (strain 6802, originally isolated from raw peanuts) and *Salmonella* Tennessee (strain K4643, originally isolated from a patient associated with consumption of contaminated peanut butter), both from L. Beuchat, University of Georgia, Athens; and *Salmonella* Montevideo (strain 691341, isolated from tahini) and *Salmonella* Mbandaka (strain 604916, isolated from sesame seeds), both from Arkansas Regional Laboratory, Jefferson. Bacteria were stored as frozen stock cultures at  $-20^{\circ}\text{C}$ . Working cultures were grown on Trypticase soy agar supplemented with 0.6% yeast extract (TSAYE; BD, Franklin Lakes, NJ) and kept at  $4^{\circ}\text{C}$ . Transfers of working cultures to fresh media were performed monthly.

**Preparation of inoculum.** Cultures were propagated and harvested as described by Keller et al. (12). Single colonies on TSAYE were transferred to 10 mL of Trypticase soy broth supplemented with 0.6% yeast extract (BD), incubated 24 h at  $37^{\circ}\text{C}$ , transferred again (100  $\mu\text{L}$ ) to the surface of TSAYE plates, and incubated 24 h at  $37^{\circ}\text{C}$ . Bacterial cells were harvested by adding 1 mL of buffered peptone water (BD) or phosphate-buffered saline (PBS; 0.5 M, pH 7.0; Fisher Scientific Co., Pittsburgh, PA) to the agar surface and then gently rubbing to suspend cells by using a disposable sterile L-shaped spreader. The cell suspension was collected and removed to a sterile 15-mL conical tube (Fisher Scientific, Fair Lawn, NJ). Suspensions were vortexed for 30 s at a speed of  $\sim 8$  to 9 (model 945404, Fisher Scientific). Each plate regularly yielded 0.4 to 0.6 mL of inoculum, with a cell density of  $10 \log \text{CFU/mL}$ . Finally, a cocktail of these four serovars was prepared by mixing an equal volume of each culture in a sterile 50-mL conical tube (Fisher Scientific). Inoculum cocktails were diluted in PBS to a cell density of  $7 \log \text{CFU/mL}$  before use in growth studies; inoculum cocktails without dilution were used in inactivation and survival studies.

**Salmonella enumeration.** Before inoculation the sesame seeds were tested to confirm that they were not contaminated with *Salmonella*. After inoculation, the sesame seeds were enumerated to determine starting *Salmonella* population levels. For enumeration, triplicate (1-g/mL) samples were individually 10-fold serially diluted in buffered peptone water or PBS, and 100  $\mu\text{L}$  of the cocktail was plated in duplicate on both TSAYE and xylose lysine desoxycholate (XLD) agars (BD). All plates were incubated at  $37^{\circ}\text{C}$  for 24 h before enumeration. Raw seed initial microbial populations were below the detection limit on both TSAYE and XLD. The limit of detection in this study was determined to be  $1.7 \log \text{CFU/g}$ . Determination of *Salmonella* populations during experimentation was carried out in the same manner.

**Evaluation of Salmonella growth on sesame seeds during soaking.** Clean white sesame seeds with and without hulls (Kevala International, LLC, Dallas, TX) were used to determine growth of *Salmonella* in the soaking experiments (Fig. 1). Seed samples were dried before inoculation in a biosafety cabinet (class II, type A2, NuAire, Plymouth, MN) for 24 h to an initial  $a_w$  of  $\sim 0.100$  as

determined using an AquaLab series 4TEV meter (Decagon Devices, Pullman, WA). Inoculation was carried out by applying 100  $\mu\text{L}$  of the diluted *Salmonella* culture ( $\sim 7 \log \text{CFU/mL}$ ) directly to each 10-g sample of dried seeds in a sterile Whirl-Pak bag (Nasco, Fort Atkinson, WI). The seeds were hand massaged for 1 min to distribute the inoculum throughout the sample. The wet sesame seeds with inoculation level of  $\sim 5 \log \text{CFU/g}$  were held in the biosafety cabinet at ambient temperature for 24 h to equilibrate the seeds to the original  $a_w$  level ( $\sim 0.100$ ).

Subsequently, 90 mL of sterile deionized water was added to each bag to soak the sesame seeds. All bags were shaken gently and placed in incubators at either 25 or  $37^{\circ}\text{C}$ . Populations of *Salmonella* were determined at 0, 18, 22, and 24 h.

**Determination of Salmonella survival on dehulled sesame seeds during roasting.** Clean, white dehulled sesame seeds (Penzeys Spices, Wauwatosa, WI) were used for inoculation in the roasting experiments (Fig. 1). Seed samples were dried in the biosafety cabinet before inoculation for 24 h. Initial  $a_w$  ( $\sim 0.100$ ) and background aerobic microbial populations after drying were determined ( $\leq 1.7 \log \text{CFU/g}$ ). The inoculation preparation used in this study was similar to the biofilm inoculation procedure described by Bowman et al. (3). A 3-mL aliquot of original cocktail culture of *Salmonella* was incubated in 270 mL of sterile deionized water containing 30 g of sesame seeds to provide a starting inoculation level of  $\sim 8 \log \text{CFU/mL}$ . The seed-water mixtures were incubated at  $25^{\circ}\text{C}$  for 24 h. To prepare enough inoculated seed samples per trial, five 300-mL mixtures in sterile glass bottles (1,500 mL total) were used, and all seeds were combined into one batch during draining. Wet inoculated seed samples were drained for 1 or 24 h using a sterile stainless steel screen in the biosafety cabinet at ambient temperature ( $23 \pm 2^{\circ}\text{C}$ ). The initial  $a_w$  of sesame seeds and inoculation level after draining were determined as indicated previously.

Inoculated seed samples were weighed into 24 aluminum pans, each containing 4 g of seeds (MB series, Ohaus Corporation, Parsippany, NJ). Seeds of this quantity in these aluminum pans made a single layer of  $\leq 5$  mm in depth. All 24 pans were placed in an air-forced oven (Heratherm, ThermoFisher Scientific, Waltham, MA) that was preheated to the desired roasting temperature. Roasting was conducted at three temperatures, 95, 110, and  $130^{\circ}\text{C}$ , for up to 90 min. The air temperature in the oven was monitored with a precision oven thermometer (DURAC Plus, H-B Instrument Company, Trappe, PA). During the roasting process, three pans of seeds were removed as quickly as possible to minimize changes in the temperature within the oven at 0, 10, 20, 30, 40, 50, 60, and 90 min of roasting. The  $a_w$  and *Salmonella* content of seeds were determined as described previously.

**Determination of Salmonella survival during refrigerated storage of tahini.** White dehulled sesame seeds previously dried 24 h to an initial  $a_w$  of  $\sim 0.100$  were used in survival experiments (Fig. 1). Two inoculation methods were used to mimic and compare natural contamination of sesame seeds before the roasting and grinding steps (method A) and postcontamination of tahini after completion of roasting and grinding steps (method B).

Method A was carried out by incubation of 6 mL of original *Salmonella* cocktail culture and 60-g quantities of sesame seeds in 540 mL of sterile deionized water. As with the preparation of inoculated seeds before roasting, soaked seeds were incubated at  $25^{\circ}\text{C}$  for 24 h and then drained and arranged in a single layer on sterile, oblong metal trays (9 by 13 in. [23 by 33 cm]; Baker's Secret, World Kitchen, LLC, Rosemont, IL). Trays were placed in the biosafety cabinet and drained and dried for 24 h. The initial  $a_w$

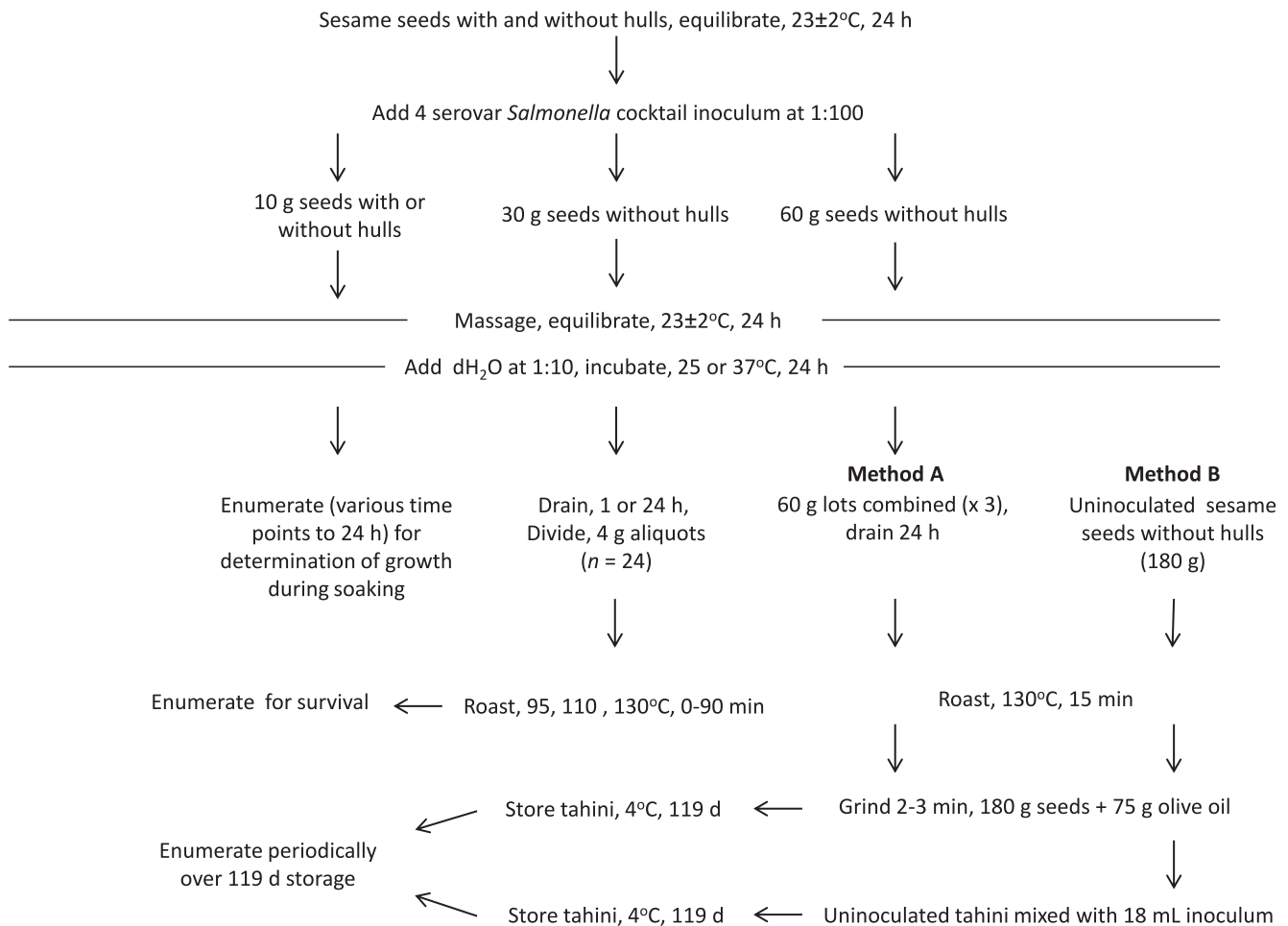


FIGURE 1. Flow diagram of tahini processing, from unhulled seeds to storage.

of sesame seeds and the inoculation level after draining (before roasting) were determined as indicated previously. To prepare enough inoculated seed samples per trial, three 600-mL seed-water mixtures were prepared in sterile glass bottles, and all seeds were combined into one batch during draining. The metal trays containing drained inoculated seed samples were placed in an air-forced oven that was preheated to 130°C for 15 min. During the roasting process, the temperature of oven air was monitored using a precision oven thermometer. The  $a_w$  and populations of *Salmonella* were determined after roasting. After roasting, the sesame seeds (180 g) were transferred from the oven to a sanitized food processor (Mini-Prep Plus, Cuisinart, East Windsor, NJ). Roasted sesame seeds were processed into tahini by grinding for 2 to 3 min with 75 mL of olive oil (Extra Virgin Olive Oil, Target Corporation, Minneapolis, MN). The  $a_w$  and populations of *Salmonella* in tahini were assayed after grinding, and the values were recorded as day 0.

For method B, 180 g of dehulled sesame seeds with initial  $a_w$  of  $\sim 0.100$  was placed in a single layer in sterile metal trays in a biosafety cabinet and dried for 24 h. The initial  $a_w$  of sesame seeds and populations of *Salmonella* were determined before and after roasting. Seed samples were roasted and processed into tahini as described in method A. The finished tahini was inoculated directly with 18 mL of original cocktail culture and mixed by alternating hand mixing and stomaching (Stomacher 400, Seward Ltd., West Sussex, UK) at 30-s intervals at 250 rpm three times each. The  $a_w$  and *Salmonella* populations in tahini were determined after inoculation, and they were recorded as day 0.

Tahini made using the two inoculation methods was divided into 10-g portions, packaged, and sealed in Whirl-Pak bags. Bags were stored at 4°C for up to 119 days. Tahini samples were assayed weekly for  $a_w$  and *Salmonella* contents, in triplicate. Three independent trials were run, with each trial beginning with new inoculum.

**Statistical analysis.** Growth kinetics,  $a_w$ , and populations change rates during inactivation and refrigerated storage were determined with Excel version 7 (Microsoft, Redmond, WA). Results were analyzed with MINITAB 17.2.1 (Minitab Inc., State College, PA) one-way analysis of variance with Tukey's analyses, and 95% confidence limits were considered.

## RESULTS AND DISCUSSION

**Growth of *Salmonella* on unhulled sesame seeds during soaking.** The first set of experiments in this study explored the growth of *Salmonella* on sesame seeds during the initial soaking period associated with tahini manufacture (Fig. 1). Soaking of raw unhulled sesame seeds for at least 12 h is frequently the preliminary step for dehulling, the process of separating seeds from hulls (13). However, it also provides an opportunity for bacterial growth on sesame seeds when large amounts of water are introduced. Whole dry sesame seeds with an initial  $a_w$  of 0.100 and an aerobic microbial background below the level of detection were used

TABLE 1. Growth ability of *Salmonella* on sesame seeds during soaking at 37°C<sup>a</sup>

Medium	Bacterial growth (log CFU/g)			
	0 h	18 h	22 h	24 h
TSAYE	2.7 ± 0.4 a A	6.6 ± 0.2 bc A	7.4 ± 0.3 cd A	7.6 ± 0.2 d A
XLD	2.3 ± 0.2 a B	5.9 ± 0.5 b B	6.9 ± 0.5 cd B	7.3 ± 0.4 cd A

<sup>a</sup> Data are population means ± standard deviations ( $n = 3$ ). Different lowercase letters indicate significant difference (95% confidence limits) within a row. Different capital letters indicate a significant difference (95% confidence limits) within a column.

in this step. The initial loading level of *Salmonella* on seeds was determined as  $\sim 5$  log CFU/g. Inoculated seeds were drained and held at ambient temperature ( $23 \pm 2^\circ\text{C}$ ) for 24 h to dry before use in growth experiments. During this drying period, the population loss of *Salmonella* was  $\sim 2.5$  log CFU/g, whereas the  $a_w$  of seeds declined gradually to  $\sim 0.100$ . The immediate drop in  $a_w$  after inoculation is likely the major cause of this initial population reduction of *Salmonella*. Previous studies have observed similar reductions in *Salmonella* populations when hydrated viable cells were introduced into low-moisture foods followed by drying (10, 11, 14, 15).

Despite this loss in population due to drying, there were still  $\sim 2.7$  log CFU/g of survivors remaining on sesame seeds. These seeds were then immersed in water, and the growth of *Salmonella* during subsequent soaking at 37°C for 24 h was determined (Table 1). At each time point between 0 and 24 h, triplicate samples were assayed for *Salmonella*. Enumeration of microbial populations on both TSAYE and XLD plates indicated all microbial populations increased from  $2.5 \pm 0.2$  to  $>7.0$  log CFU/g within 24 h. Because there was no significant difference ( $P > 0.05$ ) in populations between 22 and 24 h, the maximum value was likely reached before 22 h. The same experiments were repeated with hulled sesame seeds and indicated no differences due to the presence of the hull. Experiments were also conducted at 25°C, which resulted in similar growth (data not shown). Growth on seeds before any thermal treatment may also result in biofilm formation that could result in greater resistance to subsequent thermal treatment (3).

**Survival of *Salmonella* on dehulled sesame seeds during thermal treatment.** In these experiments, inoculated dehulled sesame seeds on which *Salmonella* was grown for 24 h before use were drained and then roasted at three temperatures, 95, 110, and 130°C, for up to 90 min (Fig. 1). Each trial at each temperature was run three times, with triplicate samples removed at each time point. Populations on TSAYE populations averaged  $0.21 \pm 0.14$  log CFU/g higher than those found on XLD. However, analysis of variance indicated no differences ( $P > 0.05$ ) between populations on media types; consequently, only data from TSAYE agar are reported. The level of *Salmonella* after draining (before roasting) was determined to be  $\sim 8.5$  log CFU/g. This value was considerably higher than that achieved by Torlak et al. (26) before similar roasting experiments. When sesame seeds were drained for 1 h before roasting, seeds remained wet and had an  $a_w$  of  $\sim 1.0$ .

This can be seen in Figure 2 for seeds roasted at 95 and 110°C. Because the  $a_w$  dropped dramatically during roasting, it was expected that the thermal resistance of *Salmonella* would increase over time, resulting in a non-log-linear reduction in populations. However, although both reduction curves seem slightly convex, at 95°C, reductions of *Salmonella* populations were unexpectedly close to linear, with an  $R^2$  value of 0.949 (Fig. 2). The calculated standard  $D$ -value at 95°C (24.7 min) is higher than the published literature values at low  $a_w$  (5). Indeed, although direct comparison to other low-moisture products is difficult, the thermal resistance observed is greater than that observed for *Salmonella* in peanut butter (11, 18, 19) or in confectionary product, seasoning, and dry pet food (17). The seemingly increased thermal resistance may have two explanations. First, the thermal transfer in the oven was slower than anticipated, despite the thin layer used ( $\leq 5$  mm, single seed layer). A second explanation is that *Salmonella* may have grown on seeds and formed a biofilm that conferred greater thermal resistance to the microorganisms than anticipated.

At 110°C, the non-log-linear decrease in surviving populations of *Salmonella* is more clearly visible (Fig. 2). Previously published research demonstrates that the heat resistance of *Salmonella* increases as the  $a_w$  decreases (1, 2, 7, 22). At 110°C, a decrease of  $>4$  log CFU/g can be observed within the first 20 min when the  $a_w$  was still  $>0.4$ . However, only  $\sim 2$  log CFU/g was lost over the next 70-min period when the  $a_w$  dropped below 0.4. By comparison,

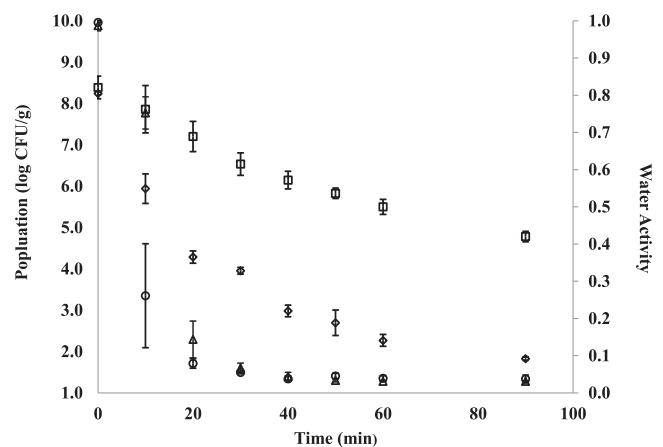


FIGURE 2. Changes in  $a_w$  and *Salmonella* populations (mean and standard deviation;  $n = 3$ ) after draining 1 h and roasting sesame seeds at two temperatures. □, *Salmonella* population when roasted at 95°C; △,  $a_w$  when roasted at 95°C; ◇, *Salmonella* population when roasted at 110°C; ○,  $a_w$  when roasted at 110°C.

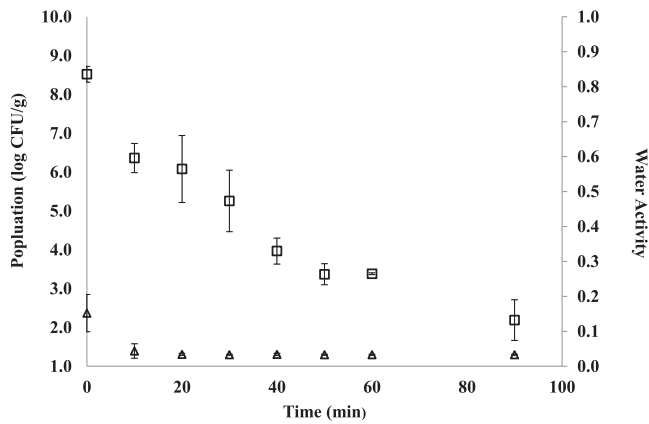


FIGURE 3. Changes in  $a_w$  and *Salmonella* populations (mean and standard deviation;  $n = 3$ ) after draining 24 h and roasting sesame seeds at 130°C. □, *Salmonella* population; △,  $a_w$ .

Torlak et al. (26) reported a loss of 6 log CFU/g in 60 min. Unfortunately, Torlak et al. (26) did not report the actual changes in  $a_w$  during roasting at each temperature examined; they only reported that the  $a_w$  started at 0.98 and dropped to 0.14 within the first 10 min. The differences in the survival between the two studies may be attributed to use of a different inoculation level, differences in the rate of reduction of the  $a_w$  during roasting, or the different *Salmonella* strains used. Changes obtained in survival with respect to product  $a_w$  were also observed when ginger was dried at 60°C (8). When ginger was dried at 60°C, *Salmonella* populations declined when the  $a_w$  of the product remained above 0.4, but they were relatively stable when the product  $a_w$  dropped below 0.4. In the current study, higher temperatures are used. However, although the population of *Salmonella* does not stabilize after the  $a_w$  drops below  $\sim 0.4$ , the rate of decline does seem to decrease even at these higher temperatures.

When roasting at 130°C, two different draining time periods were used. When seeds were allowed to drain for 1 h before roasting, the  $a_w$  at the beginning of the roasting period remained high ( $\geq 0.95$ ) and *Salmonella* populations quickly decreased below detectable limits (1.7 log CFU/g) within the first 10 min (data not shown). However, when the draining period was extended to 24 h, the seeds dried sufficiently such that the  $a_w$  was reduced to  $\leq 0.2$  before roasting. Initial populations were not affected by longer draining or drying; differences between starting populations for the seeds roasted at the three temperatures were not significantly different ( $P = 0.286$ ). Subsequent roasting of these dried seeds resulted in significantly slower declines in the surviving *Salmonella* populations (Fig. 3). Again, reduction rates were close to log linear, with an  $R^2$  value of 0.8952. Using these data, the calculated  $D_{130^\circ\text{C}}$ -value was 15 min. This thermal reduction would seem to be somewhat slower than that observed by Torlak et al. (26), who found a 5-log loss at 130°C after 35 min of roasting. Once again, Torlak et al. (26) did not correlate changes in  $a_w$  with population. However, they noted that the  $a_w$  fell from 0.98 to 0.14 within the first 10 min and that rates of decline were quicker during this period. The length of roasting time

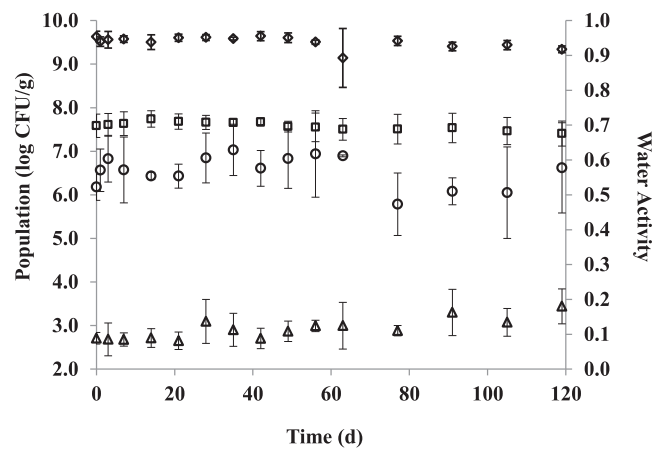


FIGURE 4. Survival of *Salmonella* (mean and standard deviation;  $n = 3$ ) during refrigerated storage of tahini. □, *Salmonella* population when inoculated before roasting; △,  $a_w$  when inoculated before roasting; ◇, *Salmonella* population when inoculated after roasting; ○,  $a_w$  when inoculated after roasting.

during which the  $a_w$  remained above 0.4 clearly affects the survival of *Salmonella* on the roasted sesame seeds. Unfortunately, ovens likely differ in heating profiles and humidity levels, with both factors affecting the time required to reduce the  $a_w$  of a product during roasting. In these experiments, no effort was made to manipulate the length of roasting time at higher  $a_w$ . However, such efforts were made in a study evaluating *Salmonella* survival during drying of ginger at 60°C (8). That study found that higher oven humidity resulted in slower decreases in  $a_w$  that, in turn, affected *Salmonella* survival. This affect may be the cause of any discrepancies in the roasting results reported by Torlak et al. (26) and those observed in the current study.

**Determination of *Salmonella* survival during refrigerated storage of tahini.** In these experiments, two inoculation methods were used to contaminate the tahini (Fig. 1). The first method attempts to mimic natural contamination (method A), allowing *Salmonella* to grow during the initial seed soaking step. In this method, inoculated seeds were soaked in sterile deionized water and incubated for 24 h to allow growth of *Salmonella* on the seeds before roasting. The inoculated roasted seeds were then processed into tahini. For the second inoculation procedure (method B), seeds were processed and *Salmonella* was added to the finished product in an effort to mimic cross-contamination. The  $a_w$  and population changes of *Salmonella* in tahini contaminated by both methods were then stored for 119 days at 4°C (Fig. 4). As noted, no statistically significant differences were found due to populations determined on media types TSAYE and XLD; consequently, populations enumerated only on TSAYE are displayed. *Salmonella* concentrations on seeds inoculated before roasting (method A) were  $8.30 \pm 0.31$  log CFU/g, but they dropped during the roasting procedure (130°C, 15 min) to  $7.59 \pm 0.27$  log CFU/g. Starting populations for *Salmonella* inoculated into tahini postroasting were  $9.63 \pm 0.13$  log CFU/g. In addition, tahini inoculated postroasting

resulted in a higher  $a_w$  than tahini made from seeds inoculated pre-roasting. For both inoculation methods, *Salmonella* populations and  $a_w$  remained constant throughout the 119-day storage period, with no significant difference in *Salmonella* populations found between the initial time point and after 119 days of storage. Because there was no discernible population change for either method, it can be concluded that the method of inoculation did not affect the survival during storage in these studies. Because both population rates seemed stable over the 119-day period, no reduction rates were calculated.

These measured rates were dramatically different than those reported by Torlak et al. (26), who found a loss of 3.3 log CFU/g over a similar period. The serotypes used in this study were not the same as those used by Torlak et al. (26); nonetheless, it is unlikely that serotype alone would account for such a dramatic difference in the survival rate. An alternative explanation is that the cultures used in this study were cultivated differently from those used in the Torlak et al. study. Torlak et al. (26) cultures were cultivated in liquid media. In the current study, both inoculation methods used involved cultivation methods that have been attributed to greater desiccation resistance in *Salmonella* (3, 11, 15, 27). Indeed, when *Salmonella* has been cultivated using one of the methods used in this study (agar grown cells), similar longevity on other low- $a_w$  products have been described under the same storage conditions. In nuts stored at 4°C, no reduction in *Salmonella* populations was observed over 550 days (27). The increased desiccation resistance and survival related to growth conditions may impact results in any challenge study. The importance of appropriate adaptation to test conditions before experimentation was emphasized by the National Advisory Committee on Microbiological Criteria for Foods in 2010 (20).

Contamination with *Salmonella* may occur at any step during industrial processing and storage of tahini (21, 23, 24). If dry raw sesame seeds, either with or without an intact hull, are contaminated with *Salmonella*, it is possible that *Salmonella* may grow and survive throughout three major processing steps (soaking, roasting, and storage) involved with the manufacture of tahini and threaten food safety and human health.

Conditions such as inoculum preparation, inoculation method, the initial  $a_w$  of sesame seeds before roasting, and roasting temperatures can affect the thermal resistance of *Salmonella* during the roasting process. Typically, the heat tolerance of *Salmonella* in low-moisture foods is higher than it is in high-moisture foods. Longer draining time creates lower  $a_w$  of seeds before roasting and may increase the heat resistance of *Salmonella* during roasting. In this study, the influence of  $a_w$  on the survival of *Salmonella* was shown in a dynamic process wherein  $a_w$  changed during thermal treatment. Survival of *Salmonella* increased as the thermal processing  $a_w$  decreased. Manufacturers should ensure sesame seeds are at a high moisture level before roasting to ensure adequate destruction of *Salmonella*.

*Salmonella* can survive in refrigerated stored tahini for at least 119 days with essentially no change in populations. Survival of *Salmonella* during refrigerated storage did not

depend on the method of inoculation (before seed soaking and roasting versus into the final product). However, the survival observed here was markedly longer than previously reported (26), indicating that inoculum preparation may influence the survival rate of *Salmonella* during storage of tahini. Cells grown on solid media (sessile cells) may survive longer than those grown in liquid culture (planktonic cells).

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