Fate of *Salmonella* during sesame seeds roasting and storage of tahini

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**A B S T R A C T**

Tahini is usually consumed without further heat treatment, and roasting of sesame seeds is the only *Salmonella* inactivation step in its traditional production process. This study examined the efficiency of the roasting process in the elimination of *Salmonella* from sesame seeds and the survival of *Salmonella* in tahini during storage. Sesame seed and tahini samples were inoculated with a cocktail of three serotypes of *Salmonella* (*S. Typhimurium*, *S. Newport* and *S. Montevideo*). Complete inactivation of *Salmonella* in sesame seeds, inoculated with 5.9 log cfu/g, was achieved by roasting at 110 °C for 60 min, 130 °C for 50 min, or 150 °C for 30 min. *Salmonella* levels in tahini (a 0.17) inoculated with 5.6 log cfu/g and stored for 16 weeks at 22 or 4 °C decreased by 4.5 and 3.3 log, respectively. Results of this study demonstrated that the standard roasting process is sufficient to inactivate *Salmonella* in sesame seeds and low water activity of tahini prevents microbial growth, but its composition allows *Salmonella* to survive for at least 16 weeks. Therefore, prevention of cross-contamination after roasting is crucial for food safety.

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1. Introduction

Foodborne outbreaks of salmonellosis are usually associated with the consumption of contaminated animal products such as eggs or poultry meat or fecally contaminated raw vegetables. However, in the last few decades, high fat-low moisture colloidal foods such as tahini have been identified several times as unusual sources of salmonellosis outbreaks (WHO, 2004; Shachar and Yaron, 2006). Presence of both high fat content and low water activity has a protective effect against the inactivation of *Salmonella* (Shachar and Yaron, 2006; Podolak et al., 2010).

Tahini, also called sesame paste, produced by milling from dehulled and roasted sesame seeds, is a popular foodstuff in Middle Eastern and eastern Mediterranean countries and is used as an ingredient in many kinds of foods such as halva and hummus (El-Adawy and Mansour, 2000; Lokumcu and Ak, 2005). Contamination of tahini with pathogens is particularly important, since it is usually consumed without any additional heat treatment (Lake et al., 2010). Furthermore, its high fat content protects the pathogen from gastric acidity resulting in a reduction of the dose–response curve (Nascimento et al., 2012). In recent years there have been a number of reports of *Salmonella* outbreaks related to the consumption of contaminated tahini. In 2001, an outbreak of *S. Typhimurium* due to contaminated halva, a traditional low-moisture confectionery produced from tahini, was reported in Sweden (Brockmann, 2001). Following this outbreak, Brockmann et al. (2004) examined in total 117 sesame seed products for the occurrence of the *Salmonella* and 11 of these were found to be contaminated. In 2002 and 2003, three outbreaks of *S. Montevideo* infection associated with tahini were reported in Australia and New Zealand (Unicomb et al., 2005). Very recently, a multistate outbreak of *Salmonella* Bovismorbillanc infections associated with tahini was reported in the USA (CDC, 2012).

Contamination of sesame seeds with *Salmonella* can occur during growth, storage or processing (Podolak et al., 2010). During growth, the crops are likely to be exposed to a wide range of microbial contamination from many sources including soil, manure, irrigation water, wild birds and animals. Further potential for microbial contamination may occur during post-harvest processing due to poor sanitation practices (Doyle and Erickson, 2008). In a comprehensive survey study conducted in the UK, the prevalence of *Salmonella* in sesame seed samples was determined as 1.7% (Willis et al., 2009). In another study, conducted in the USA, 177 sesame seed samples were investigated for *Salmonella* contamination and 20 (11%) samples were found to be contaminated (Van Doren et al., 2012).

Roasting of sesame seeds is a basic operation for the production of tahini. Sesame seeds are roasted in order to promote the flavor, desired color and changes in texture which ultimately increase the overall palatability of the product (Kahyaoglu and Kaya, 2006). Several temperature–time combinations were reported for roasting of sesame seeds during tahini production. Ozcan and Alkul (1994) reported that sesame should be roasted at 110–150 °C for 2.5–3 h. The optimum roasting range for production of tahini to obtain the desired color and texture was given as 155–170 °C for 40–60 min (Kahyaoglu and Kaya, 2006). Brockmann et al. (2004) concluded that the *Salmonella* should
not survive during the sesame seeds roasting. Therefore, they also concluded that the likely cause of the Salmonella outbreaks linked to sesame seed products was cross contamination of the products after the heat treatment. However, these conclusions are based on the results of studies performed on matrices other than sesame seeds.

There are no published data on the efficiency of the roasting process in the elimination of Salmonella from sesame seeds and the survival of Salmonella in tahini during storage. Therefore, the objective of this study was to assess the fate of Salmonella during the sesame seeds roasting for 60 min at 110 °C, 130 °C and 150 °C and storage of tahini for 16 weeks at 22 and 4 °C.

2. Material and methods

2.1. Salmonella strains

A cocktail of three S. enterica serotypes was used for inoculation of sesame seed and tahini samples: S. Typhimurium, S. Newport and S. Montevideo. These three serotypes have already been involved in outbreaks of salmonellosis associated with the consumption of oilseeds and sesame seed products.

Lyophilized cultures of S. Typhimurium (ATCC 14028) and S. Newport (ATCC 6962) were supplied from Microbiologics Inc. (Saint Cloud, USA). Culture of S. Montevideo (ATCC 5747) was kindly provided by National Public Health Agency (Ankara, Turkey). Stock cultures of microorganisms were stored in Brain Heart Infusion Broth (Merck, Darmstadt, Germany) supplemented with 20% glycerol at −18 °C. Working cultures were grown on Nutrient Agar (Merck) slants and kept at 4 °C.

2.2. Inoculum preparation

Each Salmonella strain was transferred into Tryptic Soy Broth (Lab M, Bury, UK) and incubated overnight at 37 °C. Then, a cocktail of the 3 strains was prepared by mixing an equal volume of each culture in the same tube. The mixed culture was centrifuged at 3600 g for 10 min at 5 °C (Hettich, Tuttingen, Germany) and washed three times with Phosphate-Buffered Saline (PBS). The final cell pellet was resuspended in PBS and the cell density of suspension was adjusted to 0.5 McFarland turbidity standard.

2.3. Inoculation of sesame seeds

The wet dehulled sesame seeds were used for inoculation since dehulling process with water is a step prior to roasting in traditional production of tahini. Nigeria cultivars white sesame seeds determined as Salmonella-negative by the reference culture method (ISO, 2002) were dehulled as follows; firstly seeds were sieved and then soaked in water at ambient temperature for 12 h. The soaked seeds were strained off and passed through a mechanical peeler for removing of hulls from seed. The hulls and other foreign materials were separated from seeds by using salt solution (1.5% NaCl). The seeds were taken from the surface of the salt solution and then washed four times with water to remove the salt (Kahyaoglu and Kaya, 2006).

Inoculation of wet dehulled sesame seeds was carried out using a mesh bag (Buchholz and Matthews, 2010). A mesh bag filled with 300 g wet dehulled seeds was immersed into Salmonella cell suspension prepared by diluting the inoculum in Maximum Recovery Diluent (Lab M) to obtain cell density of approximately 10^7 cfu/mL. The bag of seed sample was agitated for 10 min by moving bag using draw string and removed from the cell suspension. Then the inoculated bag of seed sample was allowed to drain for 30 min on a stainless steel screen within a biosafety cabinet ( Faster, Ferrara, Italy). After inoculation, the initial inoculation level and water activity (Novasina, Lachen, Switzerland) of sesame seeds were determined.

2.4. Roasting of sesame seeds

The inoculated seed sample was divided into six portions. Sesame seed portions were arranged, as far as was possible, in a single layer in pyrex petri dishes and then dishes were placed in a forced-air oven (Nüve, Ankara, Turkey) which was pre-heated to the respective roasting temperature. Roasting was carried out at three different temperatures (110, 130 and 150 °C) for 60 min. During the roasting, temperature of oven air was checked with K-type thermocouple connected to digital thermometer (Fluke, Everett, WA, USA). Every 10 min during the roasting process, one portion was removed as quickly as possible to minimize changes in the temperature of the oven air, cooled to room temperature and assayed for Salmonella and water activity.

2.5. Inoculation of tahini

Commercially produced tahini sample, containing 58.2% crude fat, 23.5% crude protein, 13.5% carbohydrate, 4.2% ash and 0.6% moisture, was kindly provided by Gesa Food Company (Konya, Turkey). It was determined as Salmonella-negative by the reference culture method (ISO, 2002). Twenty five grams of tahini sample was placed in sterile stomacher bags and inoculated with 200 μl of the inoculum. Inoculated sub-samples in stomacher bags were gently mixed by hand squeezing for 2 min. Then, bags were sealed and stored at 4 °C or 22 °C for up to 16 weeks. Inoculated tahini sample was assayed for Salmonella at the beginning and end of weeks 1, 2, 4, 8 and 16 during the storage period.

2.6. Salmonella assay

Salmonella enumeration was performed by plate count technique on Xylose Lysine Deoxycholate (XLD) Agar (Lab M). Initial suspensions were prepared with adding 225 ml Buffered Peptone Water (BPW, Lab M) into stomacher bags containing 25 g sample. Sample suspensions were homogenized using a stomacher (IUL, Barcelona, Spain) and additional ten-fold dilutions were made by Peptone Salt Diluent (Merck). Then, in total 1 ml of initial suspension and dilutions were surface plated on three plates of XLD agar. After incubation at 37 °C for 24 h, characteristic colonies with a black center on plates were counted and Salmonella counts were calculated as log cfu/g. Occasionally, characteristic colonies were confirmed using biochemical identification test system (Microgen Bioproducts, Camberley, UK).

The enrichment method (ISO, 2002) was performed concurrently with the enumeration method starting from initial suspensions in order to achieve a detection limit of 1 cfu/25 g. After incubation of initial suspensions at 37 °C for 22 h. 0.1 ml preenrichment BPW was added to 10 ml Rappaport Vassiliadis Soy (RVS) broth (Lab M), and 1 ml was added to 10 ml Muller-Kaufmann Tetrathionate-Novobiocin (MKTTn) broth (bioMérieux, Marcyl l'Etoile, France). RVS broth was incubated at 41.5 °C for 24 h and MKTTn broth was incubated at 37 °C for 24 h. Each selective enrichment broth was streaked onto XLD agar. Following incubation at 37 °C for 24 h, presumptive Salmonella colonies were confirmed.

2.7. Statistical analysis

Three independent trials were conducted. Results were analyzed by one-way analysis of variance (ANOVA) using statistical software (SPSS Inc., Chicago, USA). Mean counts were compared using the Duncan grouping test at P < 0.05.

3. Results and discussion

3.1. Survival of Salmonella in sesame seeds subjected to roasting

Thermal inactivation curves of a cocktail of three serotypes of Salmonella in inoculated sesame seeds roasted at three different
temperatures (110, 130 and 150 °C) for up to 60 min are given in Fig. 1. The initial inoculation level of Salmonella determined as 5.9 log cfu/g was decreased by 1.7, 2.1 and 2.9 log after 10 min of roasting at 110, 130 and 150 °C, respectively. Reductions in the counts of Salmonella ranging from 2.9 to 4.4 log were achieved after 20 min of roasting. Enumeration results indicated that reductions in the levels of Salmonella at 150 °C after 10 and 20 min were significantly (P < 0.05) higher than those at 110 and 130 °C. Roasting at 110 and 130 °C for 30 min yielded reductions of 3.8 and 4.8 log respectively, whereas Salmonella was not detected after 30 min roasting at 150 °C. Complete inactivation of Salmonella in sesame seeds was achieved after 60 min roasting at 110 °C. These results showed that all treatments were sufficient to produce over 5 log reduction in Salmonella counts during the 60 min of roasting. A thermal treatment protocol capable of reducing the Salmonella level in oilseeds and nuts by a minimum 5 log is generally accepted as sufficient to provide an appropriate level of consumer protection (GMA, 2009).

Thermal inactivation results obtained were in accordance with the previous studies dealing with the inactivation of Salmonella in similar matrices during the roasting. Nascimento et al. (2012) reported that the complete reduction of a pool of five Salmonella serotypes in cocoa beans inoculated at level of 6 log cfu/g was achieved after 30 min at 120 °C and 40 min at 110 °C. They also reported that the type of matrix, the process temperature and the initial count significantly (P < 0.05) influenced the Salmonella resistance. Doyle (2009) demonstrated that the roasting of peanuts at 129 °C for 45 min, at 146 °C for 15 min, and at 163 °C for 10 min caused the reductions of 4.3, 4.9 and 5.3 log in Salmonella counts, respectively. Beuchat and Mann (2011) achieved 5 log cfu/g reduction of Salmonella in inoculated pecan halves by roasting performed at 140 °C for 20 min.

Enumeration results revealed that reduction in the viable cell numbers achieved after the first 10 min at each of the three different temperatures were significantly (P < 0.05) higher than those achieved after further 10 min intervals of the roasting process. Similarly, Archer et al. (1998) reported that thermal inactivation curves of S. Weletvedren in flour were biphasic, indicating an initial rapid decline in the viable cell numbers during the first 5–10 min followed by slower death rates. This period coincided with a rapid decrease in the water activity of all the samples tested. In this study, the water activity of inoculated sesame seeds was decreased from 0.98 up to 0.14 during the first 10 min of roasting. Izurieta and Komitopoulou (2012) studied the effect of moisture on heat resistance of Salmonella in cocoa and hazelnut shells and reported that the addition of moisture (7% w/w) markedly reduced D<sub>100°C</sub> values of S. Oranienburg and S. Enteritidis ranging between 2 and 4.5 min in the two matrices. A study conducted by Liu et al. (1969) demonstrated that D<sub>100°C</sub> Value of S. Senftenberg in simulated naturally contaminated feed at 5% moisture level was about three times as great as at the respective moisture level of 10%.

Increase in the thermal resistance of Salmonella during the roasting process can be explained by cell protein–water interactions. Vibration of the water molecules during heating causes breakage of disulfide and hydrogen bonds in the surrounding proteins, altering the three-dimensional configuration of the proteins. Vibration effect and protein denaturation decrease while water content of sesame seeds decreases during the roasting. Additionally, a decrease in water content induces a conformational modification (rigidification) of proteins due to decrease in number of dipoles between polar groups in the peptide chains of proteins and the dipoles of the protein interact (Earnshaw et al., 1995; Laroche et al., 2005).

3.2. Survival of Salmonella in tahini

Survival curves of a cocktail of three serotypes of Salmonella in inoculated tahini during storage at room and refrigerator temperatures are shown in Fig. 2. The initial inoculation level of Salmonella in tahini determined as 5.6 log cfu/g decreased by 3.3 and 4.5 log during the storage at 4 and 22 °C for 16 weeks, respectively. At the end of the storage period at 4 and 22 °C, cell counts of Salmonella in tahini were determined as 2.3 and 1.1 log cfu/g, respectively.

Our results revealed that Salmonella serotypes tested did not grow, but survived in tahini during the 4 months of storage at both room and refrigerator temperatures. The ability of Salmonella to survive in low water activity environment of tahini sample (a<sub>w</sub> = 0.17) can be attributed to several mechanisms including accumulation of betaine via specific transporters (Abbe and Wouters, 1999), accumulation of proline (Neidhardt, 1987), modification of the outer membrane (Rychlik and Barrow, 2005) and the function of r<sup>2</sup> and s<sup>2</sup> regulated genes (McMeechan et al., 2007). Previously, several studies demonstrated that Salmonella has the ability to survive in high fat-low moisture colloidal foods like tahini during the extended period of storage (Podolak et al., 2010). Burnett et al. (2000) evaluated that survival characteristics of a five-serotype mixture of Salmonella in commercial peanut butters and peanut butter spreads. They were able to detect Salmonella from peanut butter and spread samples after 24 weeks of storage. Park et al. (2008) documented the viability of S. Tennessee in commercial brands of peanut butter for 14 d. In another study (Kotzekidou, 1998), the survival of S. Enteritidis in halva was investigated and the microorganism was found to be capable of surviving up to 8 months’ storage.

Reduction values obtained indicated that viability of Salmonella serotypes at 4 °C was significantly higher compared to at 22 °C. It was previously documented that the storage temperature affected the viability of Salmonella in colloidal food products. A study by Holliday et al. (2003) demonstrated that counts of Salmonella decreased more rapidly in butter, yellow fat spreads, and margarine stored at 21 °C, compared to those at 4.4 °C. Burnett et al. (2000) obtained considerably lower Salmonella counts from peanut butter and spreads stored at 21 °C.
compared to those at 5 °C. Similarly, Park et al. (2008) reported a significant difference between levels of Salmonella in peanut butter samples stored at 4 and 22 °C.

In addition to extrinsic factors, viability of Salmonella in high fat-low moisture colloidal foods is influenced by the sizes of water droplets which affect nutrient availability for microbial growth (Park et al., 2008). Salmonella cells inoculated into colloidal foods tend to aggregate or clump within or near the water phase (Burnett et al., 2000). Therefore, a finer emulsion provides a more limited environment and a lower quantity of nutrients available in a droplet for microbial growth (Catteau, 1985).

4. Conclusions

Thermal inactivation curves of a cocktail of three serotypes of Salmonella indicate that the standard roasting condition is sufficient to inactivate Salmonella in contaminated sesame seeds. However, it should be noted that negative consumer attitude towards dark color tahini (Kahyaoglu and Kaya, 2006) may lead producers to prefer different roasting conditions, which may be insufficient for the elimination of Salmonella. Therefore, Salmonella contamination in tahini may be attributed to both insufficient roasting of contaminated sesame seeds and to contamination after heat treatment due to poor hygienic conditions during grinding, packaging and transport.

Results of the storage experiment showed that Salmonella counts in tahini considerably reduce during an extended storage period; however, results also revealed that Salmonella can survive in tahini for at least 16 weeks. It was previously documented that small numbers of Salmonella in tahini can cause a large outbreak of salmonellosis (Unicomb et al., 2005). Therefore, in order to minimize the risk of salmonellosis relating to the consumption of contaminated tahini, good hygiene practices, such as implementation of equipment disinfection and environmental hygiene control protocols, should be employed during the production process to control various risk factors that may lead to cross contamination.

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