Survival and inhibition of *Staphylococcus aureus* in commercial and hydrated tahini using acetic and citric acids

Amin N. Olaimat a,⁎, Anas A. Al-Nabulsi b, Tareq M. Osaili b, Murad Al-Holy a, Mutamed M. Ayyash c, Ghadeer F. Mehyar d, Ziad W. Jaradat e, Mahmoud Abu Ghousha a

a Department of Clinical Nutrition and Dietetics, Faculty of Allied Health Sciences, Hashemite University, P.O. Box 150459, Zarqa 13115, Jordan
b Department of Nutrition and Food Technology, Jordan University of Science and Technology, P.O. Box 3030, Irbid, Jordan
c Department of Food Science, United Arab Emirates University, Al Ain, United Arab Emirates
d Department of Nutrition and Food Technology, Faculty of Agriculture, The University of Jordan, 11942, Amman, Jordan
e Department of Biotechnology and Genetic Engineering, Jordan University of Science and Technology, P.O. Box 3030, Irbid, Jordan

**Abstract**

Tahini (sesame paste) is a low-moisture ready-to-eat food that has been linked to foodborne outbreaks and recalls. The objectives of this study were to investigate the behavior of *Staphylococcus aureus* in commercial and hydrated tahini at 10, 21 and 37 °C and to inhibit *S. aureus* in these products by 0.1, 0.3 and 0.5% acetic or citric acid. *S. aureus* was able to survive in commercial tahini with reductions of 3.3, 1.6 and 0.7 log10 CFU/g at 37, 21 and 10 °C, respectively; while it grew in hydrated tahini with an increase of 3.9, 3.0 and 1.8 log10 CFU/ml at 37, 21 and 10 °C, respectively, by 28d. Citric or acetic acid at ≤ 0.5% reduced *S. aureus* in commercial tahini by ≥ 2.3 log10 CFU/ml by 28d compared to control at all of the tested temperatures. However, acetic and citric acid were more inhibitory at 37 and 10 °C, respectively. In hydrated tahini, viable *S. aureus* cells were not detected in the presence of 0.5 or 0.3% acetic acid after 7 and 14d, respectively, at both 21 and 37 °C; and after 14 and 28d, respectively, at 10 °C. Acetic acid at 0.1% also reduced *S. aureus* numbers to undetectable levels after 14 and 28d at 21 and 37 °C, respectively. *S. aureus* cells were also not detected in the presence of 0.5% citric acid by 21d at all of the tested temperatures, or 0.1 and 0.3% citric acid by 28 and 21d, respectively at 21 °C. Acetic and citric acids could be used in tahini or tahini-based products to reduce the potential risk associated with *S. aureus*.

© 2017 Elsevier Ltd. All rights reserved.

**1. Introduction**

Low water activity (aw) food products are either naturally low in free moisture or prepared by drying methods which remove water or adding large amounts of salt or sugar to reduce the available free water for microbial growth (Beuchat et al., 2011; 2013). Usually, these food products have long shelf life and are stable for several years because they are unable to support the microbial growth. However, post process contamination with foodborne pathogens which can survive under such conditions for long time in these products may pose a risk to consumers (Finn, Condell, McClure, Amézquita, & Fanning, 2013). *Staphylococcus aureus* is one of the most important foodborne pathogens which have the ability to grow and survive in low water activity foods (aw~0.85) (Notermans & Heuvelman, 1983; Stewart et al., 2002) and it has caused large outbreaks linked to dry foods such as powdered skim milk (Asao et al., 2003).

Tahini is an example on the low-moisture ready-to-eat food that do not require any further processing such as cooking which may eliminate pathogens if present prior to the consumption. Tahini can be used commercially and at household to prepare many popular ready-to-eat tahini-based products including halva, hummus, various salad dressings, baba ghanoush, mutabbel and tarator sauce (Lake, King, Cressey, & Gilbert, 2010; Unicomb et al., 2005; Abu-Jdayil, Al-Malah, & Asoud, 2002). Although the available water (aw~0.16—0.25) for microbial growth in tahini is low, the high fat content (57–65% wt) enhances the survival of pathogenic and spoilage organisms for long periods (Lake et al., 2010). Recently, the number of outbreaks and recalls linked to tahini has significantly increased (Unicomb et al., 2005; CDC, 2012; 2013; Canadian Food Inspection Agency, 2013). Yamani and Isa (2006) isolated *S. aureus* from all tahini samples collected from industries in Jordan.
It has been found that *Salmonella*, *Listeria innocua* and *E. coli* O157:H7 were able to survive in tahini (Al-Nabulsi et al., 2014; Torlak, Sert, & Serin, 2013). Therefore, it is highly important to search for methods to control potential pathogens in Tahini.

Organic acids are generally recognized as safe (GRAS) antimicrobials which have been used in a variety of food products as preservatives to control microbial growth and improve sensory properties (Cortesi, Panebianco, Giuffrida, & Anastasio, 2009). In a previous works, acetic acid or citric acid significantly reduced the viability of *S. Typhimurium* in tahini (Al-Nabulsi et al., 2014) and *S. aureus* in eggplant dip but citric acid was not inhibitory against *S. Typhimurium* and *E. coli* O157:H7 in eggplant dip (Osaili et al., 2015) or in hummus (Al-Holy, Al-Qadiri, Lin, & Rasco, 2006). Acetic and citric acids showed a good inhibitory effect against *S. aureus in vitro* or in food system. Citric acid was the second most effective among several organic acid tested against clinical and food *S. aureus* isolates (Kim, Yoo, Jung, Heu, & Lee, 2012). A washing solution containing 2% acetic acid reduced numbers of *S. aureus on beef surface by 1.6 log<sub>10</sub> CFU/g (Raffari et al., 2009). To the best of our knowledge, no studies are available on the control of *S. aureus in tahini using organic acids*. Therefore, the objectives of the current study were to investigate the survival and growth behavior of *S. aureus* in commercial tahini and hydrated tahini as a tahini-based products model at different storage temperatures (10, 21 and 37 °C) and to control *S. aureus* in commercial tahini and hydrated tahini using acetic and citric acids at different concentrations.

2. Materials and methods

2.1. Preparation of bacterial culture

In the current study two *S. aureus* strains (ATCC 25923 and ATCC 261217) were used. The strains were kept in Brain Heart Infusion (BHI, Oxoid Ltd, Basingstoke, UK) broth containing 20% glycerol at −40 °C. A loopful of the frozen cultures were streaked on plates of Baird parker agar (BP agar, Oxoid) and then incubated for 24 h at 37 °C. Thereafter, a typical colony was grown in BHI broth at 37 °C for 24 h. To fully activate the culture, three sequential transfers were conducted in BHI broth and a final transfer in BHI broth was performed prior to the experiment. The culture was allowed to grow at 37 °C for 24 h to harvest the cells in the stationary phase and 5 ml of the two *S. aureus* strains were combined to form a cocktail containing 9 log<sub>10</sub> CFU/ml. Thereafter, the mixture was 10-fold serially diluted in 0.1% peptone water to give an approximate final concentration of 6 log<sub>10</sub> CFU/g or 4 log<sub>10</sub> CFU/ml in commercial tahini or hydrated tahini, respectively.

2.2. Preparation of tahini

Tahini paste (protein 22.6% and fat 57.1%, aw 0.30) was purchased from a local grocery store. The tahini was checked for the presence of *S. aureus* prior to starting the experiments and found to be *S. aureus* free. The survival of *S. aureus* was investigated in tahini as is and in diluted (hydrated tahini) as well to resemble its uses in different food settings in the real life. Hydrated tahini was prepared by adding 45 ml of sterile distilled water to 5 g tahini in a sterile plastic cup.

Tahini and hydrated tahini were mixed separately with sterile spautula and 50 g sample size of each was transferred into sterile 100 ml sterile plastic cups. Acetic acid and citric acid (Sigma-Aldrich, St. Louis, MO, US) were used as antimicrobial agents against *S. aureus* in tahini and hydrated tahini. Both of the acids were added to tahini and hydrated tahini at 0.0, 0.1, 0.3 and 0.5% (v/w) with gentle mixing by means of sterile spautula. Tahini and hydrated tahini were inoculated with 1 ml of *S. aureus* cocktail to bring about the final concentration of *S. aureus* cells to 6.0 or 4.0 log<sub>10</sub> CFU/ml in tahini and hydrated tahini, respectively. The samples were stored at 10, 21 and 37 °C for 28d.

2.3. Microbiological enumeration

To enumerate *S. aureus* survivors, tahini and hydrated tahini were sampled at 0, 1, 3, 7, 14, 21, and 28 d of storage. A 5 ml sample was taken using a sterile syringe from each treatment and diluted in 45 ml of 0.1% peptone water. Thereafter, the samples were homogenized in sterile stomacher bags for 2 min by means of a stomacher (Stomacher 400 Seward Ltd., London, UK). Ten-fold serial dilution of the homogenized samples was conducted. The thin layer (TL) method was used to recovery injured *S. aureus* cells. This method involves applying a thin layer of Tryptic Soy Agar (TSA, Oxoid) to the surface of already solidified BP agar in a plate. After cooling and solidification of the top TSA layer; 100 μl aliquots were spread plated onto the TSA layer (Osaili et al., 2010). Plates were incubated aerobically for 24h at 37 °C. Colonies typical of *S. aureus* on TSA/BP agar were enumerated.

2.4. Measurements of pH and water activity

pH values of commercial tahini and hydrated tahini was conducted at the beginning and at the end of the storage period. pH was determined using a pH meter (Cyberscan 500, Eutech Instr., Singapore). aw was measured using an aw meter (Hygrolab, Rotronic Instr. Corp, Huntington, NY, US).

2.5. Statistical analysis

All data reported in the current study are average value of two independent experiments and three replicates of each experiment. Differences among treatments (concentrations of each acid (0.1%, 0.3%, 0.5% acetic acid; and 0.1%, 0.3%, 0.5% citric acid) and control, at 3 different temperatures (10, 21, and 37 °C) were analyzed at each time interval by Tukey’s test using JMP 10.0 software from SAS. Significant differences between treatments were attributed when p value was <0.05.

3. Results and discussion

3.1. Survival and growth of *S. aureus* in commercial and hydrated tahini

Numbers of *S. aureus* gradually decreased in commercial tahini at all temperatures. The reductions after 28d of storage at 37, 21 and 10 °C were 3.3, 1.6 and 0.7 log<sub>10</sub> CFU/g, respectively (Fig. 1). In contrast, *S. aureus* gradually grew in hydrated tahini, and numbers increased by 3.9, 3.0 and 1.8 log<sub>10</sub> CFU/ml at 37, 21 and 10 °C, respectively, at 28d (Fig. 2). The reduction in *S. aureus* numbers in commercial tahini is due to the low water activity (aw = 0.33) and high fat content (57% w/w), however, *S. aureus* is well adapted to survival in low water activity environments. Similar results were observed by Al-Nabulsi et al. (2014) and Torlak et al. (2013) who reported that different *Salmonella* serovars survived in commercial tahini. When tahini was diluted (aw = 0.95), the numbers of *S. aureus* increased and this in agreement with previous studies which were done under the same experimental condition where *S. Typhimurium*, *Listeria innocua* and *E. coli* O157:H7 grew in hydrated tahini (Al-Nabulsi et al., 2013; 2014).
3.2. Effect of citric acid against S. aureus in commercial or hydrated tahini

The weak organic acids have been used as food preservatives for centuries. Acetic and citric acids are commonly used in preparation of tahini-based products such as salad dressings, baba ghanoush, mutabbel (eggplant dip) and tarator sauce. In the current study, the antimicrobial activity of organic acids tested was affected by acid...
type, tahini form and temperature (Figs. 1 and 2). However, citric acid was more effective at lower temperatures compared to 37 °C.

The addition of citric acid (up to 0.5%) to tahini reduced *S. aureus* numbers by only <0.6 log_{10} CFU/ml compared to control (without acid) at 37 °C by 28d and the bacterial reductions increased at 21 °C where it ranged between 0.3 and 1.3 log_{10} CFU/ml. However, the maximum *S. aureus* reduction was observed at 10 °C where the citric acid reduced count by 1.2–2.1 log_{10} CFU/ml compared to

![Fig. 2. Antimicrobial activity of citric acid against *S. aureus* in diluted tahini at 37, 21 or 10 °C. The error bars represent the standard deviations between replicates.](image-url)
control. It should be noted that the extent of reduction in *S. aureus* count increased as the temperature increased and apparently other factors besides citric acid such as low water activity contributed to this bacterial reduction (Fig. 1).

To our knowledge, this is the first study to investigate the inhibition of *S. aureus* in tahini. Nonetheless, another study by Al-Nabulsi et al. (2014) indicated that citric acid at 0.1–0.5% reduced numbers of *S. Typhimurium* in commercial tahini by 2.5–3.8
log_{10} CFU/g, by 28 d of storage at 10 to 37 °C, respectively. In contrast, Bornemeier, Peters, and Albrecht (1997) reported that the addition of 10% citric acid to mayonnaise-based surimi salad reduced *S. aureus* by only 1 log_{10} CFU/g after 8 d at 10 °C, while no antimicrobial activity at 5% acetic acid was observed. It is evident that the antimicrobial activity of organic acids is affected by different factors including pH, temperature, food composition, acid concentration, ionic strength and bacterial strains (Doores, 2005, Fig. 4. Antimicrobial activity of acetic acid against *S. aureus* in diluted tahini at 37, 21 or 10 °C. The error bars represent the standard deviations between replicates.)
In hydrated tahini, citric acid was more effective against *S. aureus* than in commercial tahini. Addition of citric acid at 0.1 and 0.3% reduced the numbers of *S. aureus* in hydrated tahini by ≥7.0 log₁₀ CFU/ml at 37 °C and by ≥3.0 log₁₀ CFU/ml at 10 °C compared to control at 28d. However, citric acid at 0.5% reduced *S. aureus* numbers to the lower limit of detection by 21d at 10 and 37 °C. It is worth mentioning that citric acid exhibited the highest antimicrobial activity at 21 °C, where no viable cells were detected when 0.1, 0.3 or 0.5% citric acid was added to hydrated tahini after 28, 21 and 14d, respectively (Fig. 3). In a study, Osaili et al. (2015) indicated that citric acid at 0.4–0.8% reduced the numbers of *S. aureus* cocktail in eggplant dip containing 14% tahini by 1.0–1.5 log₁₀ CFU/ml at 10 and 21 °C after 10d while at 4 °C. *S. aureus* was reduced by 3.2–3.6 log₁₀ CFU/ml after 15d. In another study, citric acid at 0.1% reduced *S. Typhimurium* numbers by 3.9–5.4 log₁₀ CFU/ml compared to the control at 28d of storage at 10 to 37 °C. However, the initial pH values of commercial or hydrated tahini was also decreased in both commercial and hydrated tahini and was lower in diluted tahini (Table 1). Although both acetic and citric acids reduced the pH of tahini, it is obvious that acetic acid was more inhibitory than citric acid toward *S. aureus* in commercial and hydrated tahini. This is probably because of the presence of higher concentration of the effective undissociated form of acetic acid. Generally, the antimicrobial activity of organic acids depends on their undissociated form, which is the form capable of penetrating bacterial cell membrane. Undissociated organic acid can easily diffuse across the bacterial cell membrane and dissociate resulting in lowering the internal pH (Doories, 2005, pp. 91–142).

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial pH</th>
<th>Final pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37 °C</td>
<td>21 °C</td>
</tr>
<tr>
<td>Control</td>
<td>6.76 ± 0.01</td>
<td>5.90 ± 0.02</td>
</tr>
<tr>
<td>0.1% acetic acid</td>
<td>6.43 ± 0.04</td>
<td>5.61 ± 0.04</td>
</tr>
<tr>
<td>0.3% acetic acid</td>
<td>6.01 ± 0.01</td>
<td>5.65 ± 0.03</td>
</tr>
<tr>
<td>0.5% acetic acid</td>
<td>5.60 ± 0.06</td>
<td>5.32 ± 0.01</td>
</tr>
<tr>
<td>0.1% citric acid</td>
<td>6.46 ± 0.03</td>
<td>5.78 ± 0.01</td>
</tr>
<tr>
<td>0.3% citric acid</td>
<td>6.12 ± 0.1</td>
<td>5.59 ± 0.08</td>
</tr>
<tr>
<td>0.5% citric acid</td>
<td>5.55 ± 0.1</td>
<td>5.27 ± 0.06</td>
</tr>
</tbody>
</table>

Values are the means of two experiments ± standard deviation.

3.3. Effect of acetic acid against *S. aureus* in commercial or hydrated tahini

*S. aureus* numbers gradually decreased to undetectable levels by the end of the storage period when using 0.3 and 0.5% acetic acid in commercial tahini at 37 °C. On the contrary, no significant reduction was observed when 0.1% acetic acid was used (Fig. 3). Acetic acid at the levels of 0.3–0.5% resulted in reducing *S. Typhimurium* numbers in commercial tahini by 0.8–1.2 log₁₀ CFU/ml at 37 °C (Al-Nabulsi et al., 2014). However, acetic acid at the level of 0.1–0.5% in commercial tahini significantly reduced numbers of *S. aureus* by 1.2–2.3 log₁₀ CFU/ml at 10 °C and by 0.7–1.6 log₁₀ CFU/ml at 21 °C by 28d compared to control (Fig. 3). Similar antimicrobial activity was observed against *S. Typhimurium* in commercial tahini where 0.1–0.5% acetic acid reduced *S. Typhimurium* by 1.0–2.1 log₁₀ CFU/ml at 21 °C and 1.0 to 1.3 log₁₀ CFU/ml at 10 °C by 28d (Al-Nabulsi et al., 2014). *S. aureus* numbers were reduced by 1.0 and 2.0 log₁₀ CFU/g in mayonnaise-based surimi salad by 5 and 10% acetic acid, respectively, after 8 d at 10 °C (Bornemeier et al., 1997).

In hydrated tahini, acetic acid showed more activity against *S. aureus*. Acetic acid at 0.5 and 0.3% reduced *S. aureus* cells to undetectable levels after 7and 14 d, respectively at both 21 and 37 °C; and after 14 and 28 d, respectively at 10 °C. Acetic acid at 0.1% also reduced *S. aureus* numbers to undetectable levels after 14 and 28 d at 21 and 37 °C respectively. But at the later concentration was less inhibitory at 10 °C and reduced *S. aureus* numbers by 2.9 log₁₀ CFU/ml compared to the control after 28d of storage (Fig. 4). This is agrees with the findings of Al-Nabulsi et al. (2014) who observed that *S. Typhimurium* cells in hydrated tahini were not detected after 7 d at 37, 21 and 10 °C, respectively, by 0.5% acetic acid; or after 7, 14, and 28 d at 37, 21 and 10 °C, respectively, by 0.3% acetic acid. It is evident that antimicrobial activity of both citric and acetic acids is higher in hydrated tahini than commercial tahini (undiluted). The later form contain high levels of fat and proteins, which may protect the bacterial cells from the inhibitory activity of organic acids.

3.4. Changes in pH of commercial or hydrated tahini

The initial pH of both commercial and hydrated tahini was 6.8 which was reduced to 5.8 to 6.2 after 28 d of storage at different temperatures. However, the initial pH values of commercial or hydrated tahini acidified with 0.1–0.5% of citric acid or acetic acid ranged from 5.3 to 6.5 where the pH reduction was proportional to the concentration of acid. During the storage, the pH of acidified tahini was also decreased in both commercial and hydrated tahini and was lower in diluted tahini (Table 1). Although both acetic and citric acids reduced the pH of tahini, it is obvious that acetic acid was more inhibitory than citric acid toward *S. aureus* in commercial and hydrated tahini. This is probably because of the presence of higher concentration of the effective undissociated form of acetic acid. Generally, the antimicrobial activity of organic acids depends on their undissociated form, which is the form capable of penetrating bacterial cell membrane. Undissociated organic acid can easily diffuse across the bacterial cell membrane and dissociate resulting in lowering the internal pH (Doories, 2005, pp. 91–142).

Mortimore and Wallace (2013) reported that the proportion of undissociated form of acetic acid is larger than this for citric acid at pH values of 3.0–7.0 which may increase the dissociation of acetic acid inside the bacterial cells which enhance its antimicrobial activity. Raftari et al. (2009) found that tested organic acids (acetic acid, lactic acid, propionic acid and formic acid) were more effective against *S. aureus* than *E. coli* O157:H7. It seems that gram-positive bacteria such as *S. aureus* are more sensitive to organic acids because they do not possess the outer membrane which may enhance these agents to enter the bacterial cell.

4. Conclusions

Tahini was implicated in causing several outbreaks of foodborne illness. The current study showed that *S. aureus* was capable of surviving in commercial tahini during the storage period used in the current study. However, *S. aureus* grew more preferably in hydrated tahini at all tested temperatures. Using organic acids resulted in a remarkable inhibitory effect against *S. aureus* in commercial and hydrated tahini. The antimicrobial activity of citric acid and acetic acid against *S. aureus* was more pronounced in the hydrated form of tahini compared to the commercial form. Acetic acid posed a noticeable inhibitory effect against *S. aureus* in commercial tahini, especially when used at concentrations higher than 0.3% at all the tested temperatures. While, citric acid generally posed more inhibitory activity at 10 °C compared to acetic acid. Complete elimination of *S. aureus* from hydrated tahini was attained before the end of the 28 d storage period when using acetic acid at all the tested levels except at 10 °C. Using acetic and citric acids as natural antimicrobial agents is helpful in controlling...
the growth of S. aureus in tahini and tahini products.

Acknowledgments

This project was financially supported by the Deanship of Research at Jordan University of Science and Technology, Irbid, Jordan. We thank Mrs. Noor Zein Elabedeen for her technical assistance.

References


