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

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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 $\log_{10}$ 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 $\log_{10}$ CFU/ml at 37, 21 and 10 °C, respectively, by 28d. Citric or acetic acid at $\leq$ 0.5% reduced *S. aureus* in commercial tahini by $\leq$ 2.3 $\log_{10}$ 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*.

1. Introduction

Low water activity (a_w) 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 (a_w~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.

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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 $\log_{10}$ CFU/g (Raftari 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 24h 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 $\log_{10}$ CFU/ml. Thereafter, the mixture was 10-fold serially diluted in 0.1% peptone water to give an approximate final concentration of 6 $\log_{10}$ CFU/g or 4 $\log_{10}$ CFU/ml in commercial tahini or hydrated tahini, respectively.

2.2. Preparation of tahini

Tahini paste (protein 22.6% and fat 57.1%, a_w 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 spatula 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 spatula. 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 $\log_{10}$ 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). a_w was measured using an a_w 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 $\log_{10}$ 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 $\log_{10}$ 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 ($a_w = 0.33$) 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 ($a_w = 0.95$), 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).