Factors affecting the viability of *Staphylococcus aureus* and production of enterotoxin during processing and storage of white-brined cheese

Anas A. Al-Nabulsi,^{1*} Tareq M. Osaili,^{1,2} Roa A. AbuNaser,¹ Amin N. Olaimat,³ Mutamed Ayyash,^{4*} Murad A. Al-Holy,³ Khaled M. Kadora,⁵ and Richard A. Holley⁶

¹Department of Nutrition and Food Technology, Jordan University of Science and Technology, Irbid 22110, Jordan

²Department of Clinical Nutrition and Dietetics, University of Sharjah, Sharjah, PO Box 27272, United Arab Emirates

³Department of Clinical Nutrition and Dietetics, Faculty of Allied Health Sciences, Hashemite University, Zarqa 13115, Jordan

⁴Department of Food, Nutrition, and Health, College of Food and Agriculture, United Arab Emirates University, Al-Ain 15551, United Arab Emirates

⁵Department of Food Laboratories, Jordan Food and Drug Administration, Amman 11181, Jordan

⁶Department of Food and Human Nutritional Sciences, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

ABSTRACT

Staphylococcus aureus is a major foodborne pathogen that causes severe disease in humans. It is commonly found in milk and dairy products, particularly in fresh brined cheese. Our aim was to investigate the behavior of *Staph. aureus* and enterotoxin production during the storage of white-brined cheese prepared with or without a starter culture and stored in a 10 or 15% NaCl brine at 10°C and 25°C for 28 d. NaCl concentration, water activity, pH, and number of *Staph. aureus* and lactic acid bacteria were determined in cheese and brine. Only 1 of 4 *Staph. aureus* strains (ATCC 439) was positive for enterotoxin production, and its production was detected in unsalted UHT milk, but not in salted milk or in any of the cheese treatments held at 37°C for 1, 3, or 7 d. *Staphylococcus aureus* grew in the cheese stored in both brines at 10°C and 25°C, regardless of the presence of a starter culture, although the latter significantly reduced *Staph. aureus* growth in cheese or its brine at 10°C. *Staphylococcus aureus* numbers were increased by 2.26 and 0.47 log₁₀ cfu/g in cheese stored in 10 and 15% NaCl brine, respectively, in the presence of starter culture, and by 2.78 and 2.96 log₁₀ cfu/g, respectively, in the absence of starter culture at 10°C. Nonetheless, the pathogen grew, but at a lower number in the brines. The salt concentration of cheese stored in 10% brine remained at approximately 5% during storage; however, in 15% brine, the salt concentration increased to almost 8% (wt/wt) by 28 d. The addition of a starter culture, high salt concentration, low tem-

perature, and pH (~5.2) had inhibitory effects on the growth of *Staph. aureus*. Moreover, lactic acid bacterial numbers increased considerably in cheese and brine by d 28. The use of starter cultures, salt (15%), and low storage temperature (10°C) reduced the growth of *Staph. aureus*, and salt may have prevented enterotoxin production in white-brined cheese.

Key words: *Staphylococcus aureus*, lactic acid bacteria, starter culture, staphylococcal enterotoxin, white-brined cheese

INTRODUCTION

White-brined cheese is a traditional cheese product in communities from North Africa, the Middle East, and the Gulf Cooperation Council countries (Ayyash et al., 2013). White-brined cheese is traditionally consumed either fresh or after ripening in a brined solution (5–20% NaCl) at room temperature (Ayyash and Shah, 2011). White-brined cheese is a perishable food that can act as a medium for the rapid growth of foodborne pathogens. It serves as an ideal microenvironment for the growth of foodborne pathogens, such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus* because of its high water activity, (a_w, 0.94–0.96), high protein (21.1%), moderate fat content (10.4%), relative high pH value (6.1–5.2), and the absence of competitive starter culture (Al-Holy et al., 2012). The binomial variation in pH values (acidic and alkaline) has an adverse effect on pathogens' growth. A similar adverse effect is reported when salt concentration is increased. The thermal heat treatments (65°C/30 min, 72°C/15 s, 135°C/3 s) are designed to inactivate the foodborne pathogens (Cebrián et al., 2016).

Staphylococcal foodborne intoxication is among the common forms of bacterial food poisoning outbreaks (Dayan et al., 2016). This organism can grow in vari-

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*Corresponding authors: anas_nabulsi@just.edu.jo and mutamed.ayyash@uaeu.ac.ae

ous foods, such as milk, cream, cream-filled pastries, butter, ham, cheeses, sausage, cooked meals, sandwich filling, meat and meat products, poultry and egg products, and salads, such as egg, tuna, chicken, potato, and macaroni (Gatadi et al., 2019). Moreover, any food, such as white-brined cheese, which requires considerable handling during preparation and is kept at warm temperatures or brined in low-salt solution after preparation, is also commonly implicated in staphylococcal food poisoning (Le Loir et al., 2003; Ahmed et al., 2019).

Currently, the World Health Organization has classified *Staph. aureus* as a high-tier priority II pathogen (WHO, 2017). In 2015, the European Food Safety Authority reported that staphylococcal toxins caused 434 out of the 4,362 (9.9%) foodborne-disease outbreaks. In the 39 strong-evidence outbreaks caused by staphylococcal toxins, cheese was the most commonly implicated food vehicle, followed by mixed food (EFSA, 2018). In a report by the Center for Disease Control and Prevention, 75 outbreaks occurred between 2009 and 2015 because of contamination with *Staph. aureus* (of those, 40% were related to *Staph. aureus* enterotoxins), with 1681 illnesses and 86 incidents of hospitalization (Dewey-Mattia et al., 2018).

Further, Singh and Prakash (2008) indicated that *Staph. aureus* was isolated from milk products, including curd and cottage cheese. Coton et al. (2010) reported that *Staphylococcus* strains (227 isolates) were obtained from cheese in France. Recently, *Staph. aureus* was found in 142 samples of 350 dairy products in Egypt, where positive rates of *Staph. aureus* detection in cheeses were up to 41% (Ahmed et al., 2019). For the first time, an oxacillin-susceptible *mecA*-positive *Staph. aureus* was detected in cheese samples in Europe (Quijada et al., 2019). The prevalence of *Staph. aureus* in Brazilian milk and cheese samples was 61% and 100%, respectively (de Oliveira et al., 2019).

It is evident that *Staph. aureus* is among the major causes of foodborne illness. In response, Adhikari et al. (2018) have monitored the *Staph. aureus* in vacuum-packed canned cheeses as influenced by storage time and temperature. Mohammadi and Hanifian (2015) have investigated the growth and enterotoxin production of *Staph. aureus* in Iranian ultra-filtered white cheese. However, information about the survival of *Staph. aureus* during the processing and storage of white-brined cheese is not available. Therefore, the objective of the current study was to investigate the behavior of *Staph. aureus* and enterotoxin production during the processing and storage of white-brined cheese in the presence or absence of a starter culture when stored at different salt concentrations and temperatures.

MATERIALS AND METHODS

Preparation of Culture

Four strains of *Staph. aureus* were employed in the current study: *Staph. aureus* ATCC 1131, ATCC 1210, ATCC 25923, and ATCC 439 (MicroBiologics Inc., St. Cloud, MN). Each culture was refreshed after thawing the frozen culture by streaking a loopful on Baird-Parker agar (**BPA**, Sigma-Aldrich, St. Louis, MO). The plates were incubated at 37°C for 18 h. Next, one colony from each isolate was grown individually in brain-heart infusion (**BHI**) broth and incubated at 37°C for 18 h. Three successive transfers were carried out to activate the culture for future experimental runs. The activated cultures were kept at 4°C and subcultured weekly during experimental periods. Meanwhile, a culture from 10 mL of BHI was centrifuged at $2,500 \times g$ for 20 min to obtain pellets that were resuspended in 1 mL of sterile 0.1% peptone water. Then, the strain was used to inoculate milk samples at a final level of approximately $5 \log_{10}$ cfu/mL.

Screening of Staphylococcal Enterotoxin Production

The production of staphylococcal enterotoxins in UHT milk containing 0, 5, or 10% NaCl was tested after 0, 1, 3, 7, 14, 21, and 28 d of incubation at 37°C using VIDAS SET2 (bioMérieux SA, Marcy-l'Etoile, France). The VIDAS SET2 provided a direct method for screening food for the presence of any of the following enterotoxins: SEA, SEB, SEC1, SEC2, SEC3, SED, and SEE. VIDAS SET2 is composed of 30 reagent strips. All these compounds are ready for immediate use, except for the SET2 concentrated extraction buffer (R1), which requires further preparation by adding 1 mL of R1 to 55 mL of demineralized water (1×55 mL).

The abovementioned *Staph. aureus* strains were screened for the ability to produce staphylococcal enterotoxins in UHT milk. The UHT milk (100 mL) was inoculated separately with each *Staph. aureus* strain to a final level of approximately $5.0 \log_{10}$ cfu/mL and incubated at 37°C for 0, 1, 3, 7, 14, 21, and 28 d. The enterotoxins were detected in UHT samples according to the manufacturer's protocol using an AOAC International (2007) method. Briefly, at specific time intervals, the pH of a 25-mL UHT milk sample was adjusted to 3.5 to 4.0 using 5 N HCl. Then, the suspension was centrifuged for 15 min at $3,000$ to $5,000 \times g$ at 18 to 25°C to recover the supernatant, and the residues were discarded. Subsequently, the pH of the supernatant was adjusted to 7.5 to 8.0 using 1 N NaOH, followed by

centrifugation at $3,000$ to $5,000 \times g$ for 15 min at 18 to 25°C to recover the supernatant. After filtration, 500 μ L of the filtrate were placed in the sample well of a VIDAS SET2 reagent strip, and results were observed after 80 min.

Production of White-Brined Cheese

White-brined cheese was prepared as described by Osaili et al. (2014) as illustrated in Figure 1. The experimental design of the current study is presented in Table 1. The full-fat cow milk was acquired from the dairy farm at Jordan University of Science and Technology (Ar-Ramtha, Jordan), pasteurized at 72°C for 15 s, and cooled to 36 to 37°C. The pasteurized milk was screened for the presence of *Staph. aureus* by taking aliquots (100 μ L) of pasteurized milk, which were surface-plated on BPA supplemented with egg yolk (Oxoid Ltd.), and incubated at 37°C for 24 to 48 h. The pasteurized milk was observed to be negative for *Staph. aureus*. The pasteurized milk was divided into 15-L batches for cheese making. The *Staph. aureus* strain ATCC 439 was added to yield an initial inoculum level of approximately $5.0 \log_{10}$ cfu/mL. In cheese treated with the starter culture, 0.5% (wt/vol) of a direct-to-vat thermophilic starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Danisco France, Paris, France) was added, and inoculated milk was kept at 37°C for 30 min. Afterward, single-strength calf rennet (VGP Pharmachem, Vic, Spain) diluted 30-fold in double-distilled water was added to the pasteurized milk and kept at 37°C for 30 to 40 min. The curd was cut into 2-cm³ cubes using stainless steel knives, and whey was drained. Then, the curd was carefully transferred from the vat to a stainless steel mold with 50 cm $\times$ 50 cm $\times$ 2 cm dimensions and pressed for 30 min. The curd was cut manually into pieces that weighed 60 to 80 g and placed in sterilized glass jars containing either 10 or 15% sterilized NaCl brine solution, then stored at either 10°C or 25°C. Samples from cheeses and brine solutions were taken at 0, 1, 3, 7, 14, 21, and 28 d of storage. Cheese making was repeated in triplicate, and 2 cheese loaves were taken from each experimental cheese to perform further analysis.

Enumeration of *Staph. aureus* and Lactic Acid Bacteria

A 25-g sample of internal section of white-brined cheese was homogenized with 225 mL of (0.1%) peptone water for 2 min using a stomacher blender model 400 (Seward Ltd., London, UK). The sample from the

brine solution was prepared by mixing 5 mL of brine solution with 45 mL of 0.1% peptone water and homogenized in a sterile stomacher bag for 2 min with the stomacher. *Staphylococcus aureus* were enumerated by surface plating of 100- μ L samples from the appropriate dilution on BPA supplemented with egg yolk (Oxoid Ltd.) and incubated at 37°C for 24 to 48 h. Lactic acid bacteria (**LAB**) were enumerated by surface plating of 100 μ L of the appropriate dilution of cheese or brine sample on de Man Rogosa Sharpe agar (Thermo Scientific, Waltham, MA) and anaerobically incubated at 37°C for 48 h.

pH Measurement

A pH meter equipped with glass body pH electrode for solid and semi-solid samples ECFG6351101B (Eutech Instrument, pH tutor, Singapore) was used to measure the pH values of white-brined cheese and brine solutions. The pH meter was calibrated with 3 buffers at pH 4, 7, and 10. Then, the electrode was immersed in the white-brined cheese or brine until the reading became stable upon which pH values were recorded.

Salt Determination by Silver Nitrate

The concentration of NaCl in cheese was determined using the procedure as described by Osaili et al. (2014). Afterward, droplets (~ 0.5 mL) of potassium chromate (K_2CrO_4 , 0.5 *N*) and 25 mL of distilled water were mixed thoroughly with ashed sample. The mixture was titrated against silver nitrate ($AgNO_3$, 0.05 *N*) until a brown color developed. The salt concentration was calculated using the following equation:

$$\text{Salt content \%} = (\text{titrated volume of } AgNO_3 \times 0.00292) / \text{weight (g)} \times 100\%.$$

Water Activity Measurement of Cheese

A sample of approximately 2 g from a cross-sectional piece of white-brined cheese was placed in a water activity meter (Hygrolab, Rotronic Instr. Corp., Huntington, NY) to measure the a_w .

Staphylococcal Enterotoxin Detection for White-Brined Cheese

A cheese sample (25 g) was added to 40 mL of demineralized water pre-incubated at 35 to 40°C. Afterward, the same steps outlined in the section for screening of staphylococcal enterotoxin production in broth were

Figure 1. Preparation of white-brined cheese with or without a starter culture and stored in a 10 or 15% NaCl brine at 10°C and 25°C for 28 d. *S. aureus* = *Staphylococcus aureus*.

followed to detect the presence of enterotoxins in white-brined cheese.

Statistical Analysis

Effects of NaCl concentration, storage temperature, and the presence or absence of the starter culture on staphylococcal growth and enterotoxin production in white-brined cheese were analyzed using SPSS software

version 19.0 (IBM Corp., Armonk, NY). The independent samples *t*-test was used to compare 2 levels of temperature. One-way ANOVA was performed to examine the effect of the variables NaCl concentration, storage time, and the presence or absence of starter culture. The Duncan post hoc test was used to determine the difference between variables groups. A *P*-value of <0.05 was considered the cutoff level for statistical significance.

Table 1. Experimental design for growth of *Staphylococcus aureus* in white-brined cheese

Treatment	<i>Staph. aureus</i>	Starter culture	NaCl (%)	Storage temperature (°C)
1	+	+	10	10
2	+	–	10	10
3	+	+	10	25
4	+	–	10	25
5	+	+	15	10
6	+	–	15	10
7	+	+	15	25
8	+	–	15	25

RESULTS

Screening for Staphylococcal Enterotoxins

We investigated production of staphylococcal enterotoxins by different *Staph. aureus* strains inoculated in UHT milk containing 0, 5, or 10% NaCl incubated at 37°C. Among the tested strains, only *Staph. aureus* ATCC 439 produced enterotoxin in UHT milk without NaCl at d 1, 3, and 7, at concentrations of 0.72, 0.33, and 0.27 IU, respectively. However, the toxin was not detected beyond 7 d (Table 2). Although the initial numbers of *Staph. aureus* in UHT milk (~ 5.5 log cfu/g) increased, reaching >8 log cfu/g, no strains could produce enterotoxins in UHT milk containing 5 or 10% NaCl. It is worth mentioning that enterotoxins were also not detected in white-brined cheese at different storage times and temperatures as described in the section below (results not shown).

Behavior of *Staph. aureus* in Cheese and Brine Solution

Tables 3 and 4 present the *Staph. aureus* populations in white-brined cheeses and brine solutions, respectively, during storage for 28 d. Growth of *Staph. aureus* was noted in white-brined cheese stored in 10% or 15% NaCl brine solution at 10°C or 25°C produced with or without starter culture (Table 3). In general, the increase in the numbers of *Staph. aureus* in cheese and brine fluctuated during storage; however, at d 28, the numbers increased significantly ($P < 0.05$) compared with the initial inoculation level.

White cheese produced without starter culture and stored in 10% NaCl at 25°C (T4) showed the highest ($P < 0.05$) growth of *Staph. aureus* with numbers increasing from 5.41 to 8.39 log₁₀ cfu/g after 28 d. However, cheese produced with the starter culture and stored in 15% NaCl at 10°C (T5) showed the lowest increase in the numbers of *Staph. aureus* after 28 d. The numbers of *Staph. aureus* increased from 4.88 log₁₀ cfu/g during

Table 2. Growth of *Staphylococcus aureus* (log cfu/mL) and *Staphylococcus* enterotoxin detection (+ or ND)¹ in UHT milk with 0% NaCl²

Strain number	Storage time (d)						
	0	1	3	7	14	21	28
ATCC 1131	5.14 ± 0.02 ^{A,a} ND	7.26 ± 0.04 ^{B,a} ND	8.11 ± 0.1 ^{C,c} ND	8.53 ± 0.02 ^{D,c} ND	8.73 ± 0.01 ^{E,c} ND	8.78 ± 0.04 ^{E,c} ND	8.70 ± 0.01 ^{E,b} ND
ATCC 1210	5.86 ± 0.01 ^{A,d} ND	7.43 ± 0.02 ^{B,c} ND	7.64 ± 0.01 ^{C,a} ND	8.22 ± 0.01 ^{D,b} ND	8.28 ± 0.01 ^{E,a} ND	8.36 ± 0.01 ^{G,a} ND	8.32 ± 0.01 ^{F,a} ND
ATCC 25923	5.63 ± 0.01 ^{A,b} ND	7.33 ± 0.01 ^{B,b} ND	7.86 ± 0.01 ^{C,b} ND	8.45 ± 0.01 ^{D,b} ND	8.50 ± 0.01 ^{D,b} ND	8.49 ± 0.02 ^{D,b} ND	8.44 ± 0.08 ^{D,a} ND
ATCC 439	5.72 ± 0.01 ^{A,c} ND	7.90 ± 0.01 ^{B,d} +	8.50 ± 0.02 ^{C,d} +	8.92 ± 0.01 ^{D,d} +	8.96 ± 0.02 ^{D,d} ND	9.02 ± 0.01 ^{E,d} ND	8.92 ± 0.03 ^{D,c} ND

^{A-C}Means in the same row with the same uppercase letters are not significantly different ($P > 0.05$).

^{a-d}Means in the same column with the same lowercase letters are not significantly different ($P > 0.05$).

¹ND = not detected.

²Values are means ± SD. Each value is the average of 2 experiments (n = 4).

Table 3. Recovery of *Staphylococcus aureus* ($\log_{10}$ cfu/g) from white-brined cheeses prepared with or without the starter culture¹

Treatment ²	Storage time (d)					
	0	1	3	7	14	21
1	5.37 ± 0.27 ^{A,a}	5.87 ± 0.66 ^{B,c}	5.84 ± 0.64 ^{B,ab}	7.01 ± 0.29 ^{C,b}	7.18 ± 0.29 ^{CD,a}	7.39 ± 0.20 ^{CD,b}
2	5.41 ± 0.28 ^{A,b}	5.44 ± 0.14 ^{A,ab}	6.72 ± 0.04 ^{B,c}	7.21 ± 0.15 ^{C,b}	8.41 ± 0.13 ^{D,c}	8.24 ± 0.21 ^{D,d}
3	5.37 ± 0.27 ^{A,a}	7.45 ± 0.24 ^{B,d}	8.16 ± 0.13 ^{C,d}	8.44 ± 0.44 ^{C,c}	8.40 ± 0.18 ^{C,c}	8.32 ± 0.18 ^{C,de}
4	5.41 ± 0.28 ^{A,b}	8.62 ± 0.11 ^{B,c}	9.12 ± 0.14 ^{C,e}	9.18 ± 0.18 ^{C,d}	9.13 ± 0.13 ^{C,d}	8.58 ± 0.27 ^{B,ef}
5	4.88 ± 0.09 ^{A,a}	5.32 ± 0.11 ^{A,b,c}	6.46 ± 0.39 ^{A,ab}	6.48 ± 0.85 ^{A,c}	7.18 ± 0.07 ^{C,a}	6.76 ± 0.51 ^{BC,a}
6	5.27 ± 0.37 ^{A,a}	5.29 ± 0.15 ^{A,a}	6.33 ± 0.12 ^{B,bc}	7.52 ± 0.10 ^{C,b}	7.70 ± 0.19 ^{C,b}	8.15 ± 0.16 ^{D,d}
7	4.88 ± 0.09 ^{A,a}	7.48 ± 0.25 ^{B,d}	8.69 ± 1.17 ^{D,de}	8.30 ± 0.40 ^{CD,c}	8.29 ± 0.26 ^{CD,c}	7.93 ± 0.14 ^{BC,c}
8	5.38 ± 0.45 ^{A,a}	8.39 ± 0.12 ^{C,e}	8.89 ± 0.07 ^{D,e}	8.97 ± 0.14 ^{EF,d}	9.20 ± 0.15 ^{F,d}	8.71 ± 0.13 ^{F,d}

^{A-F}Means from each sampling time in the same row with the same uppercase letters are not significantly different ($P > 0.05$).

^{a-f}Means from each sampling time in the same column with the same lowercase letters are not significantly different ($P > 0.05$).

¹Values are means ± SD. Each value is the average of 2 experiments ($n = 4$).

²Treatments (all include *Staphylococcus aureus*): (1) with starter culture, 10% NaCl, 10°C; (2) without starter culture, 10% NaCl, 10°C; (3) with starter culture, 10% NaCl, 25°C; (4) without starter culture, 10% NaCl, 25°C; (5) with starter culture, 15% NaCl, 10°C; (6) without starter culture, 15% NaCl, 10°C; (7) with starter culture, 15% NaCl, 25°C; (8) without starter culture, 15% NaCl, 25°C.

Table 4. Recovery of *Staphylococcus aureus* ($\log_{10}$ cfu/g) from brine solution of white cheese prepared with or without the starter culture¹

Treatment ²	Storage time (d)					
	0	1	3	7	14	21
1	3.01 ± 0.25 ^{A,b}	4.36 ± 0.55 ^{B,b}	4.93 ± 0.73 ^{C,d}	5.38 ± 0.39 ^{C,b}	5.53 ± 0.72 ^{C,a}	4.55 ± 0.07 ^{C,b}
2	3.17 ± 0.09 ^{A,b}	3.99 ± 0.18 ^{B,ab}	5.09 ± 0.19 ^{C,c}	5.13 ± 0.15 ^{C,b}	6.05 ± 0.20 ^{D,b}	6.41 ± 0.32 ^{E,c}
3	3.01 ± 0.25 ^{A,b}	6.54 ± 0.42 ^{B,d}	7.13 ± 0.18 ^{C,f}	7.76 ± 0.59 ^{D,d}	7.98 ± 0.63 ^{D,d}	8.16 ± 0.52 ^{D,d}
4	3.62 ± 0.49 ^{A,c}	6.82 ± 0.28 ^{B,d}	8.26 ± 0.12 ^{C,b}	9.29 ± 0.11 ^{E,f}	8.58 ± 0.20 ^{D,e}	8.43 ± 0.21 ^{CD,d}
5	2.34 ± 0.27 ^{A,a}	3.93 ± 0.53 ^{B,ab}	3.74 ± 0.62 ^{B,a}	4.50 ± 0.03 ^{BC,a}	5.54 ± 0.37 ^{D,a}	4.75 ± 0.57 ^{C,a}
6	3.02 ± 0.05 ^{A,b}	3.80 ± 0.46 ^{B,a}	4.61 ± 0.16 ^{C,b}	5.33 ± 0.23 ^{D,b}	6.07 ± 0.25 ^{E,b}	6.13 ± 0.19 ^{E,c}
7	2.34 ± 0.27 ^{A,a}	5.28 ± 0.31 ^{B,c}	6.50 ± 0.46 ^{C,c}	6.65 ± 0.29 ^{C,c}	6.61 ± 0.33 ^{C,c}	6.56 ± 0.29 ^{C,c}
8	3.90 ± 0.51 ^{A,c}	6.59 ± 0.17 ^{D,b}	7.85 ± 0.13 ^{D,g}	8.44 ± 0.12 ^{E,e}	8.42 ± 0.38 ^{E,de}	8.29 ± 0.18 ^{E,d}

^{A-F}Means from each sampling time in the same row with the same uppercase letters are not significantly different ($P > 0.05$).

^{a-h}Means from each sampling time in the same column with the same lowercase letters are not significantly different ($P > 0.05$).

¹Values are means ± SD. Each value is the average of 2 experiments ($n = 4$).

²Treatments (all include *Staphylococcus aureus*): (1) with starter culture, 10% NaCl, 10°C; (2) without starter culture, 10% NaCl, 10°C; (3) with starter culture, 10% NaCl, 25°C; (4) without starter culture, 10% NaCl, 25°C; (5) with starter culture, 15% NaCl, 10°C; (6) without starter culture, 15% NaCl, 10°C; (7) with starter culture, 15% NaCl, 25°C; (8) without starter culture, 15% NaCl, 25°C.

the first 14 d of storage to reach $7.18 \log_{10}$ cfu/g, but viability decreased until the end of the storage period to $5.35 \log_{10}$ cfu/g. The effect of different treatments on the growth of *Staph. aureus* in cheese was inconsistent among all treatments. At 10°C , in the absence of the starter culture, the growth of *Staph. aureus* significantly increased ($P < 0.05$) in 10% and 15% NaCl by 2.78 and $2.96 \log_{10}$ cfu/g, respectively, compared with the initial inoculum level (Table 3). Furthermore, at 10°C the growth of *Staph. aureus* in the presence of starter culture increased in cheese stored in 15% and 10% NaCl brine solution by 0.47 and $2.26 \log_{10}$ cfu/g, respectively. However, the effect of starter culture on growth was inconsistent and not significant at 25°C (Table 3). Moreover, the growth of *Staph. aureus* was higher ($P < 0.05$) in cheese stored in 10% NaCl compared with growth at 15% NaCl. For instance, the growth in cheese stored in 10% NaCl at 10°C with starter culture (T1) was higher than the growth in cheese stored in 15% NaCl at 10°C with starter culture (T5). The final variable investigated was temperature. *Staphylococcus aureus* generally grew more in higher temperatures. For example, cheese stored in 10% NaCl at 25°C with the starter culture (T3) had a higher *Staph. aureus* population compared with cheese stored at 10°C (T1).

Staphylococcus aureus cells directly contaminated the sterile brine solutions in which they were stored, because approximately $3.0 \log_{10}$ cfu/mL (ranging from $1.9\text{--}4.1 \log_{10}$ cfu/mL) was recovered directly after immersion of cheese in the brine solution (Table 4). The growth of *Staph. aureus* in the brine solution increased ($P < 0.05$) in all treatments during the storage period. Similar trends were observed in brine where the maximum growth occurred in 10% brine solution stored at 25°C without the starter culture ($3.62\text{--}8.39 \log_{10}$ cfu/mL), whereas the minimum growth was noted in 15% brine solution stored at 10°C with starter culture ($2.34\text{--}4.31 \log_{10}$ cfu/mL; Table 4). The effect of different treatments on the growth of *Staph. aureus* in brine was that the starter culture and salt reduced the growth, whereas greater temperature enhanced it.

Growth of LAB in Cheese and Brine Solution

Table 5 shows the numbers of LAB (starter and non-starter) in white-brined cheese, and Table 6 shows the numbers of LAB in the brine solution. Starter and non-starter LAB numbers increased by 0.35 to $3.24 \log_{10}$ cfu/g in white cheese stored in 10 or 15% NaCl brine after 28 d at 10°C or 25°C (Table 5). Likewise, the numbers of starter and non-starter LAB in the brine solutions increased by 0.45 to $5.06 \log_{10}$ cfu/mL by 28 d at 10°C or 25°C (Table 6). The increase in viability of

LAB was significant ($P < 0.05$) up to d 14; afterward, the counts were maintained or slightly decreased ($P > 0.05$) until the end of storage time. The maximum growth was evident in samples stored in 10% NaCl at 10°C without starter culture (T4; $8.24 \log_{10}$ cfu/g in cheese and $8.20 \log_{10}$ cfu/mL in brine), while the minimum growth occurred in samples stored in 15% NaCl at 10°C with the starter culture (T5; $5.28 \log_{10}$ cfu/g in cheese and $2.78 \log_{10}$ cfu/mL in brine). We observed that the starter culture suppressed the growth of *Staph. aureus* in cheese. As shown in Table 5, the LAB count was lower in cheese stored in 10% NaCl at 10°C with the starter culture (T1) than the count in cheese stored in 10% NaCl at 10°C without the starter culture (T2). Furthermore, the growth of LAB was higher in cheese stored in 10% NaCl compared with growth at 15%. For instance, the LAB population in cheese stored in 10% NaCl at 10°C with the starter culture (T1) was higher than the LAB population in cheese stored in 15% NaCl at 10°C with the starter culture (T5; Table 5). On the contrary, temperature positively affected growth; LAB grew more in higher temperatures. For example, cheese stored in 10% NaCl at 25°C (T3) had higher numbers of LAB compared with cheese stored in 10% NaCl at 10°C (T1). Based on these data, LAB counts were affected positively by lower salt concentration and higher temperatures.

Salt Concentration, pH, and Water Activity of Cheese

The pH values of the white-brined cheeses produced with the starter culture were lower ($P < 0.05$) than those of cheeses produced without the starter culture (Table 7). Table 7 shows that the initial pH of white-brined cheeses produced with the starter culture was approximately 5.66 and dropped to $5.16\text{--}5.33$ after 28 d of storage. However, the initial pH of white-brined cheeses produced without the starter culture was 6.72 and dropped to 5.41 to 6.31 after 28 d of storage (Table 7). The salt concentration in white-brined cheese at 1 d in 10 and 15% NaCl solution was 4.73 to 4.99% and 6.04 to 6.45% NaCl (wt/wt), respectively. After 28 d of storage, a slight change occurred in the white-brined cheeses stored in 10% NaCl, whereas the salt content of cheese samples stored in 15% brine increased by 1.27% to 2.16% (Table 8).

There was a decrease in a_w of cheese in all treatments in a range from 0.06 to 0.11 at d 28 (Table 9). The a_w was less than optimal in all treatments at all storage times for *Staph. aureus* growth and SE production, which is 0.98, according to (Tatini, 1973). In addition, the a_w was higher for cheese in 10% NaCl compared with 15% NaCl, at 10°C compared with 25°C , and in

Table 5. Recovery of lactic acid bacteria ($\log_{10}$ cfu/g) from white-brined cheese prepared with or without starter culture¹

Treatment ²	Storage time (d)						
	0	1	3	7	14	21	28
1	5.11 ± 0.26 ^{A,ab}	5.74 ± 0.53 ^{B,b}	6.29 ± 0.10 ^{C,b}	6.42 ± 0.25 ^{C,b}	6.72 ± 0.45 ^{DE,b}	6.88 ± 0.29 ^{E,b}	6.54 ± 0.27 ^{C-E,b}
2	5.89 ± 0.29 ^{A,c}	5.76 ± 0.19 ^{A,b}	6.77 ± 0.19 ^{B,c}	7.28 ± 0.12 ^{C,cd}	8.15 ± 0.19 ^{D,d}	8.19 ± 0.17 ^{D,f}	7.41 ± 0.33 ^{C,d}
3	5.11 ± 0.26 ^{A,ab}	6.67 ± 0.06 ^{B,c}	6.87 ± 0.18 ^{BC,c}	7.06 ± 0.20 ^{C,c}	7.59 ± 0.47 ^{D,c}	7.85 ± 0.36 ^{DE,c}	8.01 ± 0.08 ^{E,e}
4	4.97 ± 0.23 ^{A,a}	5.89 ± 0.29 ^{B,b}	7.88 ± 0.39 ^{C,d}	8.37 ± 0.18 ^{D,e}	8.98 ± 0.09 ^{E,e}	8.30 ± 0.17 ^{D,f}	8.24 ± 0.32 ^{D,e}
5	4.92 ± 0.02 ^{A,a}	5.02 ± 0.91 ^{A,a}	5.48 ± 0.29 ^{A,ab}	6.05 ± 0.25 ^{C,a}	5.80 ± 0.46 ^{BC,a}	5.37 ± 0.23 ^{AB,a}	5.28 ± 0.26 ^{AB,a}
6	5.73 ± 0.60 ^{A,bc}	5.71 ± 0.40 ^{A,b}	6.16 ± 0.12 ^{B,b}	7.47 ± 0.08 ^{D,d}	7.39 ± 0.21 ^{D,c}	7.23 ± 0.11 ^{CD,c}	7.05 ± 0.10 ^{C,c}
7	4.92 ± 0.02 ^{A,a}	6.58 ± 0.27 ^{B,c}	6.29 ± 0.22 ^{BC,b}	6.59 ± 0.53 ^{BC,b}	6.98 ± 0.43 ^{C,b}	7.61 ± 0.06 ^{D,d}	7.50 ± 0.24 ^{D,d}
8	5.73 ± 0.6 ^{A,bc}	6.61 ± 0.01 ^{B,c}	8.73 ± 0.0 ^{D,e}	8.69 ± 0.35 ^{CD,ef}	8.88 ± 0.1 ^{D,e}	8.31 ± 0.16 ^{C,f}	7.00 ± 0.01 ^{B,c}

^{A-F}Means from each sampling time in the same row with the same uppercase letters are not significantly different ($P > 0.05$).

^{a-f}Means from each sampling time in the same column with the same lowercase letters are not significantly different ($P > 0.05$).

¹Values are means ± SD. Each value is the average of 2 experiments ($n = 4$).

²Treatments (all include *Staphylococcus aureus*): (1) with starter culture, 10% NaCl, 10°C; (2) without starter culture, 10% NaCl, 10°C; (3) with starter culture, 10% NaCl, 25°C; (4) without starter culture, 10% NaCl, 25°C; (5) with starter culture, 15% NaCl, 10°C; (6) without starter culture, 15% NaCl, 10°C; (7) with starter culture, 15% NaCl, 25°C; (8) without starter culture, 15% NaCl, 25°C.

Table 6. Recovery of lactic acid bacteria ($\log_{10}$ cfu/mL) from brine solution with white-brined cheese prepared with or without the starter culture¹

Treatment ²	Storage time (d)						
	0	1	3	7	14	21	28
1	3.14 ± 0.27 ^{A,b}	4.04 ± 0.96 ^{B,b}	4.04 ± 0.25 ^{B,a}	4.48 ± 0.09 ^{B,ab}	4.43 ± 0.75 ^{B,b}	4.14 ± 0.28 ^{B,b}	4.10 ± 0.22 ^{B,b}
2	4.58 ± 0.11 ^{A,d}	4.65 ± 0.22 ^{A,cd}	4.97 ± 0.06 ^{B,b}	5.17 ± 0.09 ^{C,a-c}	5.92 ± 0.11 ^{E,c}	5.93 ± 0.29 ^{E,c}	5.61 ± 0.23 ^{D,d}
3	3.14 ± 0.28 ^{A,b}	5.98 ± 0.25 ^{B,e}	6.33 ± 0.16 ^{C,d}	5.62 ± 0.96 ^{B,cd}	7.48 ± 0.82 ^{D,e}	8.42 ± 0.45 ^{E,de}	8.21 ± 0.14 ^{E,f}
4	4.58 ± 0.11 ^{A,d}	6.92 ± 0.03 ^{B,f}	7.45 ± 0.07 ^{C,e}	8.89 ± 0.08 ^{F,e}	8.97 ± 0.15 ^{F,g}	8.68 ± 0.25 ^{E,e}	8.20 ± 0.12 ^{D,f}
5	2.33 ± 0.07 ^{A,a}	3.15 ± 0.15 ^{A,a}	4.29 ± 0.38 ^{C,a}	4.19 ± 1.59 ^{C,a}	3.76 ± 0.95 ^{BC,a}	2.58 ± 0.26 ^{A,a}	2.78 ± 0.32 ^{A,a}
6	3.70 ± 0.21 ^{A,c}	4.34 ± 0.21 ^{B,bc}	4.98 ± 0.19 ^{C,b}	5.36 ± 0.12 ^{D,b-d}	5.44 ± 0.31 ^{D,c}	5.78 ± 0.23 ^{E,c}	5.68 ± 0.10 ^{E,d}
7	2.33 ± 0.07 ^{A,a}	4.93 ± 0.49 ^{B,d}	5.80 ± 0.82 ^{BC,c}	6.25 ± 1.43 ^{C,d}	6.47 ± 0.13 ^{C,d}	5.75 ± 0.12 ^{BC,c}	4.95 ± 1.50 ^{B,c}
8	4.34 ± 0.21 ^{A,d}	6.51 ± 0.08 ^{B,f}	7.66 ± 0.22 ^{D,e}	8.41 ± 0.22 ^{E,e}	8.18 ± 0.12 ^{E,f}	8.16 ± 0.13 ^{E,d}	7.37 ± 0.31 ^{C,e}

^{A-F}Means from each sampling time in the same row with the same uppercase letters are not significantly different ($P > 0.05$).

^{a-f}Means from each sampling time in the same column with the same lowercase letters are not significantly different ($P > 0.05$).

¹Values are means ± SD. Each value is the average of 2 experiments ($n = 4$).

²Treatments (all include *Staphylococcus aureus*): (1) with starter culture, 10% NaCl, 10°C; (2) without starter culture, 10% NaCl, 10°C; (3) with starter culture, 10% NaCl, 25°C; (4) without starter culture, 10% NaCl, 25°C; (5) with starter culture, 15% NaCl, 10°C; (6) without starter culture, 15% NaCl, 10°C; (7) with starter culture, 15% NaCl, 25°C; (8) without starter culture, 15% NaCl, 25°C.

Table 7. pH values with different treatments of white-brined cheese during storage for 28 d¹

Treatment ²	Storage time (d)						
	0	1	3	7	14	21	28
1	5.66 ± 0.07 ^{A,a}	5.70 ± 0.27 ^{A,a}	5.65 ± 0.23 ^{A,a}	5.56 ± 0.25 ^{A,a}	5.53 ± 0.27 ^{A,a}	5.49 ± 0.28 ^{A,a}	5.30 ± 0.12 ^{A,a}
2	6.72 ± 0.21 ^{A,b}	6.65 ± 0.15 ^{A,b}	6.80 ± 0.44 ^{A,b}	6.70 ± 0.32 ^{A,b}	6.59 ± 0.30 ^{A,b}	6.36 ± 0.07 ^{A,b}	6.30 ± 0.03 ^{A,c}
3	5.66 ± 0.07 ^{C,a}	5.67 ± 0.12 ^{C,a}	5.62 ± 0.14 ^{C,a}	5.50 ± 0.05 ^{BC,a}	5.35 ± 0.09 ^{AB,a}	5.28 ± 0.12 ^{AB,a}	5.16 ± 0.07 ^{A,a}
4	6.72 ± 0.21 ^{C,b}	6.52 ± 0.01 ^{C,b}	6.61 ± 0.18 ^{C,b}	6.46 ± 0.035 ^{BC,b}	6.42 ± 0.01 ^{BC,b}	6.22 ± 0.15 ^{B,b}	5.91 ± 0.01 ^{A,b}
5	5.66 ± 0.07 ^{D,a}	5.68 ± 0.15 ^{D,a}	5.57 ± 0.05 ^{CD,a}	5.55 ± 0.06 ^{BC,a}	5.45 ± 0.03 ^{A-C,a}	5.38 ± 0.07 ^{AB,a}	5.33 ± 0.03 ^{A,a}
6	6.72 ± 0.21 ^{B,b}	6.68 ± 0.19 ^{B,b}	6.76 ± 0.35 ^{B,b}	6.74 ± 0.35 ^{B,b}	6.65 ± 0.28 ^{B,b}	6.53 ± 0.28 ^{AB,b}	5.94 ± 0.07 ^{A,b}
7	5.66 ± 0.07 ^{C,a}	5.62 ± 0.09 ^{C,a}	5.57 ± 0.09 ^{BC,a}	5.52 ± 0.09 ^{BC,a}	5.47 ± 0.08 ^{BC,a}	5.40 ± 0.04 ^{B,a}	5.21 ± 0.01 ^{A,a}
8	6.72 ± 0.21 ^{B,b}	6.63 ± 0.11 ^{B,b}	6.74 ± 0.35 ^{B,b}	6.60 ± 0.21 ^{B,b}	6.49 ± 0.18 ^{B,b}	5.46 ± 0.18 ^{A,a}	5.32 ± 0.12 ^{A,a}

^{A-D}Means from each sampling time in the same row with the same uppercase letters are not significantly different ($P > 0.05$).

^{a-c}Means from each sampling time in the same column with the same lowercase letters are not significantly different ($P > 0.05$).

¹Values are means ± SD. Each value is the average of 2 experiments ($n = 4$).

²Treatments (all include *Staphylococcus aureus*): (1) with starter culture, 10% NaCl, 10°C; (2) without starter culture, 10% NaCl, 10°C; (3) with starter culture, 10% NaCl, 25°C; (4) without starter culture, 10% NaCl, 25°C; (5) with starter culture, 15% NaCl, 10°C; (6) without starter culture, 15% NaCl, 10°C; (7) with starter culture, 15% NaCl, 25°C; (8) without starter culture, 15% NaCl, 25°C.

Table 8. Salt concentration (%) in different treatments of white-brined cheese during storage for 28 d¹

Treatment ²	Storage time (d)						
	0	1	3	7	14	21	28
1	0.00 ± 0.00 ^A	4.73 ± 0.04 ^{B,a}	5.03 ± 0.06 ^{C,a}	5.38 ± 0.06 ^{D,a}	5.56 ± 0.06 ^{D,a}	5.69 ± 0.08 ^{D,b}	4.67 ± 0.33 ^{B,a}
2	0.00 ± 0.00 ^A	4.99 ± 0.04 ^{BC,b}	5.53 ± 0.02 ^{BC,b}	5.29 ± 0.88 ^{BC,a}	5.35 ± 0.14 ^{BC,a}	5.66 ± 0.24 ^{C,b}	4.51 ± 0.18 ^{B,a}
3	0.00 ± 0.00 ^A	4.84 ± 0.04 ^{B,ab}	5.08 ± 0.04 ^{C,a}	5.47 ± 0.06 ^{D,a}	5.70 ± 0.02 ^{D,b}	5.32 ± 0.22 ^{D,a}	5.05 ± 0.04 ^{BC,a}
4	0.00 ± 0.00 ^A	4.73 ± 0.08 ^{B,a}	5.18 ± 0.10 ^{BC,a}	5.63 ± 0.12 ^{CD,a}	5.82 ± 0.02 ^{D,b}	5.09 ± 0.18 ^{B,a}	4.74 ± 0.51 ^{B,a}
5	0.00 ± 0.00 ^A	6.36 ± 0.04 ^{B,de}	6.62 ± 0.04 ^{C,d}	6.71 ± 0.04 ^{C,b}	6.92 ± 0.04 ^{D,c}	7.48 ± 0.02 ^{E,c}	7.79 ± 0.12 ^{F,b}
6	0.00 ± 0.00 ^A	6.17 ± 0.061 ^{B,cd}	6.48 ± 0.08 ^{C,d}	6.83 ± 0.16 ^{B,b}	6.92 ± 0.12 ^{D,c}	7.43 ± 0.18 ^{E,c}	7.60 ± 0.02 ^{E,b}
7	0.00 ± 0.00 ^A	6.04 ± 0.20 ^{B,c}	6.26 ± 0.06 ^{B,c}	6.78 ± 0.06 ^{C,b}	7.15 ± 0.20 ^{D,d}	7.67 ± 0.16 ^{E,d}	8.20 ± 0.08 ^{F,c}
8	0.00 ± 0.00 ^A	6.45 ± 0.04 ^{B,e}	7.43 ± 0.18 ^{C,e}	7.89 ± 0.02 ^{D,b}	8.29 ± 0.24 ^{E,d}	8.27 ± 0.14 ^{E,d}	7.72 ± 0.18 ^{CD,b}

^{A-F}Means from each sampling time in the same row with the same uppercase letters are not significantly different ($P > 0.05$).

^{a-c}Means from each sampling time in the same column with the same lowercase letters are not significantly different ($P > 0.05$).

¹Values are means ± SD. Each value is the average of 2 experiments ($n = 4$).

²Treatments (all include *Staphylococcus aureus*): (1) with starter culture, 10% NaCl, 10°C; (2) without starter culture, 10% NaCl, 10°C; (3) with starter culture, 10% NaCl, 25°C; (4) without starter culture, 10% NaCl, 25°C; (5) with starter culture, 15% NaCl, 10°C; (6) without starter culture, 15% NaCl, 10°C; (7) with starter culture, 15% NaCl, 25°C; (8) without starter culture, 15% NaCl, 25°C.

Table 9. Water activity with different treatments of white-brined cheese during storage for 28 d¹

Treatment ²	Storage time (d)						
	0	1	3	7	14	21	28
1	0.98 ± 0.00 ^B	0.94 ± 0.01 ^{AB,b-d}	0.93 ± 0.01 ^{AB,a-c}	0.93 ± 0.01 ^{AB,b}	0.92 ± 0.01 ^{A,a}	0.93 ± 0.02 ^{A,ab}	0.92 ± 0.03 ^{A,a}
2	0.98 ± 0.00 ^D	0.94 ± 0.01 ^{BC,ab}	0.95 ± 0.01 ^{C,bc}	0.92 ± 0.01 ^{BC,ab}	0.93 ± 0.02 ^{BC,a}	0.90 ± 0.01 ^{A,a}	0.90 ± 0.01 ^{A,a}
3	0.98 ± 0.00 ^B	0.94 ± 0.01 ^{AB,bc}	0.92 ± 0.03 ^{A,ab}	0.92 ± 0.01 ^{A,ab}	0.93 ± 0.02 ^{A,a}	0.92 ± 0.02 ^{A,a}	0.91 ± 0.02 ^{A,a}
4	0.98 ± 0.00 ^E	0.95 ± 0.01 ^{CD,cd}	0.96 ± 0.01 ^{D,c}	0.92 ± 0.01 ^{B,ab}	0.93 ± 0.01 ^{BC,a}	0.90 ± 0.01 ^{A,a}	0.90 ± 0.01 ^{A,a}
5	0.98 ± 0.00 ^C	0.94 ± 0.01 ^{AB,ab}	0.92 ± 0.01 ^{AB,a-c}	0.92 ± 0.01 ^{AB,ab}	0.91 ± 0.01 ^{A,ab}	0.93 ± 0.01 ^{A,ab}	0.91 ± 0.01 ^{A,ab}
6	0.98 ± 0.00 ^F	0.95 ± 0.01 ^{E,d}	0.94 ± 0.01 ^{D,bc}	0.91 ± 0.01 ^{BC,ab}	0.92 ± 0.01 ^{BC,a}	0.90 ± 0.01 ^{B,a}	0.88 ± 0.01 ^{A,a}
7	0.98 ± 0.00 ^{C,a}	0.93 ± 0.01 ^{B,a}	0.89 ± 0.02 ^{A,ab}	0.90 ± 0.01 ^{A,ab}	0.90 ± 0.02 ^{A,ab}	0.89 ± 0.01 ^{A,ab}	0.87 ± 0.02 ^{A,a}
8	0.98 ± 0.00 ^E	0.93 ± 0.01 ^{CD,ab}	0.94 ± 0.01 ^{D,bc}	0.91 ± 0.02 ^{A-C,ab}	0.92 ± 0.01 ^{BC,a}	0.89 ± 0.01 ^{A,a}	0.87 ± 0.01 ^{A,a}

^{A-F}Means from each sampling time in the same row with the same uppercase letters are not significantly different ($P > 0.05$).

^{a-d}Means from each sampling time in the same column with the same lowercase letters are not significantly different ($P > 0.05$).

¹Values are means ± SD. Each value is the average of 2 experiments ($n = 4$).

²Treatments (all include *Staphylococcus aureus*): (1) with starter culture, 10% NaCl, 10°C; (2) without starter culture, 10% NaCl, 10°C; (3) with starter culture, 10% NaCl, 25°C; (4) without starter culture, 10% NaCl, 25°C; (5) with starter culture, 15% NaCl, 10°C; (6) without starter culture, 15% NaCl, 10°C; (7) with starter culture, 15% NaCl, 25°C; (8) without starter culture, 15% NaCl, 25°C.

the presence of the starter culture compared with its absence (Table 9).

DISCUSSION

In the current study, we noted that all *Staph. aureus* strains grew in UHT milk containing 0, 5, and 10% NaCl at 37°C and did not produce any enterotoxins; however, strain ATCC 439 produced enterotoxins in UHT milk without NaCl during the first 7 d of storage. No enterotoxins were detected in any experimental white-brined cheeses (data not shown). This suggested that *Staph. aureus* ATCC 439 did not produce enterotoxins during growth in the experimental cheeses. Production of enterotoxins is genetically regulated; thus, strains that cannot produce enterotoxins may not harbor the specific gene to express enterotoxins (Bystron et al., 2006). These results supported Bystron et al. (2006), who reported that 32 of the 99 examined *Staph. aureus* strains were found to be enterotoxin producers. Atanassova et al. (2001) found that 28.6% of *Staph. aureus* strains isolated from fresh meat were enterotoxigenic and harbored one or more enterotoxin genes. Furthermore, the environmental growth conditions such as low pH, presence of LAB, and salt concentration may affect expression of staphylococcal enterotoxin genes. Similarly, Novick (2003) found that the presence of 1.0 M NaCl or a sub-inhibitory concentration of clindamycin reduced the transcription of *sae*, the virulence regulator of *Staph. aureus*, which is responsible for enterotoxin production.

Therefore, the production of enterotoxins is not necessarily associated with staphylococcal growth, especially in food. *Staphylococcus aureus* growth and enterotoxin production depend on different factors such as the presence of starter culture, the temperature, salt content, pH values, a_w , atmospheric pressure, and nutritional factors (Valero et al., 2009). There is a difference in the optimal temperatures required for growth and production of enterotoxins, which are 37°C and 40°C to 45°C, respectively. Similarly, staphylococcal enterotoxins were not detected in Iranian ultra-filtered white cheese stored at 8°C and 25°C. This was attributed to the inhibitory effect of the starter culture on the growth of *Staph. aureus* and enterotoxin production (Mohammadi and Hanifian, 2015). Similar to our findings, Viçosa et al. (2019) detected enterotoxins in their experimental broth (model medium) and but not in fresh cheeses.

The present study demonstrated that the numbers of *Staph. aureus* ATCC 439 increased significantly in all treatments, with a higher growth rate in cheese produced without the starter culture and stored in 10% NaCl at 25°C. Moreover, a reduced growth rate was noted in cheese produced with the starter culture and

stored in 15% NaCl at 10°C after 28 d. The reduced growth rate may be explained by the reduced pH of 5.66 in cheese produced with the starter culture compared with the pH of 6.72 in cheese without the starter culture. This is consistent with many previous reports which found that the viability of *Staph. aureus* ATCC 439 was reduced in different dairy products following inoculation with the starter cultures (Stecchini et al., 1991; Arqués et al., 2006; Adhikari et al., 2018). The inhibitory effect of the starter culture may be due to the drop in pH as a result of lactic acid production and to other metabolites, such as antibiotic substances and the production of hydrogen peroxide (Vernozy-Rozand et al., 1998; Alomar et al., 2008; Le Marc et al., 2009). The inconsistent effect of the starter culture on *Staph. aureus* ATCC 439 in experimental cheeses stored at 25°C may be attributed to the ability of *Staph. aureus* to resist starter culture influence on pH change and nutrient availability and grow in the cheeses stored at 25°C compared with 10°C.

Salt has 3 primary functions in cheese: it acts as a preservative, enhances safety, and contributes directly to salty flavor (Fox et al., 2017). As previously mentioned, *Staph. aureus* has the potential to survive in concentrations of up to 15% NaCl (Elahi and Fujikawa, 2019b). In the present study, *Staph. aureus* ATCC 439 grew in salt concentrations of 10% and 15% due to its halotolerant nature. Furthermore, the growth was lower in 15% NaCl compared with the growth in 10% NaCl. Such behavior of *Staph. aureus* ATCC 439 was expected on the basis of similar results being reported in several previous studies. Elahi and Fujikawa (2019a) also reported that the increase in NaCl concentration reduced *Staph. aureus* growth, where 5% NaCl had little effect, whereas concentrations of 10 to 20% showed an apparent inhibitory effect against the organisms.

The growth rate of *Staph. aureus* ATCC 439 in cheese or brine solution in the present study was higher at 25°C compared with 10°C. Similarly, Mohammadi and Hanifian (2015) found that the *Staph. aureus* numbers in the cheese stored at 25°C were higher than those stored at 8°C. Moreover, a combined effect of temperature and NaCl on the growth of *Staph. aureus* ATCC 439 was observed in this study. This was supported by the results reported by Elahi and Fujikawa (2019a), who have reported that the growth of *Staph. aureus* boundaries in BHI increased with a temperature increase and limited with salt concentration. Our findings revealed that higher NaCl concentration negatively affected the growth of *Staph. aureus* in cheese or brine keeping solution, whereas the growth was positively influenced by higher temperature. Therefore, white-brine cheese should be stored in NaCl concentrations higher than 15% at refrigerated temperatures.

Starter and non-starter LAB are responsible for the production of lactic acid, which decreases the pH of the curd and whey. Lactic acid production is directly proportional to the increase in LAB numbers. In contrary with the results of this study, LAB growth was higher in samples produced with the starter culture of Iranian ultra-filtered white cheese (Mohammadi and Hanifian, 2015), Feta-type cheese (Madureira et al., 2011), and batches of sausage (Essid and Hassouna, 2013). According to previous studies, the growth of the starter LAB during cheese manufacture increased within 1 d, dominating the microflora of cheese during ripening. Subsequently, the majority dies off and lyses relatively rapidly. The growth of starter LAB is negatively influenced by NaCl concentration (Fox et al., 2017), which is reflected in the results of this study, where 15% NaCl had lower LAB counts. Non-starter LAB are found in high numbers ($>10^8$ cfu/g) in all freshly made cheese. Within the first few weeks of ripening, non-starter LAB grow relatively quickly to high numbers ($\sim 10^8$ cfu/g); however, they lyse very slowly in cheese. Their growth depends on the ripening temperature, which supports the higher LAB count noted at higher temperatures in this study. Moreover, the tolerance of non-starter LAB to salt and acid, and their ability to grow in the absence of oxygen implies that they should grow well in cheese. In contrast, starter LAB are sensitive to salt; therefore, they are not expected to survive in salted cheese (Fox et al., 2017). Although non-starter LAB grew in minimal amounts in pasteurized milk, they could grow in white cheese and might affect its quality, either positively or negatively. Therefore, LAB are considered a major uncontrolled factor in white cheese manufacture (Crow et al., 2001; Briggiler-Marcó et al., 2007).

The pH values obtained in this study were typical of soft white-brined cheese (Osaili et al., 2014). One of the most effective factors to inhibit the growth of *Staph. aureus* is to acidify the cheese by the addition of starter cultures (Turhan and Kaletunc, 1992; Medved'ová et al., 2009). That was evident in the results of this study, where pH values were lower in the presence of the starter culture compared with its absence. Results from another study by Erkmén (1995) supported this observation; pH was lower in the presence of *Streptococcus cremoris* in the Turkish Feta cheese examined. In the present study, the pH values of both cheese and brine were lower at lower NaCl concentrations. This was expected because the higher salt level affected LAB growth, microbial lactose metabolism, and the development of proteolytic and lipolytic activities during ripening (Osaili et al., 2012). The drop in pH in cheeses produced without starter culture may be attributed to organic acids produced by non-starter culture

LAB. Similar results have been reported by Osaili et al. (2014).

In brine-salted cheeses, NaCl concentration is influenced by the concentration of salt in the brine, temperature, and time immersed in the brine solution (Fox et al., 2017). The average salt content in this study increased ($P < 0.05$) after 1 d of storage. The increase in salt content was greater ($P < 0.05$) in cheeses brined in 15% NaCl compared with 10% NaCl. Likewise, it increased in Turkish Feta cheese from 6.77% to 7.88% during the storage period (Erkmen, 1995). The salt content likely increased in cheese, whereas the moisture content decreased during storage time due to the net movement of Na^+ and Cl^- from the brine into the cheese as a consequence of the difference in osmotic pressure between the brine and the moisture phase of the cheese, both as a result of dehydration (Erkmen, 1995; Fox et al., 2017). The a_w of food depends on its moisture content and the concentration of salt (Fox et al., 2017). The relationship between NaCl concentration and a_w on the growth of *Staph. aureus* is inversely proportional. That is, increased concentration of NaCl decreases the a_w , thereby decreasing the growth of *Staph. aureus* and SE production. This is most likely due to differences in moisture and salt content between the different concentrations of NaCl (Ibrahim et al., 1981; Notermans and Heuvelman, 1983). In conclusion, brine solution and storage temperature had major effects on *Staph. aureus* growth in the white-brined cheese. Enterotoxin production by *Staph. aureus* ATCC 439 was inhibited in stored white-brined cheese. Nonetheless, it is highly recommended that the best hygienic manufacturing practices and storage conditions be maintained to control the growth of *Staph. aureus* in case of contamination.

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ORCIDS

- Anas A. Al-Nabulsi <https://orcid.org/0000-0002-9592-055X>
 Roa A. AbuNaser <https://orcid.org/0000-0002-8520-7515>
 Mutamed Ayyash <https://orcid.org/0000-0002-9312-500X>
 Murad A. Al-Holy <https://orcid.org/0000-0001-8627-0391>
 Richard A. Holley <https://orcid.org/0000-0001-7583-805X>