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Effects of storage temperatures on survival and enterotoxin production of *Staphylococcus aureus* in Turkish white pickled cheese

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ABSTRACT

Aims: Turkish white pickled cheese is the most consumed cheese type in Turkey and it is an important food to be evaluated in terms of food safety. In this study we investigated the behavior (survival and production of enterotoxin) of *Staphylococcus aureus* (*S. aureus*) NCTC 10654 in Turkish white pickled cheeses, which were ripened at 4 °C and 12 °C for 90 days.
Methodology and results: Counting of microorganisms was carried out by conventional methods on appropriate media. Detection of enterotoxins was performed by double-sandwich ELISA technique and gene region responsible for enterotoxin production by reverse transcription-PCR (RT-PCR). The counts of *S. aureus* decreased (p<0.05) in all of the cheese samples during ripening, where they decreased by 10^2 (CFU/g) at the end of the 90-day ripening period. The reduction in the *S. aureus* count was 2.5 times lower in cheeses ripened at the higher temperature, but the temperature was determined that had no significant effect on *S. aureus* survival (p >0.05). Staphylococcal enterotoxin could not be detected in the cheeses during ripening. Staphylococcal enterotoxin (SE) B mRNA was detected in cheese samples on days 1, 15, and 30 of ripening by RT-PCR. The SEB mRNA expression levels had differed according to the storage temperature.
Conclusion, significance and impact of study: This study showed that enterotoxin B producing *S. aureus* decreased in Turkish white pickled cheese stored at different temperatures and it could not produce enterotoxins, possibly due to factors such as type and nature of the cheese, and the conditions of production and activity of the starter culture.

Keywords: Enterotoxin, *Staphylococcus aureus*, storage temperature, white cheese

INTRODUCTION

Staphylococcus aureus (S. aureus) is an opportunistic pathogen, which can lead to various diseases such as skin lesions, septicemia, and meningitis. The natural habitat of *S. aureus* is humans and other warm-blooded animals. Food workers who carry enterotoxigenic *S. aureus* can contaminate food via infected skin lesions and act as asymptomatic carriers. In addition, dairy products made from pasteurized or raw milk derived from animals with subclinical mastitis are intrinsically contaminated, which may lead to staphylococcal outbreaks (Hennekinne *et al.*, 2012). *S. aureus* produces 22 different types of staphylococcal enterotoxin (SE), which are resistant to external environmental conditions (heat treatment, pH, other bacteria, etc.), and all of these enterotoxins possess superantigenic activity, where they are encoded by different gene regions in *S. aureus*. SEA, SEB, SEC, and SED are the enterotoxins that are isolated most frequently from food. SEB is considered to be a “biological weapon” and it is the most frequent SE type found in dairy products according to previous studies (Otto, 2014; Santos *et al.*, 2014). *S. aureus* can cause food poisoning by producing these toxins when its abundance exceeds 10^5 log CFU (Colony Forming Units)/mL.

Cheese is the most important product of the dairy industry because of its nutritional value and unique flavor. Many different types of cheese are produced in various countries. Turkish white pickled cheese is a type of soft cheese that is consumed most widely in Turkey, and similar feta style cheeses can be found in other countries. Turkish white pickled cheese is produced by coagulating milk from cows, sheep, or goats, or a mixture in suitable proportions (Seckin *et al.*, 2017). The Cheese Communique of the Turkish Food Codex for Turkish white pickled cheese stipulates that the ripening period should be at least 90 days. Moreover, the cheese should be kept at temperatures below 10 °C during storage, transport,

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and when on sale in the market according to the same standard ((TFC), 2015). In fact, the storage temperatures in the market range between 4 ℃ and 12 °C.

Cheese was previously classified as a “safe food” but since the 1980s, there have been reports that infections and intoxications may occur due to the consumption of cheese contaminated with pathogenic microorganisms and/or their toxins in various production stages. Thus, there is a public health risk and possible huge economic losses because of quality defects in cheese. The milk obtained from animals with subclinical mastitis contains large amounts of *S. aureus*. Staphylococcal poisoning can be caused by persistent contamination with *S. aureus* after the pasteurization of milk, insufficient activity in the starter cultures used for the production of cheese, and unsuitable conditions during storage (Hennekinne *et al.*, 2012). *S. aureus* growth and enterotoxin production in milk can occur within a few hours, but it is known that they depend on many factors in cheese such as the type and nature of the cheese, the production type and conditions, and the activity of the starter culture. Thus, Pexara *et al.* (Pexara et al., 2012) reported that the *S. aureus* count reached 6–7 log CFU/g in a model feta cheese within a short period of time (6–8 h). In particular, among SEA, SEB, SEC, and SED, it was found that SED was detected only when the bacterial count was 7–9 log CFU/g in the feta cheese matrix. A high abundance of *S. aureus* in cheese facilitates the development of resistance to factors with inhibitory effects on this bacterium. In addition, *S. aureus* is more readily inhibited when there is a greater abundance of competitive microorganisms in milk. Many studies (Selcuk, 1991; UNLITURK et al., 1994; Yücebay, 1994) have investigated the survival of *S. aureus* in Turkish white pickled cheese, but none have determined the survival of *S. aureus* and its production of toxins in Turkish white pickled cheese stored at different temperatures using classical and molecular methods.

MATERIALS AND METHODS

Preparation of inoculum cultures

S. aureus NCTC 10654 can produce SEB and it was used in this study, where it was obtained from 19 Mayıs University (Samsun, Turkey). The culture was grown in 50 mL of brain hearth infusion broth (BHI; Oxoid, London, UK) at 37 °C for 24 h. Overnight cultures of *S. aureus* were pelleted by centrifugation (2000 x g for 15 min), before being resuspended in the same volume of 0.1% peptone water, and this treatment was repeated three times. The bacterial cells obtained were enumerated by serial dilution and spread plating on Baird Parker agar (BPA, Merck, Darmstadt, Germany), followed by incubation for 24 h at 37 °C. The cells were diluted in 0.1% peptone water to obtain the desired inoculum level (10^6 CFU/mL) before adding to the model pasteurized milk.

Cheese production

Turkish white pickled cheese was prepared from pasteurized (65 °C for 30 min) milk in three trials. The cheese was inoculated with 10^6 CFU/mL of *S. aureus* in 60 L of pasteurized and cooled (32 °C) cow’s milk. Next, a lyophilized starter culture comprising a mixture of lactic acid bacteria (LAB) strains (*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, and *Streptococcus thermophilus*; 1:1:1; Mystarter CT 201, İstanbul, Turkey) was added to the pasteurized milk. The starter culture inoculum was added at 0.9% (v/w) to pasteurized milk, according to the manufacturer’s recommendations. In addition, the milk was inoculated with toxigenic *S. aureus* at 10^6 CFU/mL. $CaCl_2$ (Riedel-de Haen, Germany) was added at 10% (v/w) in order to restore the disrupted ionic calcium balance. Commercial rennet (Trakya, İstanbul, Turkey) with a strength of 1/8000 was added at a dose of 3.0 g/100 L milk to pasteurized milk and coagulation occurred within about 90 min at room temperature. After coagulation, the coagulum was cut into 1 cm^3 cubes and left to drain for 30 min. After draining the whey, the cheese curds were placed in boxes. The boxes were lined with cheesecloth and the curd was pressed for about 3 h at room temperature. The curd was cut into equal pieces weighing 250-300 g and placed into 12 g NaCl/100 g (w/v) pasteurized brine. The experimental cheeses were then packed into plastic containers and stored at about 4 °C or 12 °C to ripen for 90 days.

Physicochemical analysis

Standard methods were used to determine the titratable acidity, dry matter, and salt contents of Turkish white pickled cheese samples (Enstitüsü, 2013). The pH values of the cheese samples were also determined using a pH meter (WTW, Weilheim, Germany).

Microbiological analysis

From each cheese, 10 g samples were obtained aseptically and homogenized with 90 mL of Ringer's solution for 2 min in a stomacher. Using this basic dilution, a series of tenfold dilutions were prepared for microbiological analysis. *S. aureus* counts were performed after storing for 1, 15, 30, 60, and 90 days. *S. aureus* populations were counted on BPA (Merck, Darmstadt, Germany) supplemented with egg yolk tellurite emulsion (Merck) (Walcher *et al.*, 2014). Total aerobic mesophilic bacteria were enumerated on Plate count agar (PCA) (Merck) after incubating at 32 °C for 24-48 h in aerobic conditions. Lactococci were counted on M17 agar (pH 7.2) (Merck, Darmstadt, Germany) after incubating at 30 °C for 3 days in aerobic conditions, whereas lactobacilli were enumerated on de Man, Rogosa and Sharpe agar (MRS) (pH 5.7) (Merck, Darmstadt, Germany) after incubation in a CO_2-enriched atmosphere using Anaerocult C in an anaerobic jar (Merck, Darmstadt, Germany) at 37 °C for 3 days (Hanifian and Khani, 2012).

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Staphylococcal Enterotoxin (SE) analysis

Turkish white pickled cheese samples were also subjected to SE analysis at the same time as the microbiological analyses. The white pickled cheeses inoculated with *S. aureus* were manufactured without a starter in the positive control. The cheese samples were examined for the presence of SEs using a Staph Enterotoxins Visual Immunoassay test kit (3M™), according to the manufacturer's recommendations. The detection limit for the test kit was 1 ng/g.

Isolation of total RNA (Ribonucleic Acid) from cheese samples

Total RNA was isolated from the cheese matrix according to the method described by (Masoud et al., 2011) with a slight modification. Briefly, 10 g of cheese was added to 90 mL of 2% w/v sodium citrate (Sigma-Aldrich, Steinheim, Germany) buffer and mixed in a stomacher (Masticator, Neutec Group Inc., Farmingdale, NY) for 2 min. Next, 50 mL of the mixture was transferred to sterilized falcon tubes and centrifuged at 300 x g at 4 °C for 15 min. Oil (supernatant) was removed and 10 mL of the aqueous solution was transferred to tubes. The supernatant was centrifuged at 5000 x g and 4 °C for 15 min, and the pellet was used for isolating the total RNA. The pellet was incubated at 37 °C for 10 min by suspending in 200 µl Tris (10 mM, pH 8), before adding 10 µL of lysozyme (Roche, Mannheim, Germany) and 50 µl lysostaphin (Sigma-Aldrich). The total RNA was then isolated using a High Pure RNA Isolation Kit (Roche) according to the manufacturer's instructions. During RNA extraction, to eliminate any possible contamination with genomic DNA (Deoxyribonucleic Acid), the total RNA extracted from the cheese matrix was treated for 1 h at room temperature with 500 U/mL of DNase (Roche).

RNA samples were checked for the presence of residual DNA and quality by electrophoresis on 1% w/v agarose gel (Merck, Darmstadt, Germany) in 1× Tris–acetate-EDTA buffer (Sigma) containing 0.50 µg/mL ethidium bromide (Sigma). The RNA concentration and purity (represented by: absorption at 260 nm/absorption at 280 nm) were assessed using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Reverse transcription and real-time qPCR

cDNA (complementary DNA) was synthesized using a First Strand cDNA Synthesis Kit (Roche). Total RNA in 10.4 µL of nuclease-free water was added to 1 µl of Anchored-oligo(dT)18 Primer (Roche). Denaturation was performed for 10 min at 65 °C. The denatured RNA mixture was added to 4 µL of 5× reverse transcriptase buffer (Roche), 0.5 µL of 40U/µl RNase inhibitor (Roche), 2 µL of 10 mM dNTP mix (Roche), and 0.5 µL reverse transcriptase (Roche) in a total volume of 18.4 µL. The PCR (polymerase chain reaction) reaction comprised 30 min at 65 °C (cDNA synthesis) and 5 min at 85 °C (enzyme denaturation). Next, the cDNA was stored at -20 °C until reverse transcription-PCR (RT-PCR).

The following primers were used for housekeeping gene: *nuc* Fw: 5'-AGCCAAGCCTTGACGAACTAAAGC-3', *nuc* Rev: 5'-GCGATTGATGGTGATACGGTT-3', and for target gene: *SEB* Fw: 5'-CCAAATAGTGACGAGTTAGG-3', *SEB* Rev: 5'-GTATGGTGGTGTAACTGAGC-3'. For RT-PCR, each 25 µL of PCR mix comprised 12.5 µL of RT^2 Real-Time SYBR Green/Fluorescein PCR Master Mix (Qiagen Inc, Valencia, CA), 5.0 µL of cDNA template, and 2.0 µL of each primer, and it was made up to a final volume of 25 µL using RNAse-free water. The RT-PCR assays were performed using a Lightcyler Nano (Roche) under the following conditions: initial denaturation at 95 °C for 3 min and 40 cycles at 94 °C for 15 sec, 57 °C for 30 sec, and 72 °C for 35 sec, where the fluorescence signal was acquired at the end of each cycle. After amplification, melting curve analysis was performed for 30 sec at 60 °C and 10 sec at 95 °C. The Ct values of each sample were transformed into relative quantities (Q) of the target gene (SEB) versus the reference gene (housekeeping gene) as follows: $2^{-\Delta\Delta CT}$.

Statistical analysis

All of the results were expressed as mean values and standard deviations based on three replicate experiments. The data were analyzed by one-way analysis of variance (ANOVA) with repeated measures. Significant differences between means were tested using Duncan's multiple comparisons test, where logarithmic transformations were applied to the microbiological data before the analysis. The tests were performed with SPSS and Infostat/F program, and significant differences were accepted at $p<0.05$. The mean gene expression values were compared using Tukey tests at $p<0.05$.

RESULTS

The pH and titratable acidity of the Turkish white pickled cheeses (Table 1) were typical according to the Turkish Food Codex ((TFC), 2015). In all of the cheese samples, the titratable acidity increased gradually and the pH decreased during ripening. Moreover, the titratable acidity increased and the pH decreased when the storage temperature increased from 4 °C to 12 °C, and these differences were significant during ripening ($p<0.05$). The mean LAB counts increased in the white cheese samples during ripening. As the LAB counts increased, the mean pH declined from 5.01 to 4.77 and from 4.82 to 4.56 at 4 °C and 12 °C, respectively. The dry matter and salt contents of the cheese samples differed significantly ($p<0.05$) during the ripening and storage periods (Table 2), but this did not affect the abundance of *S. aureus*.

The changes in the *S. aureus* counts in Turkish white pickled cheeses produced from inoculated milk during the 90-day storage period are shown in Figure 1. For the cheese samples stored at 4 °C and 12 °C, the *S. aureus* counts increased to 10^7 CFU/g on the first day ($p<0.05$), before decreasing to 10^2 CFU/g at the end of ripening.

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Table 1: Mean composition of cheese samples ripened for 90 days at 4°C and 12°C.

Parameters	Storage temperature (°C)	Count (time(days))				
		1	15	30	60	90
Titratable acidity (LA%)	4	0.86±0.05^{f}	1.02±0.07^{e}	1.14±0.06^{de}	1.27±0.08^{bcd}	1.35±0.06^{ab}
	12	1.02±0.07^{e}	1.21±0.09^{cd}	1.34±0.03^{abc}	1.44±0.04^{a}	1.31±0.15^{abc}
pH	4	5.01±0.06^{f}	4.90±0.07^{e}	4.84±0.05^{ed}	4.79±0.04^{cd}	4.77±0.03^{cd}
	12	4.82±0.03^{d}	4.74±0.04^{c}	4.64±0.03^{b}	4.59±0.02^{ab}	4.56±0.04^{a}
Dry matter (%)	4	34.11±0.72^{e}	36.01±0.16^{d}	37.26±0.21^{c}	38.59±0.40 ^{ab}	39.49±0.13^{a}
	12	37.39±0.14^{c}	38.29±0.85^{bc}	38.39±1.45^{bc}	38.99±1.20^{b}	41.98±0.34^{a}
Salt (%)	4	2.51±0.12^{f}	2.61±0.11^{f}	3.08±0.16^{d}	3.04±0.09^{d}	3.29±0.06^{c}
	12	2.84±0.05^{e}	3.87±0.13^{b}	3.89±0.05^{b}	3.77±0.07^{b}	4.16±0.05^{a}

$^{a-f}$ Means in the same row but different superscripts differ significantly (P<0.05); The standard deviation is reported for each measurement.

Table 2: Effects of temperature on the total viable counts, *lactococci,* and *lactobacilli* in cheese samples during storage for 90 days

Population	Storage temperature (°C)	Count (time(days))				
		1	15	30	60	90
TAMB	4	7.58±0.20^{d}	7.35±0.04^{c}	6.97±0.01^{b}	6.96±0.06^{b}	6.83±0.09^{ab}
	12	7.80±0.28^{e}	7.61±0.07^{de}	6.99±0.07^{b}	6.97±0.06^{b}	6.74±0.01^{a}
Lactococci	4	7.24±0.34^{g}	7.01±0.06^{fg}	6.83±0.21^{fg}	6.76±0.26^{fg}	6.69±0.31^{f}
	12	7.24±0.29^{g}	7.10±0.23^{fg}	6.96±0.19^{fg}	6.73±0.40^{fg}	6.65±0.40^{f}
Lactobacilli	4	6.62±0.18^{n}	5.66±0.20^{ijk}	5.65±0.20^{ij}	5.48±0.11^{i}	5.46±0.03^{i}
	12	7.00±0.08°	6.05±0.12^{lm}	6.08±0.07^{m}	5.88±0.04^{klm}	5.85±0.01^{jkl}

TAMB: Total Aerobic Mesophylic Bacteria; $^{a-m}$ Means in the same row but different superscripts differ significantly (p<0.05); The standard deviation is reported for each measurement.

The effect of temperature on the *S. aureus* counts was not significant (p>0.05), but the reduction in the *S. aureus* counts was 2.5 times lower in cheeses ripened at the high temperature compared with the low temperature, which was probably due to the different pH of the cheese stored at 12 °C.

According to Enzyme-Linked Immunosorbent Assay test (ELISA), SEB was not detected in any of the maturation stages of the Turkish white pickled cheeses at all temperatures.

Total bacterial RNA was extracted from the cheese matrix during ripening for 90 days. However, *S. aureus* genomic DNA could only be extracted on days 1, 15, and 30 of ripening by RT-PCR, which was probably due to the sensitivity of the RNA extraction method and the commercial kit. Genomic DNA was not detected by gel electrophoresis when we determined the quality of the total RNA extracted from the cheese samples.

We compared the *S. aureus* SEB mRNA levels in cheese samples ripened at two different temperatures.

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The predicted effects of the cheese storage temperature and time on SEB production are shown in Figure 2. According to this model, the total quantity of SEB produced by *S. aureus* during cheese ripening decreased when the temperature increased from 4 °C to 12 °C.

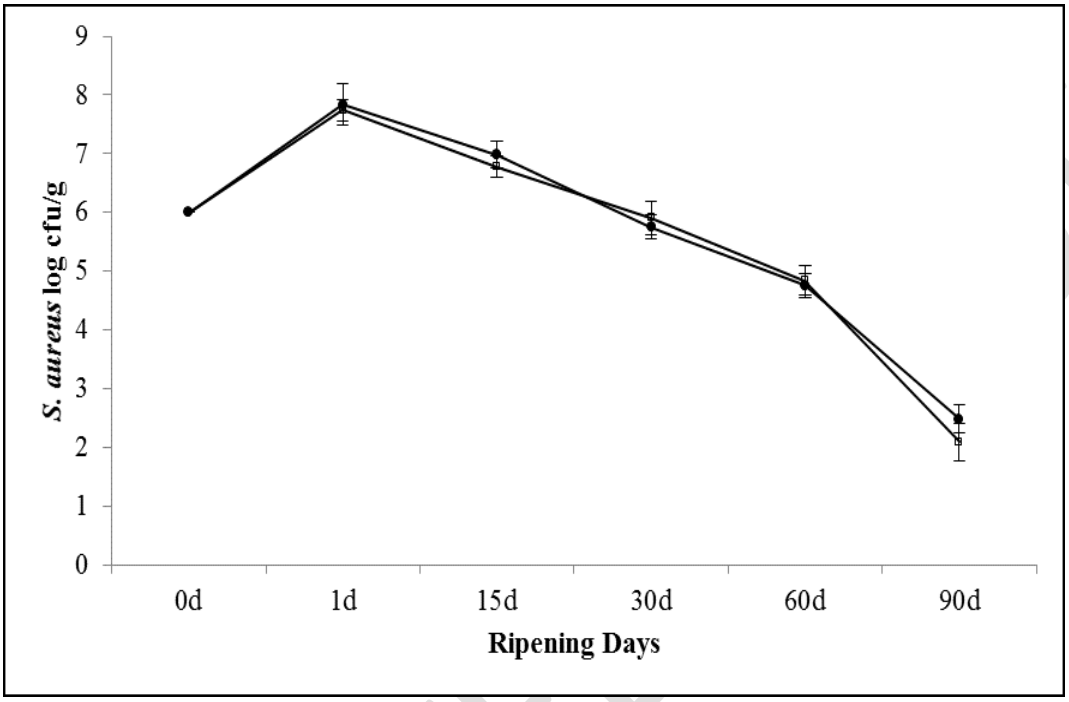

Figure 1: Growth of *S. aureus* NCTC 10654 FDA in cheese samples at 4 °C (●) and 12 °C (□). Each value represents the mean based on triplicate measurements and the bars denote the standard deviation.

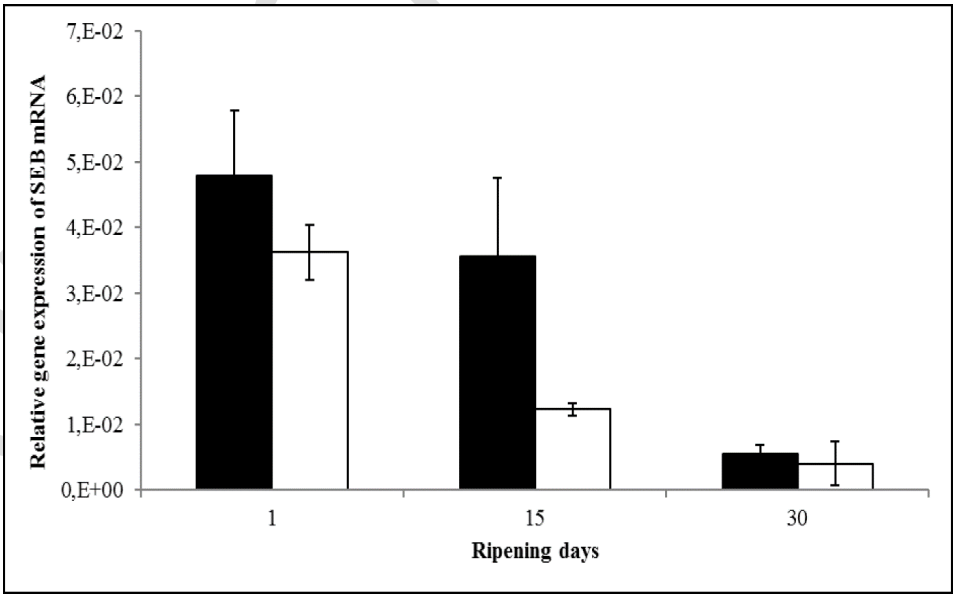

Figure 2: Relative *SEB* mRNA gene expression levels at 4 °C (■) and 12 °C (□). Each value represents the mean based on triplicate measurements and the bars denote the standard deviation.

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DISCUSSION

We observed that the pH decreased as the storage temperature of the cheese samples increased, which was probably caused by accelerated acid production and the catabolism of proteins and other nutrients in the cheeses at 12 °C. As expected, the acidity increased according to the decrease in the pH. The effects of temperature on the cheese matrix are similar to those reported by (le P et al., 2012). During brining, cheese loses moisture into the brine, which decreases the dry matter content. A warmer temperature decreases the viscosity of water and also leads to more salt penetration at high temperatures. The initial increase in the *S. aureus* counts was probably due to the favorable environment for the growth of *S. aureus* in the Turkish white pickled cheese production conditions. The *S. aureus* counts decreased during the 90-day ripening period but *S. aureus* was never completely inhibited throughout the ripening period. The decreases in the *S. aureus* counts during the ripening period may be attributable to the combined effect of different microbial inhibitors such as LAB, low pH, NaCl, and low water activity. The inhibitory effect of LAB on *S. aureus* is due to the metabolic end products of LAB, including organic acids, diacetyl, hydrogen peroxide, and bacteriocins (Gaya *et al.*, 1988). *S. aureus* is relatively resistant to salt, low water activity, and low pH, but they could have antagonistic effects on *S. aureus* during cheese production (Cretenet *et al.*, 2011). (Erkmen, 1995)) found that the *S. aureus* counts increased by 2 to 3 log in feta cheeses during the first 24 h after artificial contamination with *S. aureus* at 10^5 to 10^7 log CFU/g, respectively. Decreases in the *S. aureus* counts determined in cheese samples have been reported previously (Bellio *et al.*, 2016; Lindqvist *et al.*, 2002; Yücebay, 1994). (Gaya et al., 1988) reported that the *S. aureus* count was five times lower in cheese cured at 10 °C for 60 days compared with cheese held at 5 °C. (Cretenet et al., 2011) also found that the virulence and metabolism of *S. aureus* were negatively affected by the presence of *Lactococcus lactis*, where this effect was stronger when the temperature increased.

The apparent lack of the enterotoxin may simply be an analytical artifact due to the loss of serological recognition when using immuno-based methods such as ELISA, but it has also been proposed that proteases produced by LAB can decrease the enterotoxin levels, or the enterotoxin may have been cell-associated and thus not detectable. However, previous findings indicate that the extracellular protease activity of microorganisms such as LAB can decrease the enterotoxin level under certain conditions (Nouaille *et al.*, 2014; Sabike *et al.*, 2014). The presence of enterotoxin in the cheese produced without the starter demonstrated the accuracy of the test for detecting the enterotoxin, thereby confirming this assumption. Similar results were obtained by (Mohammadi and Hanifian, 2015) who found that a 5 log CFU/mL inoculum of *S. aureus* did not produce enterotoxin in Iranian ultrafiltered white cheese. The growth and production of enterotoxin by *S. aureus* depend on many factors, particularly the production technique and cheese processing conditions, including the use of a lactic acid starter culture (Otero *et al.*, 1993). The lactic acid generated by LAB reduces the pH and inhibits the growth of *S. aureus* during the fermentation of dairy products. Furthermore, most strains fail to produce detectable SE at low pHs (Paulin *et al.*, 2012; Sihto *et al.*, 2016). SEB production may have occurred in our cheese samples but the amounts were not detectable. In Camero cheese, Olarte et al. (2000) showed that the growth of *S. aureus* was higher in batches without the starter, but they did not detect any SE. In fresh cheeses, it might be concluded that the growth of *S. aureus* and thus SE production is likely in the absence of competitive flora, or with reduced activity. Similar results were reported by (Otero et al., 1993), and (Bachmann and Spahr, 1995) who determined the enterotoxins in different types of cheese.

The effects of technological parameters used in Turkish white pickled cheese production, including the ripening temperature, brine concentration, and starter cultures, on growth and enterotoxin production by *S. aureus* are important in terms of public health. In this study, we determined whether the storage temperature parameters caused any changes in the genes related to enterotoxin production.

The differences between ripening days were significant ($p<0.05$) and they were mainly associated with the pH and LAB activity. SEB mRNA was detected in the cheese samples, but the amount of SEB produced was too low to be quantified using the enterotoxin detection method. The cheese ripening time also affected the SEB mRNA levels in the samples. In addition, (Omoe et al., 2002) reported that *S. aureus* strains did not produce detectable amounts of some SEs despite the detectable transcription of the corresponding SE genes. However, 10- to 100-fold induction of SEB and SEC expression were found after entry into the stationary growth phase during the growth of *S. aureus* CIM441 and CIM479 in BHI broth by (Derzelle et al., 2009), although this induction was not detected in the cheese matrix by (Nouaille et al., 2009). The SEB gene is usually found on a plasmid or genomic island, and its expression depends on the agr system. Indeed, there is a close link between quorum sensing and SE production, where multiple genes are involved in the regulatory mechanism (Duquenne *et al.*, 2016). The maximum agr-dependent activation occurs during the late exponential growth phase. The minimum cell density predicted to promote specific agr-controlled expression was reported as $1.2x10^8$ *S. aureus* CFU/mL (Wright and Holland, 2003), which probably explains the different SEB expression pattern detected in the present study.

CONCLUSION

In this study, we showed that SEB production can be prevented in response to environmental changes driven by the type and nature of the cheese, the production conditions, and the starter culture activity. In addition, the SEB gene expression level varied according to the

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storage temperature. Overall, our results indicate that the ripening temperature did not influence the growth of *S. aureus* or SE production. The starter culture used is critical for both the production of enterotoxin by *S. aureus* and the quality characteristics of Turkish white pickled cheese. Our results suggest that ripening Turkish white pickled cheese for 90 days can decrease the growth of *S. aureus* given a starter culture with sufficient activity.

Further research is required to determine the effects of the storage temperature on *S. aureus* in different types of cheese using RT-PCR, as well as understanding how the cheese production parameters affect the agr system.

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