J. Dairy Sci. 104:2719–2734
https://doi.org/10.3168/jds.2020-19308

Antagonistic effects of *Lactobacillus reuteri* against *Escherichia coli* O157:H7 in white-brined cheese under different storage conditions

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ABSTRACT

This study aimed to investigate the survival of the foodborne pathogen *Escherichia coli* O157:H7 in white-brined cheeses as influenced by the presence of *Lactobacillus reuteri*. The white cheeses were made from pasteurized bovine milk inoculated with *E. coli* O157:H7 (cocktail of 3 strains) to achieve ~5 $\log_{10}$ cfu/g with absence or presence of *Lb. reuteri* (~6 $\log_{10}$ cfu/g). Cheese samples were brined in 10% or 15% NaCl solution and stored at 10°C and 25°C for 28 d. The white-brined cheeses were assessed for salt content, pH, water activity (A_w), and numbers of *E. coli* O157:H7, *Lb. reuteri*, nonstarter lactic acid bacteria (NSLAB), yeasts, and molds. Results showed that *E. coli* O157:H7 survived in cheese stored in both brine solutions at 10°C and 25°C regardless of the presence of *Lb. reuteri*. A substantial reduction was observed in cheese stored in 10% NaCl brine at 25°C, followed by cheese stored in 15% NaCl brine at 10°C by 2.64 and 2.16 $\log_{10}$ cfu/g, respectively, in the presence of *Lb. reuteri* and by 1.02 and 1.87 $\log_{10}$ cfu/g, respectively, in the absence of *Lb. reuteri* under the same conditions. The pathogen in brine solutions survived but at a lower rate. Furthermore, the growth of *Lb. reuteri* and NSLAB were enhanced or slightly decreased in cheese and brine by 28 d, respectively. The salt concentrations of cheese ranged from 4 to 6% and 5 to 7% (wt/wt), during 28-d ripening in 10 and 15% brine, respectively. Values of pH and A_w slightly increased at d 1 after exposure to brine and reached 4.69 to 6.08 and 0.91 to 0.95, respectively, in all treatments. Therefore, the addition of *Lb. reuteri* can be used as a biopreservation method to inhibit the survival of *E. coli* O157:H7 in white-brined cheese when combined with the appropriate temperature, NaCl level, and storage time.

Key words: white-brined cheese, biopreservation, *Escherichia coli* O157:H7, *Lactobacillus reuteri*

INTRODUCTION

White-brined cheese is a popular traditional product in Mediterranean regions. It is classified as a soft to semi-hard cheese because it has a moisture content between 45 and 55% (Al-Holy et al., 2012; Osaili et al., 2012). White-brined cheese can be prepared by coagulation of various types of milk, including ovine, buffalo, bovine, caprine, or their mixtures, using the enzyme rennet (Ross et al., 2002).

Several favorable factors such as solids consistency, buffering capacity, fat and protein content, absence of starter culture, high water activity ($\mathbf{A_w}$), and other multiple factors support bacterial growth in cheese (Karimi et al., 2011; Ortakci et al., 2012; Osaili et al., 2014). As with other cheeses, white-brined cheese may be contaminated by *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* spp., and other foodborne pathogens at any stage of production (Temelli et al., 2006). Cheese handlers, cheese cloth, cheese-making equipment, and utensils are potential sources of cheese contamination.

The most recent report by the European Food Safety Authority (2018) disclosed that around 6,000 cases of infection by Shiga toxin–producing *E. coli* occurred each year from 2013 to 2017. *Escherichia coli* O157:H7 has been isolated from different types of cheese including cottage cheese (Singh and Prakash, 2008), Mexican fresh cheese (Torres-Vitela et al., 2012), white-brined cheese and its brine (Osaili et al., 2014), and other

Received July 17, 2020.
Accepted November 3, 2020.
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cheese varieties (Kousta et al., 2010; Pal et al., 2016). Moreover, *E. coli* O157:H7 was associated with a multistate outbreak in the US related to Gouda cheese, in which 15 hospitalized cases were reported, with complications as serious as hemolytic uremic syndrome (CDC, 2010). Earlier, in 2000, 55 patients were sickened by *E. coli* O157:H7 after consumption of fresh, unaged cheese curds (CDC, 2000). These cheese-related outbreaks highlight the importance of developing methods to reduce the viability of pathogenic microorganisms or to control their growth. Consequently, different methods have been used to eliminate pathogens in cheeses, such as thermal processing (Angiolillo et al., 2014), antimicrobial additives (Younis et al., 2017), and nanopacking films (Jafarzadeh et al., 2019; Zhang et al., 2019).

Several preservative techniques (heating, chemical preservation, high-pressure processing, irradiation, ozone, and CO_2) have been used to inhibit or kill foodborne pathogens in food products. Biopreservation is a well-regarded technique to inhibit and control various foodborne pathogens in dairy products, especially cheeses (Asare et al., 2018). This approach employs naturally-produced compounds to control foodborne pathogens in a wide range of food products, including cheeses (Medina and Nuñez, 2011).

Biopreservation by microorganisms, especially with lactic acid bacteria (**LAB**), has been developed by utilizing bacterial cells or their metabolites to suppress the growth of undesirable microorganisms (Bagci et al., 2019; Pinto et al., 2020). In addition to organic acids, bacteriocins (short peptides), are prominent antimicrobial compounds produced by LAB (Mesa-Pereira et al., 2018). It has been reported that antimicrobial activities of these biopreservative compounds against foodborne pathogens are highly dependent on the food matrix (Silva et al., 2018).

Lactobacillus reuteri can be used as a biopreservative to obstruct the growth of foodborne pathogens due to the production of hydroxypropionaldehyde (reuterin; Asare et al., 2020; Greppi et al., 2020). Reuterin is a nonpeptide, proteolysis-resistant, broad pH range–tolerant compound that has antimicrobial activity against fungi, parasites, and different gram-positive and gram-negative bacteria (including *E. coli* O157:H7) in many food products including cheeses (Garde et al., 2016; Ortiz-Rivera et al., 2017b; Langa et al., 2018). Moreover, numerous studies have examined the feasibility and the inhibitory effects of *Lb. reuteri* against a wide range of pathogenic bacteria in various food products (Gómez-Torres et al., 2014, 2016; Montiel et al., 2015). For instance, reuterin-producing *Lb. reuteri* INIA-P572 was employed as a biopreservative in semi-hard ewe milk cheese with no negative effects on its sensory characteristics (Gómez-Torres et al., 2016). To the best of our knowledge, no information is available on using *Lb. reuteri* as a biopreservative in white-brined cheese. Therefore, this study aimed to evaluate the inhibitory effect of *Lb. reuteri* against *E. coli* O157:H7 in white-brined cheese stored in 10% or 15% NaCl brine solution at 10°C or 25°C for 28 d.

MATERIALS AND METHODS

Bacterial Strains and Culture Preparation

E. coli O157:H7. Three verocytotoxigenic-negative, nonpathogenic *E. coli* O157:H7 strains (O627, O628, and 3581) obtained from the culture collection at the Department of Nutrition and Food Technology at Jordan University of Science and Technology (**JUST**) were used in this study (Osaili et al., 2014). The cultures, stored in 20% glycerol, were kept at −40°C. To propagate these cultures, a loopful of each thawed culture was streaked on sorbitol MacConkey agar with cefixime tellurite supplement (Oxoid, Basingstoke, UK) and incubated at 37°C for 24 h. A single colony of each strain was transferred in tryptone soy broth (**TSB**, Oxoid). Then, *E. coli* O157:H7 strains were subcultured twice in TSB at 37°C for 18 h. For experimental use, a final transfer was carried out in TSB at 37°C for 18 h to refresh the culture. Overnight cultures of *E. coli* O157:H7 strains were centrifuged at 4,000 × *g* for 15 min at 4°C. After discarding the supernatant, the pellets were resuspended in 10 mL of sterile 0.1% peptone water (Oxoid). Afterward, 1 mL from each strain was combined in 100 mL of sterile 0.1% peptone water to achieve approximately 7 to 8 $\log_{10}$ cfu/mL with equal concentrations of each strain.

Lb. reuteri. Five strains of *Lb. reuteri* (SS730, S3608, MM-2, CF2, and RC14) obtained from the culture collection at the Department of Nutrition and Food Technology at JUST were used in this study. The *Lb. reuteri* strains were kept at −40°C in de Man, Rogosa, and Sharpe broth (**MRS**, Oxoid) with 20% glycerol. To propagate these cultures, a loopful of each thawed culture was grown on MRS agar (Oxoid) and incubated anaerobically using a CO_2 generating kit (AnaeroGen, Oxoid) at 37°C for 24 h. A single colony of each strain was transferred in MRS broth. Then, *Lb. reuteri* strains were subcultured twice in MRS broth at 37°C for 24 h anaerobically. For experimental use, a final transfer was carried out in MRS broth anaerobically at 37°C for 24 h to refresh the culture.

Overnight cultures of *Lb. reuteri* strains were centrifuged at 8,000 × *g* for 10 min at 4°C, the supernatant

was discarded, and the pellets were resuspended in 1 mL of sterile 0.1% peptone water (Oxoid) to achieve a concentration of about 9 to 10 $\log_{10}$ cfu/mL. After that, the cocktail culture was used to inoculate white-brined cheese at a final level of ~6 $\log_{10}$ cfu/g.

White-Brined Cheese Processing

The experimental design and cheese processing procedure are presented in Table 1 and Figure 1, respectively. White-brined cheese was prepared at the Food Safety laboratory in the Department of Nutrition and Food Technology at JUST, according to Al-Nabulsi et al. (2020). Each treatment was prepared using 15 L of full-fat raw bovine milk, which was supplied from the dairy plant at JUST. The milk was pasteurized at 72°C for 15 s, followed by testing for the presence of *E. coli* O157:H7 according to the International Organization for Standardization method 11866-1 (ISO, 2005), and it was found to be free of this pathogen. Following pasteurization, the milk was cooled to 37°C. Cocktail cultures of *Lb. reuteri*, nonpathogenic *E. coli* O157:H7, or both, were added to the cheese milk to yield a final initial inoculum level of ~6 $\log_{10}$ cfu/g and ~5 $\log_{10}$ cfu/g of cheese, respectively. Afterward, at 35°C, a single-strength microbial rennet (Dairy Connection, Inc. Madison, WI) diluted (1:10) by sterile distilled water was added to the inoculated pasteurized milk and allowed to set for 30 to 40 min until coagulation was completed. The curd was cut into cubes of 1 to 2 cm^3 and stirred for 10 min to improve whey expulsion. The cut curd was carefully transferred from the vat to a sterile stainless-steel mold measuring 50 × 50 × 2 cm in length, width, and height, respectively, and pressed for 30 min (Figure 1). Thereafter, the pressed curd was cut manually using a sterile steel knife into 25-g pieces that were then placed in sterilized glass jars containing either 10 or 15% (wt/vol) sterilized NaCl brine solution in a ratio of 1:4 (cheese:brine). Cheese samples were stored either at 10°C or 25°C for 28 d (Table 1). Experimental cheeses were sampled at 0, 1, 3, 7, 14, 21, and 28 d. Day 0 cheese and brine solutions were sampled immediately before placing cheeses into brine solutions.

Water Activity Measurements

Approximately 2 g from the cross-sectional area of white-brined cheese was used to measure the A_w directly with an A_w meter (Hygrolab, Rotronic Instrument Corp., Huntington, NY).

Salt Determination

The NaCl contents of the white-brined cheeses were determined using the procedure described by Al-Nabulsi et al. (2020). Briefly, a 1- to 1.5-g cheese sample was held in a muffle furnace (Labtech Co. Ltd., Namyangjy, South Korea) at 550°C for 8 h. The sample's ash was thoroughly mixed with 25 mL of deionized-distilled water. Then, 0.05 *N* $AgNO_3$ (Alpha Chemika, Mumbai, India) was used for titration after drops of 0.5 *N* potassium chromate were added as an indicator. The salt concentration was calculated using the following equation:

$$\text{Salt content}\,(\%) = \frac{\text{titrated volume of } AgNO_3\,(\text{mL}) \times 0.00292}{\text{mass of sample}\,(\text{g})} \times 100.$$

Table 1. Experimental design used for studying survival of *Escherichia coli* O157:H7 in white-brined cheese stored in 10 or 15% brine solution and made with (+) or without (−) *Lactobacillus reuteri*

Treatment	*E. coli* O157:H7[1]	*Lb. reuteri*[2]	% Brine	Storage temperature
1	−	+	10	10°C
2	+	−	10	10°C
3	+	+	10	10°C
4	−	+	10	25°C
5	+	−	10	25°C
6	+	+	10	25°C
7	−	+	15	10°C
8	+	−	15	10°C
9	+	+	15	10°C
10	−	+	15	25°C
11	+	−	15	25°C
12	+	+	15	25°C

[1]Inoculum level: ~5 $\log_{10}$ cfu/g of white-brined cheese.
[2]Inoculum level: ~6 $\log_{10}$ cfu/g of white-brined cheese.

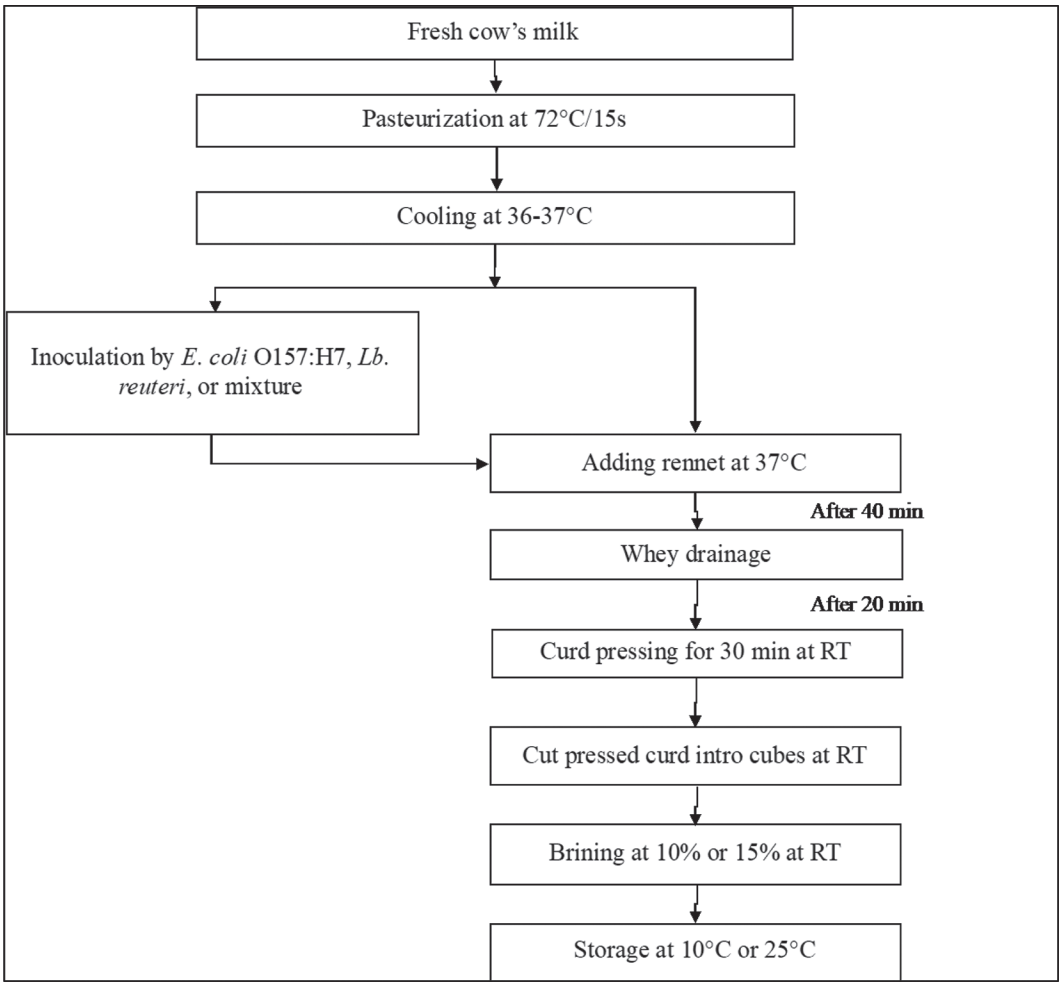

Figure 1. White-brined cheese processing with *Lactobacillus reuteri* and *Escherichia coli* O157:H7. RT = room temperature (23–25°C).

pH Measurements

A pH meter (pH Tutor, Eutech Instruments, Singapore) was used to measure the pH of white-brined cheese and brine samples. The electrode was immersed in cheese or brine samples, and the reading was the mean of 3 experiments from 2 different random places in cheese or brine.

Microbiological Analysis

Cheese and brine samples were analyzed for viability of *E. coli* O157:H7, *Lb. reuteri*, total nonstarter LAB (**NSLAB**), yeasts, and molds. Five-gram samples from a cross-sectioned piece of white-brined cheese or 5 mL of brine were initially diluted with 45 mL of 0.1% peptone water and homogenized in a sterile stomacher bag for 2 min using a stomacher model 400 (Seward Ltd., London, UK). To enumerate *E. coli* O157:H7, 100 μL from an appropriate dilution was withdrawn and surface plated on cefixime tellurite-supplemented agar (Oxoid), overlaid with TS agar to allow recovery of injured *E. coli* O157:H7 cells, and incubated at 37°C for 24 h aerobically. The *Lb. reuteri* numbers were determined by withdrawal of 100 μL from an appropriate dilution into an empty Petri dish, and then liquefied MRS agar supplemented with penicillin G (AppliChem GmbH, Darmstadt, Germany) was poured in the Petri dishes containing the samples and mixed thoroughly before anaerobic incubation using a CO_2 generating kit (AnaeroGen, Oxoid) at 37°C for 48 h. For NSLAB, a 100-μL sample was plated on *Lactobacillus*-selective agar and incubated aerobically at 30°C for 5 d (Rogosa et al., 1951). The numbers of yeasts and molds were enumerated by surface plating 100 μL on potato dextrose agar and incubating aerobically at 25°C for 5 d.

Statistical Analysis

All experiments were conducted in triplicate, and 2 replicates for each measurement were performed (n = 6) unless otherwise stated. Data were analyzed using SPSS version 22.0 (2013; IBM Corp., Armonk, NY). Values are presented as means ± standard deviations. One-way ANOVA was used to test the effects of different treatment factors (brine NaCl concentration, storage temperature, storage time, and presence or absence of *Lb. reuteri*) on bacterial numbers. Duncan's multiple range test was performed to compare means at $P < 0.05$.

RESULTS

A_w of White-Brined Cheese

The A_w values of cheese in 10% brine solution were 0.94 to 0.95 directly after processing, decreasing slightly to 0.91 in some treatments after 28 d of storage at 10°C or 25°C (Table 2). Conversely, A_w values of cheeses stored in 15% brine solution ranged from 0.92 to 0.95 and remained constant ($P > 0.05$) during the 28-d storage (Table 2).

Salt Content

In cheese stored in 10% brine solution, the salt content ranged from 4.09 to 6.06% after 1 d of storage and slightly increased to reach 4.48 to 6.74% after 28 d storage (Table 3). In cheese stored in 15% brine solution, the salt content was between 5.46 and 7.29% after 1 d of storage and reached 5.31 to 7.74% after 28 d of storage (Table 3).

pH Measurements

White-brined cheeses and their brine were sampled to measure pH during 28 d, as indicated in Tables 4 and 5, respectively. The pH values for all cheese and brine treatments were reduced by 0.10 to 1.80 and 0.04 to 1.90 units, respectively, after 28 d. A greater reduction in pH was observed at the higher temperature (25°C) and lower brine concentration (10%). At d 28, the pH values between cheese samples differed significantly ($P < 0.05$), regardless of the presence of *E. coli* O157:H7 or *Lb. reuteri*, except treatments 3 and 9 (Table 4). Table 5 shows that pH values of the brine solution changed significantly ($P < 0.05$) during storage, except in treatments 8 and 9.

Behavior of E. coli O157:H7 and Lb. reuteri in White-Brined Cheese

The numbers of *E. coli* O157:H7 in cheese made without *Lb. reuteri* and stored in 10% NaCl brine at 10°C did not change significantly during the study. The initial numbers of *E. coli* O157:H7 were reduced from 6.01 to 5.87 $\log_{10}$ cfu/g after 28 d (Figure 2A). However, a significant ($P < 0.05$) reduction in *E. coli* O157:H7 populations was observed in cheeses made with *Lb. reuteri*. The numbers of *E. coli* O157:H7 were significantly reduced by 1.54 $\log_{10}$ cfu/g in cheese stored under the same conditions (Figure 2A).

Table 2. Water activity (A_w) of white-brined cheese during storage[1]

Treatment[2]	Storage time (d)						
	0[3]	1	3	7	14	21	28
1	$0.95 \pm 0.03^{BC,c}$	$0.95 \pm 0.01^{BC,d}$	$0.95 \pm 0.01^{BC,bc}$	$0.94 \pm 0.01^{A,bc}$	$0.95 \pm 0.01^{C,c}$	$0.94 \pm 0.01^{AB,bc}$	$0.95 \pm 0.00^{ABC,c}$
2	$0.95 \pm 0.01^{A,bc}$	$0.94 \pm 0.01^{A,bcd}$	$0.92 \pm 0.04^{A,ab}$	$0.94 \pm 0.01^{A,bc}$	$0.93 \pm 0.02^{A,abc}$	$0.93 \pm 0.03^{A,abc}$	$0.94 \pm 0.01^{A,bc}$
3	$0.94 \pm 0.01^{A,bc}$	$0.92 \pm 0.03^{A,ab}$	$0.93 \pm 0.01^{A,abc}$	$0.92 \pm 0.04^{A,b}$	$0.92 \pm 0.04^{A,ab}$	$0.92 \pm 0.04^{A,ab}$	$0.91 \pm 0.06^{A,a}$
4	$0.95 \pm 0.00^{ABC,c}$	$0.96 \pm 0.01^{C,d}$	$0.95 \pm 0.01^{ABC,c}$	$0.95 \pm 0.01^{AB,c}$	$0.94 \pm 0.01^{A,bc}$	$0.95 \pm 0.01^{BC,c}$	$0.95 \pm 0.01^{ABC,c}$
5	$0.95 \pm 0.01^{B,ab}$	$0.95 \pm 0.02^{B,d}$	$0.93 \pm 0.01^{AB,abc}$	$0.94 \pm 0.01^{B,c}$	$0.94 \pm 0.01^{B,abc}$	$0.93 \pm 0.02^{AB,abc}$	$0.91 \pm 0.02^{A,ab}$
6	$0.94 \pm 0.01^{A,bc}$	$0.93 \pm 0.01^{A,bcd}$	$0.92 \pm 0.02^{A,abc}$	$0.94 \pm 0.02^{A,bc}$	$0.93 \pm 0.03^{A,abc}$	$0.93 \pm 0.02^{A,ab}$	$0.94 \pm 0.01^{A,abc}$
7	$0.95 \pm 0.00^{B,c}$	$0.94 \pm 0.01^{A,bcd}$	$0.94 \pm 0.01^{A,abc}$	$0.94 \pm 0.01^{A,bc}$	$0.94 \pm 0.15^{A,abc}$	$0.93 \pm 0.01^{A,abc}$	$0.94 \pm 0.01^{AB,abc}$
8	$0.93 \pm 0.02^{A,ab}$	$0.92 \pm 0.02^{A,ab}$	$0.91 \pm 0.03^{A,a}$	$0.93 \pm 0.00^{A,bc}$	$0.91 \pm 0.03^{A,a}$	$0.92 \pm 0.01^{A,ab}$	$0.92 \pm 0.02^{A,abc}$
9	$0.92 \pm 0.01^{AB,a}$	$0.90 \pm 0.01^{AB,a}$	$0.94 \pm 0.01^{B,bc}$	$0.88 \pm 0.05^{A,a}$	$0.92 \pm 0.02^{AB,ab}$	$0.91 \pm 0.02^{AB,a}$	$0.92 \pm 0.03^{B,abc}$
10	$0.95 \pm 0.00^{B,c}$	$0.95 \pm 0.01^{AB,d}$	$0.94 \pm 0.02^{AB,abc}$	$0.93 \pm 0.01^{A,bc}$	$0.94 \pm 0.01^{AB,abc}$	$0.94 \pm 0.01^{AB,bc}$	$0.94 \pm 0.01^{AB,abc}$
11	$0.93 \pm 0.02^{A,ab}$	$0.94 \pm 0.02^{A,cd}$	$0.92 \pm 0.02^{A,abc}$	$0.93 \pm 0.01^{A,bc}$	$0.92 \pm 0.02^{A,ab}$	$0.93 \pm 0.02^{A,ab}$	$0.95 \pm 0.01^{A,abc}$
12	$0.92 \pm 0.01^{A,a}$	$0.92 \pm 0.02^{A,abc}$	$0.93 \pm 0.01^{A,abc}$	$0.92 \pm 0.03^{A,bc}$	$0.93 \pm 0.01^{A,abc}$	$0.93 \pm 0.02^{A,abc}$	$0.93 \pm 0.01^{A,abc}$

[A–C]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 ($P > 0.05$).

[1]Values are the means of 3 experimental units (n = 3) ± SD.

[2]Treatments as described in Table 1.

[3]Day 0 is immediately before adding to brine solution.

Table 3. Salt content of white-brined cheese during storage[1]

Treatment[2]	Storage time (d)						
	0[3]	1	3	7	14	21	28
1	ND^{A,a}	4.09 ± 0.81^{B,a}	5.48 ± 2.29^{B,a}	4.78 ± 0.80^{B,a}	5.66 ± 0.67^{B,ab}	5.73 ± 1.96^{B,ab}	5.65 ± 1.79^{B,a}
2	ND^{A,a}	5.75 ± 0.02^{B,ab}	6.77 ± 3.30^{B,a}	5.47 ± 3.80^{B,a}	6.02 ± 2.50^{B,ab}	5.49 ± 2.10^{B,ab}	4.60 ± 1.61^{B,a}
3	ND^{A,a}	6.06 ± 0.23^{B,abc}	7.04 ± 0.04^{B,a}	7.11 ± 0.64^{B,a}	6.75 ± 2.20^{B,ab}	6.78 ± 1.02^{B,ab}	6.74 ± 1.41^{B,a}
4	ND^{A,a}	5.75 ± 0.01^{B,abc}	5.68 ± 1.90^{B,a}	5.83 ± 0.64^{B,a}	4.60 ± 2.20^{B,ab}	6.75 ± 1.10^{B,ab}	6.49 ± 0.71^{B,a}
5	ND^{A,a}	5.33 ± 2.20^{B,abc}	7.36 ± 0.35^{B,a}	5.36 ± 2.41^{B,a}	3.73 ± 0.58^{AB,a}	5.32 ± 3.50^{B,ab}	4.48 ± 2.21^{B,a}
6	ND^{A,a}	5.00 ± 1.80^{B,ab}	7.23 ± 0.25^{B,a}	6.75 ± 0.45^{B,a}	6.01 ± 2.05^{B,ab}	6.49 ± 2.01^{B,ab}	6.35 ± 0.88^{B,a}
7	ND^{A,a}	5.46 ± 0.22^{BC,abc}	6.91 ± 1.40^{CD,a}	6.57 ± 0.65^{BCD,a}	6.87 ± 0.82^{CD,ab}	6.97 ± 0.17^{D,ab}	5.31 ± 0.01^{B,a}
8	ND^{A,a}	7.10 ± 0.42^{B,c}	8.20 ± 0.48^{B,a}	7.67 ± 2.00^{B,a}	7.31 ± 0.39^{B,b}	6.11 ± 1.20^{B,ab}	6.66 ± 0.01^{B,a}
9	ND^{A,a}	6.91 ± 0.49^{B,bc}	7.80 ± 0.01^{B,a}	7.51 ± 0.13^{B,a}	6.72 ± 1.03^{B,ab}	7.02 ± 1.50^{B,ab}	7.74 ± 0.19^{B,a}
10	ND^{A,a}	6.38 ± 0.14^{B,bc}	5.99 ± 1.90^{B,a}	6.32 ± 0.42^{B,a}	5.91 ± 1.61^{B,ab}	8.19 ± 0.44^{B,b}	6.94 ± 3.30^{B,a}
11	ND^{A,a}	6.83 ± 0.01^{B,bc}	7.32 ± 1.01^{B,a}	6.91 ± 3.03^{B,a}	6.63 ± 1.51^{B,ab}	4.21 ± 0.99^{B,a}	5.56 ± 2.54^{B,a}
12	ND^{A,a}	7.29 ± 0.25^{B,c}	6.51 ± 0.92^{B,a}	7.64 ± 0.30^{B,a}	6.04 ± 0.01^{B,ab}	6.84 ± 1.50^{B,ab}	6.10 ± 0.04^{B,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 ($P > 0.05$).

[1]Values are the means of 3 experimental units (n = 3) ± SD.

[2]Treatments as described in Table 1.

[3]Day 0 is immediately before adding to brine solution. ND = not detected at lower limit of <0.1 g of NaCl/g of cheese.

With respect to *Lb. reuteri*, the initial numbers were reduced significantly in cheese made without *E. coli* O157:H7, from 6.22 to 5.14 $\log_{10}$ cfu/g after 28 d. However, *Lb. reuteri* declined to a lesser extent after half of the storage period in the presence of *E. coli* O157:H7, and ranged from 6.19 to 5.72 $\log_{10}$ cfu/g at d 28, as illustrated in Figure 2A.

The numbers of *E. coli* O157:H7 in cheese made without *Lb. reuteri* and stored in 10% NaCl at 25°C decreased significantly from 6.14 to 5.12 $\log_{10}$ cfu/g after 28 d. A substantial reduction in *E. coli* O157:H7 numbers was noted when *Lb. reuteri* was added under the same conditions. The viability of *E. coli* O157:H7 decreased significantly from 5.81 to 3.17 $\log_{10}$ cfu/g after 28 d (Figure 2B). The numbers of *Lb. reuteri* in cheese treatments made without *E. coli* O157:H7 showed a steady level within narrow margins over a time interval of 28 d, which was between 6.22 and 6.26 $\log_{10}$ cfu/g. Whereas in the presence of *E. coli* O157:H7, the numbers of *Lb. reuteri* significantly ($P < 0.05$) increased from 6.19 to 8.31 $\log_{10}$ cfu/g after storage for 28 d (Figure 2B).

The initial numbers of *E. coli* O157:H7 in cheese made without *Lb. reuteri* were significantly reduced, from 6.29 to 4.42 $\log_{10}$ cfu/g, after 28-d storage in 15% NaCl at 10°C. It is notable that the reduction was higher in the presence of *Lb. reuteri*, where the viability of *E. coli* O157:H7 was reduced by 2.16 $\log_{10}$

Table 4. pH values of white-brined cheese during storage[1]

Treatment[2]	Storage time (d)						
	0[3]	1	3	7	14	21	28
1	6.48 ± 0.29^{D,a}	6.08 ± 0.11^{BC,abc}	6.19 ± 0.16^{BC,c}	6.23 ± 0.09^{CD,c}	6.06 ± 0.11^{BC,d}	5.92 ± 0.45^{AB,c}	5.70 ± 0.07^{A,ef}
2	6.30 ± 0.06^{C,a}	6.24 ± 0.15^{BC,bc}	6.15 ± 0.04^{BC,c}	6.15 ± 0.13^{BC,c}	6.09 ± 0.23^{BC,d}	6.00 ± 0.29^{B,c}	5.64 ± 0.24^{A,ef}
3	6.18 ± 0.14^{AB,a}	6.23 ± 0.08^{AB,bc}	6.21 ± 0.08^{AB,c}	6.29 ± 0.06^{B,c}	6.21 ± 0.09^{A,bd}	6.09 ± 0.06^{A,cd}	6.08 ± 0.16^{A,g}
4	6.48 ± 0.29^{E,a}	6.20 ± 0.12^{D,bc}	5.64 ± 0.34^{C,a}	5.20 ± 0.12^{B,a}	4.92 ± 0.20^{A,a}	4.88 ± 0.12^{A,a}	4.69 ± 0.17^{A,a}
5	6.30 ± 0.06^{F,a}	6.04 ± 0.19^{E,abc}	5.59 ± 0.21^{D,a}	5.28 ± 0.26^{C,a}	5.08 ± 0.28^{BC,ab}	5.02 ± 0.14^{AB,a}	4.80 ± 0.18^{A,a}
6	6.18 ± 0.14^{C,a}	5.99 ± 0.14^{C,a}	5.79 ± 0.42^{C,ab}	5.32 ± 0.59^{B,a}	4.91 ± 0.15^{A,a}	4.75 ± 0.15^{A,a}	4.94 ± 0.16^{A,ab}
7	6.45 ± 0.35^{B,a}	6.07 ± 0.13^{A,abc}	6.07 ± 0.08^{A,bc}	6.13 ± 0.12^{A,c}	6.16 ± 0.16^{A,d}	6.10 ± 0.14^{A,cd}	5.90 ± 0.21^{A,fg}
8	6.23 ± 0.09^{B,a}	6.25 ± 0.20^{B,c}	6.27 ± 0.08^{B,c}	6.18 ± 0.09^{B,c}	6.19 ± 0.09^{B,d}	6.21 ± 0.17^{B,cd}	5.92 ± 0.38^{A,fg}
9	6.21 ± 0.02^{AB,a}	6.22 ± 0.05^{B,bc}	6.25 ± 0.05^{B,c}	6.26 ± 0.06^{B,c}	6.19 ± 0.06^{A,bd}	6.32 ± 0.13^{B,d}	6.07 ± 0.16^{A,g}
10	6.45 ± 0.35^{E,a}	6.11 ± 0.15^{D,abc}	5.77 ± 0.07^{C,ab}	5.58 ± 0.22^{BC,b}	5.38 ± 0.34^{AB,bc}	5.36 ± 0.26^{AB,b}	5.17 ± 0.23^{A,bc}
11	6.23 ± 0.09^{C,a}	6.03 ± 0.23^{C,ab}	6.08 ± 0.12^{C,bc}	5.67 ± 0.17^{B,b}	5.33 ± 0.22^{A,bc}	5.47 ± 0.34^{AB,b}	5.31 ± 0.35^{A,cd}
12	6.21 ± 0.02^{D,a}	6.05 ± 0.10^{CD,abc}	5.99 ± 0.26^{BCD,bc}	5.64 ± 0.45^{ABC,b}	5.43 ± 0.57^{A,c}	5.32 ± 0.35^{A,b}	5.53 ± 0.51^{AB,de}

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

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

[1]Values are the means of 3 experimental units (n = 3) ± SD.

[2]Treatments as described in Table 1.

[3]Day 0 is immediately before adding to brine solution.

cfu/g after 28 d (Figure 3A). Nevertheless, the initial numbers of *Lb. reuteri* in cheese made without *E. coli* O157:H7 decreased from 5.94 to 5.06 $\log_{10}$ cfu/g after 28 d. In relation to cheese made in the presence of *E. coli* O157:H7, the numbers of *Lb. reuteri* significantly decreased from 6.19 to 5.2 $\log_{10}$ cfu/g at 28 d (Figure 3A). The numbers of *E. coli* O157:H7 significantly decreased from 6.33 to 5.22 $\log_{10}$ cfu/g. In contrast, the numbers of *Lb. reuteri* remained around 6 $\log_{10}$ cfu/g after 28 d of storage in 15% NaCl at 25°C (Figure 3B). In cheese made with both *E. coli* O157:H7 and *Lb. reuteri*, the initial numbers of *E. coli* O157:H7 significantly decreased from 5.76 $\log_{10}$ cfu/g and reached 4.14 $\log_{10}$ cfu/g after 28 d. Meanwhile, the numbers of *Lb. reuteri* showed a significant increase from 6.19 to 7.79 $\log_{10}$ cfu/g after 28 d (Figure 3B).

Behavior of E. coli O157:H7 in Cheese Brine. The survival of *E. coli* O157:H7 in cheese brine made with or without *Lb. reuteri* and containing 10% or 15% NaCl held at 10°C or 25°C for 28 d was investigated. *Escherichia coli* O157:H7 cells contaminated the sterile brine solutions by ~4.0 $\log_{10}$ cfu/mL, which was detected after d 1 of immersion of cheese in the brine (Table 6). *Escherichia coli* O157:H7 populations in the brine either did not change or significantly decreased in treatments during storage. Maximum *E. coli* O157:H7 survival occurred in 10% NaCl brine at 25°C without *Lb. reuteri* (6.91 $\log_{10}$ cfu/mL) at d 1, which declined to 5.72 $\log_{10}$ cfu/mL at d 28, whereas the minimum survival was observed in 15% NaCl brine held at 10°C with *Lb. reuteri* and ranged from 3.97 $\log_{10}$ cfu/mL at d 1 to 2.59 $\log_{10}$ cfu/mL after d 28 of storage.

Behavior of Lb. reuteri in Cheese Brine. The survival of *Lb. reuteri* in cheese brine containing 10% or 15% NaCl made with or without *E. coli* O157:H7 and stored at 10°C or 25°C for 28 d was investigated (Table 7). Performance of *Lb. reuteri* in the brine was similar to that found in cheese, but with lower numbers. At 25°C, *Lb. reuteri* numbers significantly increased from 4.96 to 5.29 $\log_{10}$ cfu/mL at d 1 to reach 5.67 to 7.84 $\log_{10}$ cfu/mL after 28 d in the presence of *E. coli* O157:H7 at both NaCl brine concentrations.

NSLAB, Yeasts, and Molds in White-Brined Cheese

Tables 8 and 9 show the NSLAB counts in the white-brined cheese and brine solution during 28 d of storage, respectively. As can be seen from Table 8, the NSLAB population increased ($P < 0.05$) in white cheeses brined in 10% and stored at 25°C compared with 10°C. The NSLAB counts in white cheeses brined in 15% and stored at 10°C decreased ($P < 0.05$) during storage compared with 25°C. Generally, yeasts and molds grew at lower levels in cheese stored in 15% NaCl brine compared with 10% NaCl, and at 10°C compared with 25°C (Table 10). Regarding the presence of *E. coli* O157:H7 and *Lb. reuteri*, the numbers of yeasts and molds decreased from 5.63 to 4.65 $\log_{10}$ cfu/g, and remained constant (~5 log cfu/g) in 15% and 10% NaCl brine, respectively, at 10°C. Their numbers remained nearly stable in cheese stored in 15% NaCl brine at 25°C in the absence of *E. coli* O157:H7. In brine samples, lower numbers of yeasts and molds were found in 15% NaCl compared with 10% NaCl, and at 10°C compared with 25°C. Furthermore, the lowest numbers were found in 15% NaCl brine containing inoculated *E. coli* O157:H7 stored at 10°C during the entire ripening period (Table 10).

Table 5. pH values of brine solution during storage[1]

	Storage time (d)						
Treatment[2]	0[3]	1	3	7	14	21	28
1	$6.49 \pm 0.24^{D,b}$	$6.24 \pm 0.03^{BC,abc}$	$6.33 \pm 0.23^{CD,e}$	$6.24 \pm 0.15^{BC,e}$	$6.10 \pm 0.05^{B,c}$	$6.07 \pm 0.18^{B,cd}$	$5.80 \pm 0.10^{A,fg}$
2	$6.36 \pm 0.09^{C,ab}$	$6.31 \pm 0.10^{C,bc}$	$6.38 \pm 0.23^{C,e}$	$6.23 \pm 0.18^{BC,e}$	$6.04 \pm 0.21^{B,c}$	$6.02 \pm 0.34^{B,c}$	$5.69 \pm 0.23^{A,ef}$
3	$6.39 \pm 0.09^{C,ab}$	$6.37 \pm 0.07^{C,c}$	$6.29 \pm 0.11^{BC,e}$	$6.35 \pm 0.11^{C,e}$	$6.28 \pm 0.10^{BC,c}$	$6.20 \pm 0.07^{B,cd}$	$6.04 \pm 0.05^{A,gh}$
4	$6.49 \pm 0.24^{D,b}$	$6.23 \pm 0.02^{D,abc}$	$5.49 \pm 0.33^{C,ab}$	$5.09 \pm 0.07^{B,a}$	$4.88 \pm 0.14^{AB,a}$	$4.91 \pm 0.22^{AB,a}$	$4.62 \pm 0.48^{A,a}$
5	$6.36 \pm 0.09^{C,ab}$	$6.10 \pm 0.31^{C,a}$	$5.45 \pm 0.34^{B,a}$	$5.12 \pm 0.14^{A,a}$	$5.00 \pm 0.21^{A,a}$	$4.92 \pm 0.20^{A,a}$	$5.07 \pm 0.19^{A,bc}$
6	$6.39 \pm 0.09^{D,ab}$	$6.19 \pm 0.11^{CD,abc}$	$5.90 \pm 0.32^{C,cd}$	$5.43 \pm 0.51^{B,a}$	$4.92 \pm 0.11^{A,a}$	$4.74 \pm 0.22^{A,a}$	$4.95 \pm 0.11^{A,b}$
7	$6.38 \pm 0.28^{C,ab}$	$6.21 \pm 0.03^{BC,abc}$	$6.32 \pm 0.15^{BC,e}$	$6.06 \pm 0.33^{AB,de}$	$6.21 \pm 0.09^{BC,c}$	$6.13 \pm 0.13^{ABC,cd}$	$5.90 \pm 0.18^{A,fgh}$
8	$6.28 \pm 0.05^{A,ab}$	$6.31 \pm 0.13^{A,bc}$	$6.32 \pm 0.05^{A,e}$	$6.25 \pm 0.10^{A,e}$	$6.28 \pm 0.19^{A,c}$	$6.30 \pm 0.05^{A,d}$	$6.22 \pm 0.09^{A,h}$
9	$6.23 \pm 0.09^{A,a}$	$6.33 \pm 0.15^{A,bc}$	$6.26 \pm 0.08^{A,e}$	$6.29 \pm 0.14^{A,e}$	$6.33 \pm 0.08^{A,c}$	$6.30 \pm 0.05^{A,d}$	$6.19 \pm 0.17^{A,h}$
10	$6.38 \pm 0.28^{D,ab}$	$6.17 \pm 0.04^{D,ab}$	$5.77 \pm 0.10^{C,bc}$	$5.46 \pm 0.13^{B,bc}$	$5.33 \pm 0.27^{B,b}$	$5.27 \pm 0.22^{AB,b}$	$5.03 \pm 0.21^{A,bc}$
11	$6.28 \pm 0.05^{C,ab}$	$6.20 \pm 0.12^{C,abc}$	$6.10 \pm 0.15^{C,de}$	$5.64 \pm 0.31^{B,bc}$	$5.32 \pm 0.21^{A,b}$	$5.43 \pm 0.29^{AB,b}$	$5.33 \pm 0.30^{A,cd}$
12	$6.23 \pm 0.09^{C,a}$	$6.18 \pm 0.10^{C,abc}$	$6.06 \pm 0.20^{C,cde}$	$5.77 \pm 0.47^{BC,cd}$	$5.44 \pm 0.56^{AB,b}$	$5.29 \pm 0.24^{A,b}$	$5.48 \pm 0.50^{AB,de}$

[A–D]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 ($P > 0.05$).

[1]Values are the means of 3 experimental units (n = 3) ± SD.

[2]Treatments as described in Table 1.

[3]Day 0 is immediately before adding to brine solution.

DISCUSSION

Chemical Properties

The salt content of cheese stored in either 10% or 15% NaCl brine increased on d 1 due to the movement of Na^+ and Cl^- ions from brine into cheese to achieve osmotic pressure balance (Ayyash et al., 2013). Moreover, salt content in all cheese samples slightly changed during 28 d, which was reflected by nearly steady A_w measurements (~0.95), but they were higher in cheese stored in 15% NaCl brine than in 10% brine and reached ~5 to 7% and ~4 to 6%, respectively, on d 28. Similar results were noted by Osaili et al. (2014), who found that the salt content of cheese stored in 15% brine was higher than that in 10% NaCl brine,

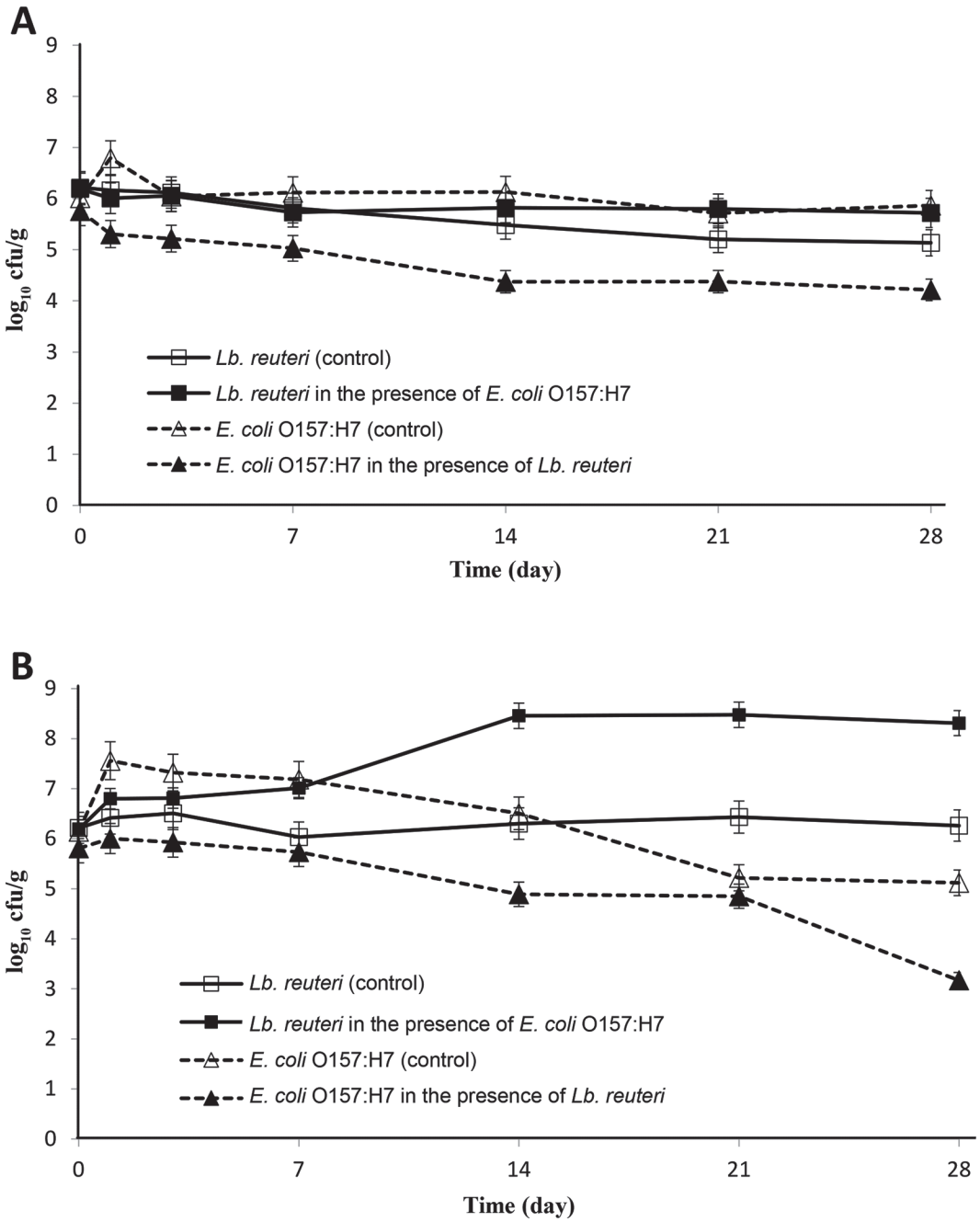

Figure 2. Survival of *Lactobacillus reuteri* and *Escherichia coli* O157:H7 in white-brined cheese stored in 10% NaCl brine at 10°C (A) and 25°C (B). Error bars represent standard deviations.

and it remained around 5% (wt/wt) in the latter after 28 d. Hickey et al. (2018) found that the salt uptake of cheese immersed in 18% or 22% NaCl brine for 60 h and vacuum stored for 26 d was the same. Moreover, salt had an effect on *Lb. reuteri* and NSLAB growth, their metabolic activities, lactic acid production, and proteolysis, and consequently had an indirect effect on cheese pH (Ulpathakumbura et al., 2016; Blaya et al., 2018). In this study, lower pH values of cheese and brine were found in 10% NaCl brine than in 15% brine, as pH ranged from 4.69 to 6.08 and 4.62 to 6.04, respectively, in 10% brine and 5.17 to 6.07 and 5.03 to 6.22, respectively, in 15% brine. Increasing salt concentration had less effect on the growth of NSLAB compared with starter cultures (Ulpathakumbura et al., 2016; Blaya et al., 2018). The numbers of NSLAB in 15% brine were slightly lower compared with 10% brine, which may be attributed to the higher salt level, because NSLAB

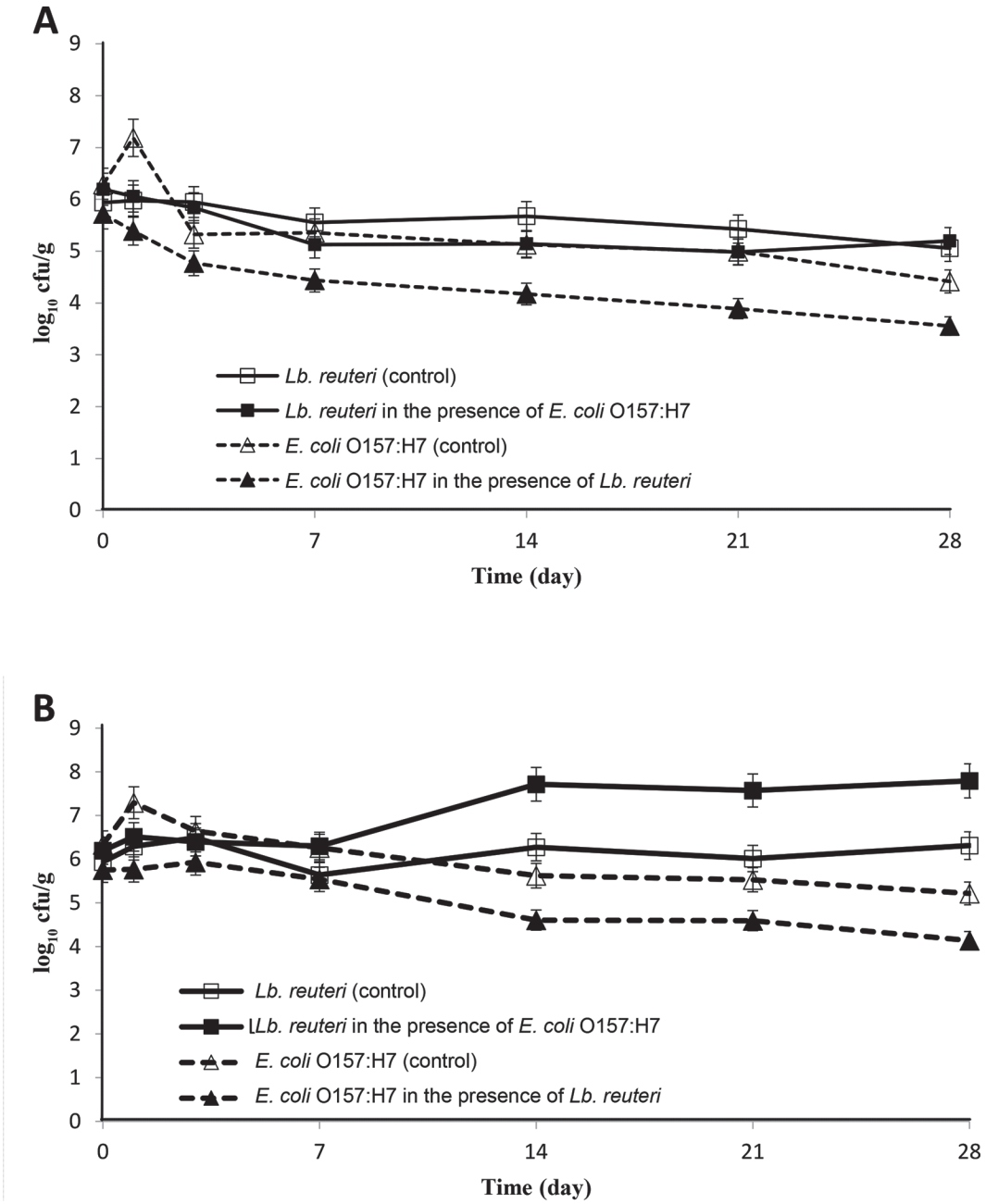

Figure 3. Survival of *Lactobacillus reuteri* and *Escherichia coli* O157:H7 in white-brined cheese stored in 15% NaCl brine at 10°C (A) and 25°C (B). Error bars represent standard deviations.

Table 6. Recovery of *Escherichia coli* O157:H7 (log_{10} cfu/mL) from brine solution during storage[1]

Treatment[2]	Storage time (d)						
	0[3]	1	3	7	14	21	28
2	ND^{A,a}	5.54 ± 0.92^{B,c}	5.10 ± 1.27^{B,a}	4.76 ± 0.05^{B,ab}	5.15 ± 0.77^{B,de}	5.07 ± 1.05^{B,bc}	5.47 ± 1.05^{B,cd}
3	ND^{A,a}	4.31 ± 0.72^{B,a}	4.51 ± 0.42^{B,a}	4.44 ± 0.00^{B,ab}	4.38 ± 0.16^{B,cd}	3.98 ± 0.94^{B,abc}	3.76 ± 0.90^{B,bc}
5	ND^{A,a}	6.91 ± 0.57^{D,d}	6.49 ± 0.52^{CD,b}	6.55 ± 1.10^{CD,c}	5.57 ± 0.52^{B,e}	5.42 ± 0.89^{B,d}	5.72 ± 0.93^{BC,d}
6	ND^{A,a}	4.41 ± 0.59^{CD,ab}	4.89 ± 0.90^{D,a}	4.80 ± 1.60^{D,ab}	4.10 ± 0.62^{CD,bc}	3.68 ± 1.30^{C,abc}	2.55 ± 0.13^{B,b}
8	ND^{A,a}	5.17 ± 1.20^{C,bc}	4.48 ± 0.43^{BC,a}	3.86 ± 0.20^{BC,ab}	4.18 ± 1.10^{BC,bc}	3.88 ± 1.60^{BC,abc}	3.28 ± 0.19^{B,b}
9	ND^{A,a}	3.97 ± 0.99^{B,a}	4.02 ± 0.48^{B,a}	3.01 ± 1.03,=^{B,a}	3.07 ± 0.55^{B,a}	2.57 ± 2.65^{B,a}	2.59 ± 2.24^{B,b}
11	ND^{A,a}	5.49 ± 0.41^{B,c}	4.85 ± 1.20^{B,a}	5.02 ± 1.90^{B,bc}	4.18 ± 0.18^{B,bc}	4.40 ± 0.41^{B,bc}	4.15 ± 1.6^{B,bcd}
12	ND^{A,a}	4.41 ± 0.63^{C,ab}	4.29 ± 0.97^{C,a}	4.32 ± 1.04^{C,ab}	3.37 ± 0.52^{B,ab}	3.46 ± 0.45^{B,ab}	ND^{A,a}

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

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

[1]Values are the means of 3 experimental units (n = 3) ± SD.

[2]Treatments as described in Table 1.

[3]Day 0 is immediately before adding to brine solution. ND = not detected (detection level was ≤1 cfu/mL).

can tolerate 4 to 6% salt in moisture (De Angelis et al., 2002). Gandhi and Shah (2015) found that the numbers and the enzymatic activity of some species of LAB (*Lb. casei*) were maintained in MRS broth with 5% NaCl after 1 wk at room temperature.

E. coli O157:H7 and *Lb. reuteri* in White-Brined Cheese

Consumer demand for white-brined cheese has grown in recent years (Salameh et al., 2016). Because of its solids consistency, high buffering capacity (pH 5.2–6.5), high fat (10.4%) and protein (21.1%) contents, absence of starter culture, and high A_w (0.94–0.96), white-brined cheese could be considered an appropriate medium for the growth of different foodborne pathogens, including *L. monocytogenes*, *S. aureus*, and *E. coli* O157:H7 (Kousta et al., 2010; Al-Holy et al., 2012; Al-Nabulsi et al., 2020). This was evident in the present study because *E. coli* O157:H7 survived in white-brined cheese stored in 10% or 15% NaCl solution at either 10°C or 25°C. In cheese stored in 10% NaCl brine, it was noted that that changes in the population of *E. coli* O157:H7 over the study period were insignificant ($P > 0.05$) at 10°C, whereas a 1.02 log_{10} cfu/g reduction was noted at 25°C after 28 d. Additionally, the *E. coli* O157:H7 populations in cheese were reduced by 1.87 and 1.11 log_{10} cfu/g at 10°C and 25°C (Figure 2), respectively, after 28 d in 15% brine solution. The reduction of *E. coli* O157:H7 may have been due to a lack of salt tolerance (Beneduce et al., 2003), although *E. coli* O157:H7 can survive exposure to levels from 2.5 to 8% NaCl (Beneduce et al., 2003; Bae and Lee, 2017).

Ozer et al. (2004) found that *E. coli* O157:H7 survived in unscalded Turkish Urfa cheese stored at 6°C, with greater reduction occurring with prolonged storage (90 d). Also, it was noted that their numbers were dramatically reduced when the salt concentration of brine was increased from 12.5 to 17.5% (wt/vol). In addition, *E. coli* O157:H7 numbers remained about 6 log_{10} cfu/g in

Table 7. Recovery of *Lactobacillus reuteri* (log_{10} cfu/mL) from brine solution of cheese during storage[1]

Treatment[2]	Storage time (d)						
	0[3]	1	3	7	14	21	28
1	ND^{A,a}	4.83 ± 0.22^{BC,abc}	4.84 ± 0.29^{BC,ab}	4.53 ± 0.39^{B,ab}	5.26 ± 1.3^{BC,b}	5.09 ± 1.71^{BC,b}	5.78 ± 1.38^{C,c}
3	ND^{A,a}	4.62 ± 0.25^{CD,ab}	4.84 ± 0.18^{D,ab}	4.68 ± 0.95^{D,ab}	4.05 ± 0.75^{BC,a}	3.93 ± 0.43^{B,a}	4.36 ± 0.62^{BCD,ab}
4	ND^{A,a}	5.29 ± 0.36^{B,d}	5.63 ± 1.50^{B,bc}	5.45 ± 1.15^{B,ab}	5.47 ± 1.14^{B,b}	5.72 ± 0.66^{B,b}	5.83 ± 0.52^{B,c}
6	ND^{A,a}	4.96 ± 0.46^{C,bcd}	4.23 ± 0.11^{B,a}	6.53 ± 1.36^{D,b}	7.84 ± 0.32^{E,c}	7.68 ± 0.45^{E,c}	7.84 ± 0.45^{E,d}
7	ND^{A,a}	5.03 ± 0.39^{C,cd}	4.47 ± 0.26^{BC,a}	3.82 ± 0.21^{B,a}	5.01 ± 0.79^{C,b}	5.33 ± 1.70^{C,b}	5.03 ± 1.12^{C,bc}
9	ND^{A,a}	4.49 ± 0.37^{DE,a}	4.53 ± 0.36^{E,a}	3.89 ± 0.74^{BCD,a}	3.72 ± 0.97^{BC,a}	3.39 ± 0.78^{B,a}	4.09 ± 0.50^{CDE,a}
10	ND^{A,a}	5.00 ± 0.32^{B,cd}	5.73 ± 0.99^{C,c}	6.05 ± 0.12^{B,b}	5.62 ± 0.47^{B,b}	5.63 ± 1.10^{B,b}	5.67 ± 0.68^{B,c}
12	ND^{A,a}	4.96 ± 0.18^{B,bcd}	4.73 ± 1.40^{B,a}	5.13 ± 3.02^{B,ab}	7.85 ± 0.35^{C,c}	7.71 ± 0.72^{C,c}	7.08 ± 0.63^{C,d}

$^{A–E}$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 ($P > 0.05$).

[1]Values are the means of 3 experimental units (n = 3) ± SD.

[2]Treatments as described in Table 1.

[3]Day 0 is immediately before adding to brine solution. ND = not detected (detection level was ≤1 cfu/g).

Table 8. Recovery of nonstarter lactic acid bacteria ($\log_{10}$ cfu/g) from white-brined cheese made with *Lactobacillus reuteri* during storage[1]

	Storage time (d)						
Treatment[2]	0[3]	1	3	7	14	21	28
1	$6.32 \pm 0.18^{A,ab}$	$6.48 \pm 0.45^{A,bc}$	$6.29 \pm 0.49^{A,ab}$	$6.19 \pm 0.26^{A,bc}$	$6.20 \pm 0.53^{A,b}$	$5.99 \pm 0.53^{A,abcd}$	$6.04 \pm 0.74^{A,abc}$
3	$6.15 \pm 0.27^{A,ab}$	$5.98 \pm 0.27^{A,a}$	$6.16 \pm 0.39^{A,ab}$	$5.88 \pm 0.27^{A,ab}$	$5.81 \pm 0.76^{A,ab}$	$5.64 \pm 0.66^{A,ab}$	$5.66 \pm 0.83^{A,ab}$
4	$6.32 \pm 0.18^{A,ab}$	$6.44 \pm 0.37^{A,bc}$	$6.54 \pm 0.55^{A,bc}$	$6.18 \pm 0.71^{A,bc}$	$6.30 \pm 0.42^{A,b}$	$6.36 \pm 0.60^{A,bcd}$	$6.46 \pm 0.57^{A,bcd}$
6	$6.15 \pm 0.27^{A,ab}$	$6.67 \pm 0.37^{A,c}$	$6.88 \pm 0.41^{A,c}$	$6.60 \pm 1.00^{A,c}$	$6.56 \pm 1.30^{A,b}$	$6.56 \pm 1.21^{A,bcd}$	$7.91 \pm 0.37^{B,e}$
7	$6.29 \pm 0.25^{C,ab}$	$6.16 \pm 0.37^{BC,ab}$	$5.77 \pm 0.35^{AB,a}$	$5.70 \pm 0.25^{AB,ab}$	$5.76 \pm 0.78^{AB,ab}$	$5.72 \pm 0.63^{AB,abc}$	$5.64 \pm 0.39^{A,ab}$
9	$6.08 \pm 0.22^{A,b}$	$5.95 \pm 0.26^{AB,a}$	$5.73 \pm 0.49^{AB,a}$	$5.33 \pm 0.28^{A,a}$	$5.26 \pm 0.91^{A,a}$	$5.26 \pm 0.54^{A,a}$	$5.34 \pm 1.40^{A,a}$
10	$6.29 \pm 0.25^{A,ab}$	$6.20 \pm 0.20^{A,ab}$	$6.34 \pm 0.50^{A,bc}$	$6.25 \pm 0.95^{A,bc}$	$6.25 \pm 1.30^{A,b}$	$6.59 \pm 0.43^{AB,cd}$	$7.11 \pm 0.20^{B,de}$
12	$6.08 \pm 0.22^{A,a}$	$6.34 \pm 0.44^{A,bc}$	$6.43 \pm 1.00^{A,bc}$	$6.49 \pm 0.87^{A,c}$	$7.46 \pm 0.23^{B,c}$	$6.69 \pm 1.67^{AB,d}$	$6.73 \pm 0.72^{AB,cd}$

[A–C]Means from each sampling time in the same row with the same uppercase letters are not significantly different ($P > 0.05$).

[a–e]Means from each sampling time in the same column with the same lowercase letters are not significantly different ($P > 0.05$).

[1]Values are the means of 3 experimental units (n = 3) ± SD.

[2]Treatments as described in Table 1.

[3]Day 0 is immediately before adding to brine solution.

Iranian white cheese made without starter culture but with 8% NaCl during 60 d storage at 4°C (Mohammadi et al., 2009). Furthermore, temperature, pH, and salt content together affected the growth of *E. coli* O157:H7 in cheese (Ozer et al., 2004). The salt content of ~6% (wt/wt) and pH from 4.69 to 5.53 (treatments 4, 5, 6, 10, 11, and 12) may explain the enhanced survival of *E. coli* O157:H7 in cheese stored at 25°C compared with 10°C in the present study. It has been stated that the lethal effect of higher temperatures is enhanced when combined with high salt content in cheese (~20%) and low pH (~4.0; Lekkas et al., 2006). In agreement with our results, Lekkas et al. (2006) found that *E. coli* O157:H7 survived better at 12°C compared with 4°C when combined with low salt concentration (4%) in an artisanal-type Galotyri cheese after 28 d. Similarly, Osaili et al. (2014) reported that the survival of *E. coli* O157:H7 was enhanced in cheese stored at 21°C compared with 10°C, with a maximum salt content of 7.43%.

At 10°C, the numbers of *Lb. reuteri* were slightly reduced ($P > 0.05$) by 1.08 and 0.88 $\log_{10}$ cfu/g when stored in 10% and 15% NaCl brine, respectively, after 28 d (Figure 2A and 3A). Similarly, the viability of *Lb. reuteri* differed insignificantly ($P > 0.05$) at ~6 $\log_{10}$ cfu/g in both brines at 25°C after 28 d (Figure 2B and 3B). The reduction might have been partially due to either the high salt content, which was ~5% (wt/wt), or to the effect of the lower temperature (10°C), which may have caused dehydration of the bacterial cells and then osmotic stress (Gandhi and Shah, 2015; Chen et al., 2019). In contrast with our results, Langa et al. (2018) reported that *Lb. reuteri* numbers were reduced by ~2 log cfu/g in semi-hard cheese stored at 12°C after 30 d. Garde et al. (2016) also noticed a reduction in *Lb. reuteri* numbers in Castellano cheese with glycerol, which reached 3.8 log when stored at 12°C for 30 d. Another study found that *Lb. reuteri* decreased to a level less than 4.5 log in semi-hard cheese containing 100 to 500 m*M* glycerol and stored at 12°C after 30 d (Martín-

Table 9. Recovery of nonstarter lactic acid bacteria ($\log_{10}$ cfu/mL) from brine of cheese made with *Lactobacillus reuteri* during storage[1]

	Storage time (d)						
Treatment[2]	0[3]	1	3	7	14	21	28
1	$ND^{A,a}$	$4.82 \pm 0.27^{BC,ab}$	$4.65 \pm 0.26^{B,a}$	$4.65 \pm 0.17^{B,a}$	5.3 ± 0.75^{Ca}	$5.18 \pm 0.97^{BC,b}$	$6.51 \pm 0.68^{D,b}$
3	$ND^{A,a}$	$4.60 \pm 0.25^{B,a}$	$4.99 \pm 0.15^{B,ab}$	$4.51 \pm 0.99^{B,a}$	$4.62 \pm 1.26^{B,a}$	$4.85 \pm 1.03^{B,ab}$	$4.87 \pm 0.98^{B,a}$
4	$ND^{A,a}$	$5.45 \pm 0.65^{B,cd}$	$5.75 \pm 1.52^{BC,abc}$	$5.01 \pm 1.2^{B,ab}$	$6.48 \pm 0.77^{CD,b}$	$6.73 \pm 0.97^{D,c}$	$6.61 \pm 0.75^{D,b}$
6	$ND^{A,a}$	$5.77 \pm 0.58^{B,d}$	$6.29 \pm 1.74^{B,c}$	$6.25 \pm 1.82^{B,c}$	$7.85 \pm 0.45^{C,d}$	$7.87 \pm 0.41^{C,d}$	$7.88 \pm 0.36^{C,c}$
7	$ND^{A,a}$	$5.00 \pm 0.34^{C,abc}$	$4.74 \pm 0.54^{BC,a}$	$4.29 \pm 0.42^{B,a}$	$4.90 \pm 0.59^{BC,a}$	$5.19 \pm 1.18^{C,b}$	$5.19 \pm 0.99^{C,a}$
9	$ND^{A,a}$	$4.54 \pm 0.37^{B,a}$	$4.72 \pm 0.32^{B,a}$	$4.23 \pm 0.36^{B,a}$	$4.51 \pm 1.06^{B,a}$	$4.19 \pm 0.737^{B,a}$	$4.69 \pm 0.85^{B,a}$
10	$ND^{A,a}$	$5.16 \pm 0.23^{B,bc}$	$6.16 \pm 0.21^{C,bc}$	$6.69 \pm 1.10^{C,c}$	$6.87 \pm 0.42^{C,bc}$	$6.89 \pm 0.89^{C,c}$	$6.52 \pm 1.13^{C,b}$
12	$ND^{A,a}$	$5.29 \pm 0.64^{B,c}$	$5.80 \pm 1.80^{B,abc}$	$6.10 \pm 1.6^{BC,bc}$	$7.55 \pm 0.38^{D,cd}$	$7.39 \pm 0.67^{D,cd}$	$6.93 \pm 0.56^{CD,b}$

[A–D]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 ($P > 0.05$).

[1]Values are the means of 3 experimental units (n = 3) ± SD.

[2]Treatments as described in Table 1.

[3]Day 0 is immediately before adding to brine solution. ND = not detected (detection level was ≤1 cfu/mL).

Table 10. Recovery of yeasts and molds ($\log_{10}$ cfu/mL) from brine of cheese made with *Lactobacillus reuteri* during storage[1]

	Storage time (d)						
Treatment[2]	0[3]	1	3	7	14	21	28
1	ND^{A,a}	4.11 ± 0.06^{B,a}	5.67 ± 0.55^{CD,bcd}	5.43 ± 0.95^{CD,ab}	5.37 ± 0.24^{C,ab}	6.19 ± 0.18^{D,bc}	6.04 ± 0.69^{CD,bc}
3	ND^{A,a}	4.52 ± 0.55^{B,ab}	5.02 ± 0.17^{BC,ab}	5.48 ± 1.20^{BCD,ab}	5.11 ± 1.70^{BC,ab}	5.94 ± 1.40^{CD,bc}	6.36 ± 1.30^{D,bc}
4	ND^{A,a}	6.43 ± 0.03^{C,d}	6.70 ± 0.29^{C,d}	6.29 ± 0.11^{BC,bc}	6.12 ± 0.19^{BC,bc}	6.15 ± 0.83^{BC,bc}	5.63 ± 0.5^{B,b}
6	ND^{A,a}	5.28 ± 0.37^{B,bc}	6.29 ± 0.67^{C,cd}	6.75 ± 1.30^{C,c}	7.17 ± 0.58^{CD,c}	7.04 ± 1.3^{CD,c}	7.9 ± 0.09^{D,d}
7	ND^{A,a}	4.80 ± 0.03^{B,ab}	5.39 ± 0.48^{BC,abc}	5.03 ± 1.10^{B,a}	5.58 ± 0.16^{BC,abc}	6.89 ± 0.35^{CD,bc}	6.46 ± 0.19^{D,bc}
9	ND^{A,a}	4.72 ± 0.69^{C,ab}	4.52 ± 0.47^{C,a}	4.91 ± 0.51^{A,c}	4.19 ± 1.10^{BC,a}	3.64 ± 0.85^{B,a}	4.71 ± 0.98^{C,a}
10	ND^{A,a}	6.57 ± 0.03^{D,d}	6.04 ± 0.34^{BCD,bcd}	6.39 ± 0.21^{CD,bc}	5.41 ± 0.78^{B,ab}	5.75 ± 0.46^{BC,b}	5.89 ± 0.36^{BC,b}
12	ND^{A,a}	5.77 ± 0.25^{B,cd}	6.12 ± 1.09^{B,cd}	6.44 ± 0.57^{B,bc}	6.37 ± 0.03^{B,bc}	6.67 ± 1.41^{B,bc}	6.79 ± 1.03^{B,c}

$^{A–D}$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 ($P > 0.05$).

[1]Values are the means of 3 experimental units (n = 3) ± SD.

[2]Treatments as described in Table 1.

[3]Day 0 is immediately before adding to brine solution. ND = not detected (detection level was ≤1 cfu/mL).

Cabrejas et al., 2017). Chen et al. (2018) reported that *Lb. reuteri* was able to survive well in drinkable yogurt, because *Lb. reuteri* numbers were maintained at ~4 $\log_{10}$ cfu/g in thin yogurt stored at 37°C for 4 wk and ~5 $\log_{10}$ cfu/g in thick yogurt stored at 28°C for 5 mo.

In cheese treatments containing both *Lb. reuteri* and *E. coli* O157:H7, it seems that the bacterial cells interacted with each other. In cheese stored at 10°C, the viability of *E. coli* O157:H7 was reduced ($P < 0.05$) by 1.54 and 2.16 $\log_{10}$ cfu/g in 10% (Figure 2A) or 15% (Figure 3A) NaCl brine, respectively, after 28 d. At 25°C, the numbers of *E. coli* O157:H7 declined ($P < 0.05$) by 2.64 and 1.62 $\log_{10}$ cfu/g when cheeses were stored in 10% (Figure 2B) or 15% (Figure 3B) NaCl brine, respectively, for 28 d. The drop in the viability of *E. coli* O157:H7 suggests that *Lb. reuteri* has strong antimicrobial activity against *E. coli* O157:H7 under those conditions (Figures 2 and 3). This may be due to the quorum-sensing system of *Lb. reuteri*, which stimulates reuterin production in environments with high target population densities (Muthukumarasamy and Holley, 2007). Another possible reason is that increased *Lb. reuteri* numbers mediated higher reuterin production (Doleyres et al., 2005). It is also possible that increased reactivity of reuterin during storage at room temperature might have induced oxidative stress of *E. coli* O157:H7 (Langa et al., 2014; Ortiz-Rivera et al., 2017a). Moreover, many studies have elucidated that salt works synergistically with reuterin and enhances its activity (Langa et al., 2014).

Reuterin activity is concentration dependent, and increasing salt concentrations can have an apparent stimulatory action on this activity at refrigeration temperatures, where reuterin stability is high (Talarico and Dobrogosz, 1989). However, the significant increase in *Lb. reuteri* numbers noted at the end of storage might have been due to the slow growth of *Lb. reuteri* (Lynch et al., 2014) in response to the antagonistic effect of other bacterial cells in a coculture environment (Gao et al., 2019). Enhanced numbers of *Lb. reuteri* in cheese stored at 10°C could have been facilitated by the presence of cold shock proteins (Chen et al., 2019). Moreover, the increase that occurred at 25°C from d 14 onward in both NaCl brines seemed to be due to the presence of compatible solutes that equalize the intracellular and extracellular osmolarities (Gaucher et al., 2019). Moreover, the brine solution that was most suitable for *Lb. reuteri* survival in the current white-brined cheese investigation was 10% (Figure 2). Gaucher et al. (2019) reported that *Lb. reuteri* survival rate was enhanced at 6% NaCl and pH 4.94 to 5.53.

Most previous studies have used either the *Lb. reuteri*-reuterin system or reuterin as a chemical additive in dairy products with storage at 4 to 10°C. However, using the *Lb. reuteri*-reuterin system is more advantageous compared with direct reuterin addition for several reasons. First, *Lb. reuteri* and glycerol are approved to use in food products, whereas reuterin has not been approved yet due to its moderate cytotoxicity (Fernández-Cruz et al., 2016; Martín-Cabrejas et al., 2017). Second, there is concern that the direct addition of reuterin to the food matrix may cause interaction with food components and reduce its biological activity (Garde et al., 2014; Arqués et al., 2015). Also, using *Lb. reuteri* itself enables continuous in situ production of reuterin in the food product in the presence of glycerol (Langa et al., 2014; Garde et al., 2016). Generally, our results regarding *E. coli* O157:H7 viability agree with the findings of other work (Langa et al., 2014; Arqués et al., 2015). However, in contrast to our findings, a previous study has reported reduction in *Lb. reuteri* numbers (Garde et al., 2016).

El-Ziney and Debevere (1998) pointed out that addition of 50 units/g of reuterin to cottage cheese reduced

E. coli O157:H7 by 3.5 $\log_{10}$ cfu/g after 3 wk, whereas increasing reuterin to 150 units resulted in its reduction to a nondetectable level after 7 d storage at 7°C. Furthermore, high-dose (6 $\log_{10}$ cfu/g) and low-dose (3 log cfu/g) inoculation levels for each of *Lb. reuteri* and *E. coli* O157:H7 cells were tested in ground beef, and it was reported that both levels of *E. coli* O157:H7 were completely inhibited by *Lb. reuteri* at different times during storage at 4°C, with the fastest reduction using the higher level of *Lb. reuteri* with the lower level of *E. coli* O157:H7 (Muthukumarasamy et al., 2003). Similar to treatment 9 in this study, a significant decrease of *Lb. reuteri* by ~3 $\log_{10}$ cfu/g was noticed in cheese containing *Lb. reuteri* and glycerol and stored at 14°C for 30 d, which represented total lactobacilli numbers (Garde et al., 2016). Moreover, this system was able to reduce *Clostridium tyrobutyricum* spores to the undetectable limit from d 30 onward (Gómez-Torres et al., 2014). Langa et al. (2018) revealed that a decrease of *Lb. reuteri* numbers by 4.25 log cfu/g was observed when cocultured with *E. coli* O157:H7 and *L. monocytogenes* in semi-hard cheese, which was much higher compared with the reduction observed in treatment 9 in the present study. Simultaneously, *E. coli* O157:H7 numbers were reduced to a nondetectable level from the first week of storage at 12°C, with little effect on *L. monocytogenes*, which was still detected on d 30. The initial increase (~1.5 $\log_{10}$ cfu/g) in the numbers of *E. coli* O157:H7 at d 1 in non–*Lb. reuteri* cheeses (Figure 2 and 3) may have been due to its shorter lag phase in the absence of *Lb. reuteri*.

NSLAB in White-Brined Cheese

Nonstarter LAB (Table 8) are able to survive pasteurization and are considered to be indigenous microflora in most cheese varieties (Garde et al., 2016; Hickey et al., 2018). In general, the enhanced survival of NSLAB especially at 25°C may be due to mesophilic lactobacilli, which are the most abundant NSLAB group, having a growth range of 25 to 30°C (Blaya et al., 2018). It has also been reported that NSLAB exhibit noticeable resistance and ability to adapt to the harsh conditions in cheeses containing high salt concentrations (De Angelis et al., 2002; Garde et al., 2016). In addition, NSLAB can utilize other energy substrates such as free amino acids instead of lactose, which could be depleted with cheese aging (Garde et al., 2016; Blaya et al., 2018). Moreover, previous researchers reported that *Lb. reuteri* did not have a negative effect on the viability of nonstarter lactobacilli (De Angelis et al., 2002; Lynch et al., 2014). Similar to the present results, Garde et al. (2016) observed that nonstarter lactobacilli were increased and the numbers of starter lactococci remained high (~7 log cfu/g) in Castellano cheese containing *Lb. reuteri* stored at 12°C from d 30 onward. By contrast, Martín-Cabrejas et al. (2017) reported that the numbers of starter culture bacteria were reduced by ~6 log cfu/g in semi-hard cheese made with *Lb. reuteri* stored at 12°C after 60 d. Similarly, it was noted that the numbers of starter culture bacteria were reduced by 6.4 log cfu/g in contaminated model cheese containing *Lb. reuteri* stored at 14°C after 60 d (Gómez-Torres et al., 2014). According to the current results, *Lb. reuteri* growth did not interfere with the growth of NSLAB in any of the cheese treatments.

Yeasts and Molds in White-Brined Cheese

Yeasts and molds are potential influencers in white-brined cheese during storage. Moreover, the yeast and mold counts are considered to be quality indicators for the white-brined cheese (Al-Nabulsi et al., 2020). In this study, the numbers of yeasts and molds were not changed in most cheese treatments, because they are acidotolerant, xerotolerant, and psychrotolerant microorganisms (Garnier et al., 2017). However, a reduction of 0.98 $\log_{10}$ cfu/g was found in cheese stored in 15% NaCl brine at 10°C with both *Lb. reuteri* and *E. coli* O157:H7 (Table 10) due to combined stress factors (Garnier et al., 2017). Yeast and mold survival was decreased because the salt content of cheese and the A_w were ~7% and 0.92, respectively, on d 28. Several studies detected high prevalence of yeasts in brined cheeses such as white-brined cheese and brined beaten cheese (Miloradovic et al., 2018). Similar to most treatments in this study, Bao et al. (2019) found that *Lb. reuteri* did not affect the fungi content (mainly *Mocus* spp.) in Furu cheese, a type of tofu cheese, stored at 20°C.

Microbial Survival in the Brine

In the present study, *Lb. reuteri* grew well in the brine in the same manner as cheese, but at lower levels, reaching ≤7.9 $\log_{10}$ cfu/mL. Likewise, *E. coli* O157:H7 survived in the brine, but in lower numbers at 10°C, and in 15% NaCl brine in the presence of *Lb. reuteri*, with numbers reaching ≤6.9 $\log_{10}$ cfu/mL and reductions of 0.1 to 4.4 after 28-d storage. This may have been caused by continuous diffusion of all components between the cheese matrix and its brine (Ayyash et al., 2013). The *E. coli* O157:H7 cells were found in both white cheese (≤2.0 $\log_{10}$ cfu/g) and brine (≤1.3 $\log_{10}$ cfu/mL). However, *E. coli* O157:H7 was not detected in 15% NaCl brine at 25°C by 28 d, perhaps due to the synergistic effects of both high salt concentration and high temperature as well as the lack of any protective components in the brine, such as fat and protein (Osaili

et al., 2014). In agreement with the present findings, Ingham et al. (2000) reported that *E. coli* O157:H7 survived in model brine (23% NaCl) stored at 8°C or 15°C for 28 d and in commercial brine (22.1, 28.0, or 29.3% NaCl) stored at 4°C or 13°C for 35 d. Osaili et al. (2014) also found that *E. coli* O157:H7 was able to survive in either 10% or 15% NaCl brine at 21°C or 10°C for 28 d, regardless of the presence of starter culture.

CONCLUSIONS

Exposure to 10% or 15% NaCl brine at 10°C or 25°C for 28 d did not affect *E. coli* O157:H7 survival in white-brined cheese. In general, lower numbers of *E. coli* O157:H7 were observed at 10°C compared with 25°C, with an additional reduction when *Lb. reuteri* was added. Greater reduction of *E. coli* O157:H7 numbers by 2.6 $\log_{10}$ cfu/g was observed in cheese with *Lb. reuteri* stored in 10% NaCl brine at 25°C for 28 d. At 10°C, the NSLAB and *Lb. reuteri* counts remained constant or slightly decreased, respectively, in white-brined cheese, but significantly increased or remained constant, respectively, at 25°C by 28 d. Survival of *E. coli* O157:H7 in white-brined cheese can be controlled by optimizing different parameters, such as NaCl concentration in the brine, temperature, and presence of *Lb. reuteri*.

ACKNOWLEDGMENTS

The authors thank Jordan University of Science and Technology (grant no. 167/2017) and the United Arab Emirates University (grant no. 31F101). The authors have not stated any conflicts of interest.

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