ABSTRACT

In addition to its nutritional and therapeutic properties, camel milk has the ability to suppress the growth of a wide range of foodborne pathogens, but there is a lack of information regarding the behavior of these pathogens in products such as yogurt produced from camel milk. The objective of the current study was to investigate the behavior of *Listeria monocytogenes* and *Escherichia coli* O157:H7 during manufacture and storage of camel yogurt. Camel milk inoculated with *L. monocytogenes* and *E. coli* O157:H7 was fermented at 43°C for 5 h using freeze-dried lactic acid bacteria (LAB) starter cultures (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) and stored at 4 or 10°C for 14 d. Camel milk inoculated with *L. monocytogenes* and *E. coli* O157:H7 without starter culture was also prepared. During fermentation, the numbers of *L. monocytogenes* and *E. coli* O157:H7 increased 0.3 and 1.6 log cfu/mL, respectively, in the presence of LAB, and by 0.3 and 2.7 log cfu/mL in the absence of LAB. During storage at 4 or 10°C, *L. monocytogenes* increased 0.8 to 1.2 log cfu/mL by 14 d in camel milk without LAB, but in the presence of LAB, the numbers of *L. monocytogenes* were reduced by 1.2 to 1.7 log cfu/mL by 14 d. Further, *E. coli* O157:H7 numbers in camel milk were reduced by 3.4 to 3.5 log cfu/mL in the absence of LAB, but *E. coli* O157:H7 was not detected (6.3 log cfu/mL reduction) by 7 d in camel yogurt made with LAB and stored at either temperature. Although camel milk contains high concentrations of natural antimicrobials, *L. monocytogenes* was able to tolerate these compounds in camel yogurt stored at refrigerator temperatures. Therefore, appropriate care should be taken during production of yogurt from camel milk to minimize the potential for postprocess contamination by this and other foodborne pathogens.

**Key words:** *Listeria monocytogenes*, *Escherichia coli* O157:H7, lactic acid bacteria, camel milk, yogurt

INTRODUCTION

*Escherichia coli* O157:H7 is a gram-negative, rod-shaped, facultative anaerobic bacterium and is the most significant enterohemorrhagic *E. coli* serotype in relation to public health. *Escherichia coli* O157:H7 produces Shiga-like toxin(s) that may cause abdominal cramps and diarrhea that can progress through invasive infection to hemorrhagic colitis, hemolytic uremic syndrome (HUS), and death (WHO, 2011). *Listeria monocytogenes* is a gram-positive, rod-shaped, facultative anaerobic bacterium that has been linked to foodborne illness outbreaks with an unusually high mortality rate of 20% (McLauchlin et al., 2004). *Escherichia coli* O157:H7 and *L. monocytogenes*, as well as other pathogenic bacteria, have been isolated from different types of milk, including milk from camels (Al All et al., 2012).

Camel milk can play the same fundamental role in the human diet as bovine milk because they contain similar amounts of the same vital nutrients (Al Haj and Al Kanhal, 2010). Camel milk is estimated to contain on average 2.9 to 5.8% lactose, 2.5 to 4.5% protein, 2.9 to 5.5% fat, 0.35 to 0.90% ash, 86.3 to 88.5% water, and 8.9 to 14.3% nonfat solids (Hashim et al. 2009). It is also reported that camel milk provides potential therapeutic properties by acting in an antidiabetic, antihypertensive, or anticarcinogenic manner (Magjeed, 2005; Agrawal et al., 2007; Quan et al., 2008). Furthermore, children who are allergic to bovine milk can...
safely consume camel milk because the 2 types of milk proteins do not antigenically cross-react (El-Agamy et al., 2009). Traditionally, fresh and fermented products of camel milk have been used for the treatment of many diseases including asthma, leishmaniasis, tuberculosis, edema (dropsy), and jaundice (Abdelgadir et al., 1998).

Camel milk is inhibitory toward both gram-negative (Salmonella Typhimurium, Escherichia coli) and gram-positive (Staphylococcus aureus, Listeria monocytogenes) bacteria (El-Agamy et al., 1992; Benkerroum et al., 2004). The ability of camel milk to inhibit these foodborne pathogens is largely due to its high concentrations of antimicrobial compounds such as immunoglobulins, lactoferrin (LF), and lysozyme compared with buffalo or other types of milk (Kappeler et al., 1991; El-Agamy, 2000; Konuspayeva et al., 2007). The concentration of immunoglobulins in raw camel milk (2.23 mg/mL) is 4 to 6 times higher than in cow and buffalo milk, respectively, whereas the LF concentration (0.17 mg/mL) is 2 and 6 times higher than that of cow and buffalo milk, respectively. Similarly, the concentration of lysozyme (1.32 µg/mL) is 4.9 and 11 times higher than in cow and buffalo milk, respectively (El-Agamy, 2000). Although the presence of antimicrobial compounds in camel milk has been studied, information about the fate of foodborne pathogens in yogurt produced from camel milk is lacking.

Processing during pasteurization at elevated temperatures (85–90°C), as well as the biochemical changes and microbial interactions that take place during milk fermentation, may be of concern because they can influence the structural stability of antimicrobials found naturally in camel milk. Paulsson et al. (1993) reported that the thermal stability of LF in water was dependent on the degree of iron saturation of LF. They found that native LF displayed 2 denaturation temperatures, 65 and 92°C, whereas apo-LF (iron-free) denatured at 71°C and halo-LF (iron-saturated) denatured at 93°C. Furthermore, they found that pasteurization did not influence the ability of native and iron-saturated LF to attach to the surface of various bacterial species and reduce their viability, whereas UHT treatment decreased ability of LF to attach and it consequently lost its antibacterial activity. Additionally, Abe et al. (1991) found that LF at neutral and basic pH was more susceptible to thermal denaturation, whereas its stability toward heating was higher at low pH. Therefore, mild heat treatments (pasteurization) that are normally used in the production of yogurt might affect the stability of the tertiary structure of some of the natural antimicrobials in camel milk, causing a reduction in their antibacterial activity. However, El-Agamy (2000) reported that camel milk proteins were more resistant to structural change during pasteurization than those present in cow or buffalo milk, and that these proteins may show antimicrobial activity against pathogens in fermented camel milk products. Therefore, the objective of the current study was to investigate the behavior of lactic acid bacteria (LAB), E. coli O157:H7, and L. monocytogenes during processing and storage of yogurt produced from camel milk and stored at 4 and 10°C.

**MATERIALS AND METHODS**

**Bacterial Strains and Culture Media**

Four mutated nonpathogenic (verotoxigenic-negative) E. coli O157:H7 strains 02-0628, 02-0304, 02-0627, and 00-3581 were obtained from Rafiq Ahmed (National Microbiology Laboratory, Public Health Agency, Canadian Science Centre for Human and Animal Health, Winnipeg, MB). The E. coli O157:H7 strains were kept individually at −40°C in brain heart infusion (BHI, Oxoid Ltd., Basingstoke, UK) broth containing 20% glycerol. Three culture transfers were performed to resuscitate each culture in BHI broth at 37°C for 24 h to reach the stationary phase before inoculation into yogurt. Then, 1 mL from each culture was pooled in a 10-mL test tube and mixed to obtain an approximately equal number of cells of each strain in the mixed culture. The mixed cocktail cultures were diluted using 0.1% (wt/vol) peptone water (Oxoid Ltd.) to give a final concentration of about 3 log_{10} cfu/mL in the inoculated camel milk.

Three strains of L. monocytogenes were used in the current study: L. monocytogenes ATCC 7644 (Micro-BioLogics Inc., St Cloud, MN) and 2 L. monocytogenes isolates from processed meat (Al-Nabulsi et al., 2015[AU2: 2015a or 2015b?]) and white brined cheese (Osaiil et al., 2012), respectively, in Jordan. These isolates were obtained from the Department of Nutrition and Food Technology at Jordan University of Science and Technology and kept frozen (−40°C) in glycerol. After resuscitation of cultures as previously described, 1 mL from each culture was pooled in a 10-mL test tube and mixed to obtain an approximately equal number of cells of each strain in the mixed culture. The mixed cocktail cultures were diluted using 0.1% peptone water to give a final concentration of about 3 log_{10} cfu/mL in the inoculated camel milk.

**Starter Culture**

Freeze-dried LAB starter culture (direct vat set, Yo-FlexX-11) containing Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus used in
fermentation and storage of camel yogurt

Yogurt Production

Yogurt made of camel milk was produced according to Hashim et al. (2009) with minor modifications. Camel milk used in this study was obtained on the day of experiments from a local farm (Al-Mafraq, Jordan). The raw milk was analyzed for \textit{E. coli\textsubscript{O157:H7}} and \textit{L. monocytogenes} before inoculation tests, and all samples were negative for both pathogens. Camel milk (2 L) was batch pasteurized at 85°C for 3 min, and then cooled immediately in a water bath to 43°C. After that, the pasteurized camel milk was divided into 4 portions (500 mL each) and poured into presterilized 1-L glass beakers covered with aluminum foil. A 1-mL sample of cocktail culture of \textit{E. coli\textsubscript{O157:H7}} or \textit{L. monocytogenes} was added to the 4 portions to yield 3 log\textsubscript{10} cfu/mL milk. Then, 1% (wt/vol) of freeze-dried yogurt starter culture (LAB) was added to 2 portions and then stirred for 2 min, and the other 2 portions of milk, inoculated with \textit{E. coli\textsubscript{O157:H7}} or \textit{L. monocytogenes}, were prepared without the addition of LAB as a control to measure the effect of LAB on viability of \textit{E. coli\textsubscript{O157:H7}} or \textit{L. monocytogenes} and to monitor pH changes during fermentation and storage (Osaili et al., 2013). The experimental design for this study is presented in Table 1. Afterward, 50 g of the resulting inoculated milk, with or without LAB, was poured into 125-mL sterile plastic cups, covered with lids, and incubated at 43°C for 4.5 to 5.5 h until the pH of the milk reached 4.6 ± 0.1 in samples inoculated with LAB. Pasteurized camel milk and fermented milk (yogurt) was then cooled to 4 or 10°C within 2 h and then stored for 14 d.

Sampling and pH Measurement

Pasteurized camel milk and fermented milk (yogurt) were sampled at 0, 1, 3, and 5 h during fermentation and at 0, 1, 3, 7, and 14 d during storage at 4 or 10°C for \textit{E. coli\textsubscript{O157:H7}}, \textit{L. monocytogenes}, and LAB, as well as for determination of pH (Osaili et al., 2013). At specified time intervals, a 5-g sample was withdrawn and diluted in 45 mL of 0.1% peptone water and homogenized in a sterile stomacher bag for 2 min with a Stomacher blender. Numbers of \textit{E. coli\textsubscript{O157:H7}} were determined by surface plating 100 µL in duplicate on the surface of MacConkey sorbitol agar (Oxoid Ltd.). After aerobic incubation at 37°C for 24 h, typical colorless colonies of \textit{E. coli\textsubscript{O157:H7}} were enumerated. Numbers of \textit{L. monocytogenes} were determined by surface plating 100 µL in duplicate on the surface of \textit{Listeria} selective PALCAM (Oxoid Ltd.) medium base containing the antimicrobial supplement. After incubating aerobically at 37°C for 24 h, typical black colonies were enumerated. Lactic acid bacteria were enumerated by surface plating 100 µL in duplicate on the surface of de Man, Rogosa, and Sharpe agar (MRS, Oxoid Ltd.). The plates were incubated anaerobically (AnaeroGen, Oxoid Ltd.) at 37°C for 48 to 72 h for LAB. To detect the presence of damaged \textit{L. monocytogenes} or \textit{E. coli\textsubscript{O157:H7}} cells at the end of fermentation and storage, samples from appropriate dilutions were directly plated on the surface of PALCAM agar overlaid with tryptic soy agar or on the surface of MacConkey sorbitol agar overlaid with tryptic soy agar, respectively (Osaili et al., 2010). The pH values of milk or yogurt samples were measured by immersing the electrode of a pH meter (Cyberscan 500, Eutech Instruments, Singapore) directly in the samples.

Statistical Analysis

All data were analyzed using SAS software (version 8.1; SAS Institute Inc., Cary, NC). Analysis of variance by the general linear model (GLM) and Duncan’s multiple range tests were used to find significant differences ($P < 0.05$) among treatments. Each value is the average of 3 experiments ($n = 6$).

Table 1. Experimental design for fermented camel milk manufacture

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Treatment</th>
<th>Starter culture</th>
<th>Fermentation temperature (°C)</th>
<th>Storage temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Escherichia coli\textsubscript{O157:H7}}</td>
<td>1</td>
<td>−</td>
<td>43</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>43</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>−</td>
<td>43</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+</td>
<td>43</td>
<td>10</td>
</tr>
<tr>
<td>\textit{Listeria monocytogenes}</td>
<td>1</td>
<td>−</td>
<td>43</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>43</td>
<td>4</td>
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<td>3</td>
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<td></td>
<td>4</td>
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</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Properties of Yogurt

The textural parameters viscosity, firmness, cohesiveness, adhesiveness, creaminess, and syneresis are the most common descriptors used to characterize the perception of yogurt (Harte et al., 2007[AU3: Add to Refs]). However, unlike other ruminant milk, camel milk does not coagulate well during fermentation to produce a firm gel-like structure (Shori et al., 2013; Al-Zoreky and Al-Otaibi, 2015). In the current study, the camel yogurt produced was soft and had a fragile heterogeneous structure. Others have also reported that the fermentation of camel milk by starter cultures did not yield firm curds (Abu-Tarboush, 1994; Attia et al., 2001; Abdel Rahman et al., 2009). To overcome this problem, Hashim et al. (2009) used gelatin, alginate, and calcium or their combination with some success and improved the firmness, appearance, smoothness, and overall acceptability ratings of plain and flavored camel yogurt. However, Al-Zoreky and Al-Otaibi (2015) had limited success in improving the firmness of camel milk yogurt using these ingredients plus other proteins or hydrocolloids. The best combination they found to minimize syneresis was 0.6% alginate plus 0.06% CaCl₂.

Changes in pH of Camel Yogurt During Fermentation and Storage

The pH values of camel milk inoculated with *L. monocytogenes* and *E. coli O157:H7* fermented for 5 h at 43°C and then stored at 4°C or 10°C with or without LAB are shown in Figure 1A and 1B. During fermentation, the pH values of camel milk inoculated with LAB decreased slightly during the first 3 h to reach 5.7 to 5.8 and this was followed by a rapid decrease to 4.5 at the end of the period allowed for fermentation. The initial delay may have been due to the presence in camel milk of higher concentrations of natural antimicrobial peptides such as LF, lysozyme, and lactoperoxidase. These peptides may have reduced lactic acid production by the starters and caused an early decline in their viability during yogurt manufacture (Attia et al., 2001). In contrast, the pH values of the camel milk inoculated with only *L. monocytogenes* or *E. coli O157:H7* remained constant during the fermentation period, and this was attributed to the lack of lactic acid production. During storage at 4°C or 10°C, the pH values of camel yogurt inoculated with LAB plus *L. monocytogenes* or *E. coli O157:H7* were further reduced (*P < 0.05*) and reached ≤4.2 by 14 d. In the absence of LAB but with the pathogens, camel milk pH increased slightly (*P > 0.05*) from 6.2 during storage to reach 6.5 to 6.9. It is apparent that storage temperature had an insignificant effect on the pH values of camel milk with or without LAB (Figure 1A and 1B).

Behavior of LAB During Fermentation and Storage of Camel Yogurt

Lactic acid bacteria are the dominant component of the microflora in fermented camel milk (Sulieman et al., 2006; Abdel Rahman et al., 2009). In the current study, behavior of the LAB (*S. thermophilus* and *Lb. bulgaricus*) during manufacture and storage of camel yogurt in the presence of *E. coli O157:H7* and *L. monocytogenes* is shown in Figure 2A and 2B. During fermentation and cooling periods, LAB numbers progressively increased in the presence of the pathogens and were about 1.3 log₁₀ cfu/mL higher when the yogurt was cooled to the desired storage temperature. Similarly, Abdel Rahman et al. (2009) found that counts of LAB increased by 2.3 to 3.5 log₁₀ cfu/mL in camel milk during 6 h of fermentation at 43°C. We noted that the reduction of LAB was significantly (*P < 0.05*) greater during storage at 10°C than at 4°C in the presence of *L. monocytogenes* (Figure 2A), whereas we observed no significant difference in the viability of LAB in the presence of *E. coli O157:H7* at either temperature. Numbers of LAB were reduced 0.6 to 1.2 log₁₀ cfu/mL after 14 d of storage at 10°C in the presence of *L. monocytogenes* compared with at 4°C (Figure 2A). In the presence of *E. coli O157:H7*, numbers of LAB gradually increased by 0.4 to 1.2 log₁₀ cfu/mL up to 7 d at 4 or 10°C, and this was followed by a reduction of ≤1.3 log₁₀ cfu/mL by 14 d (Figure 2B). Like LAB, *L. monocytogenes* can grow at refrigeration temperatures but *E. coli O157:H7* cannot. It is possible that competition between *L. monocytogenes* and LAB for available nutrients reduced the numbers of LAB during storage (Wimpfheimer et al., 1990).

Behavior of *L. monocytogenes* During Fermentation and Storage of Camel Yogurt

Dairy products have been linked to several human outbreaks of illness caused by *L. monocytogenes* (CDC, 2014). In general, *L. monocytogenes* has the ability to grow under a range of environmental stresses and harsh conditions that may involve fermentation to low pH (Al-Nabulsi et al., 2015[AU4: 2015a or 2015b?]). The fate of *L. monocytogenes* during fermentation (5 h), cooling (2 h), and storage (14 d) of camel yogurt in the presence or absence of LAB is shown in Figures 3A and 3B. No damaged *L. monocytogenes* cells were found on agar overlay plates. During fermentation and cooling,
the counts of \( L. \text{monocytogenes} \) in camel milk were not different in the presence or absence of LAB, and an increase of 0.3 to 1.0 log_{10} cfu/mL in the viability of \( L. \text{monocytogenes} \) was noted during storage at 4°C (Figure 3). On the other hand, by 14 d, \( L. \text{monocytogenes} \) viability was significantly \((P < 0.05)\) reduced 1.2 to 1.7 log_{10} cfu in the presence of LAB at 4 or 10°C. However, in the absence of LAB, \( L. \text{monocytogenes} \) grew by 0.8 to 1.2 log_{10} cfu/mL within 14 d. Benkerroum et al. (2004) reported that \( L. \text{monocytogenes} \) numbers did not change in raw camel milk stored at 4 or 20°C during a 48-h period. In the current study, a major reduction in \( L. \text{monocytogenes} \) numbers was observed during the first 24 h of storage, which might be attributed to the antimicrobial effects of LAB against \( L. \text{monocytogenes} \) (Cleveland et al., 2001; Osaili et al., 2014). Why this did not occur during fermentation is unclear but it may be related to pH. Thus, the reduction in viability of \( L. \text{monocytogenes} \) may have been due to the reduced pH or bacteriocins, which are able to suppress the growth of \( L. \text{monocytogenes} \) and are known to be produced by LAB (Cleveland et al., 2001; Osaili et al., 2014). Al-Otaibi and El-Demerdash (2013) found that, in camel milk fermented by \( S. \text{thermophilus} \) and \( Lb. \text{bulgaricus} \) at 37°C for 12 h, the total aerobic count was reduced by 1.4 log cfu/mL after 21 h at 4°C. It seems that the antimicrobial activity of LAB against \( L. \text{monocytogenes} \) was not affected by storage temperature because the

\[ \text{Figure 1. Changes in pH values of fermented camel milk inoculated with Listeria monocytogenes (A) or Escherichia coli O157:H7 (B) in the absence (–––) or presence (-----) of lactic acid bacteria (LAB) during fermentation, cooling, and storage at 4°C or 10°C.} \]
difference between *L. monocytogenes* numbers at 4 or 10°C was not significant. Moreover, the activity of thermostable natural antimicrobials present in camel milk such as lactoferrin (Abe et al., 1991; Paulsson et al., 1993; El-Agamy, 2000) may also contribute to the reduction in viability of *L. monocytogenes*.

**Behavior of *E. coli* O157:H7 During Fermentation and Storage of Camel Yogurt**

Serious illness outbreaks have been reported from consumption of dairy products, including cheeses and yogurt manufactured from cow and sheep milk.

![Behavior of lactic acid bacteria in fermented camel milk incubated at 43°C in the presence of *Listeria monocytogenes* (A) or *Escherichia coli* O157:H7 (B) for 5 h and stored at 4°C (■) or 10°C (▲) for 14 d. [AU8: For Figs 2 to 4, what do error bars indicate?]
contaminated with *E. coli* O157:H7 (WHO, 2011). The fate of *E. coli* O157:H7 in camel yogurt during fermentation and storage in the presence or absence of LAB is shown in Figures 4A and 4B. Counts of *E. coli* O157:H7 increased by 1.6 and 2.7 log_{10} cfu/mL, respectively, in the presence and absence of LAB at the end of fermentation at 43°C. No significant changes were observed during cooling. However, the viability of *E. coli* O157:H7 was reduced significantly (*P* < 0.05) with or without LAB, but reductions were greater (*P* < 0.05) in the presence of LAB during storage at 4 or 10°C. The numbers of *E. coli* O157:H7 in camel yogurt were reduced by 3.4 to 3.5 log_{10} cfu/mL in the absence of LAB at 4 or 10°C. No injured *E. coli* O157:H7 cells (<3 cfu/mL) were found on agar overlay plates at the end of storage. It is worth noting that Benkerroum et al. (2004) reported that numbers of *E. coli* O78:K80 did not change in raw or pasteurized camel milk stored at 4°C for 48 h. In the present work, after 7 d, *E. coli* O157:H7 cells were not detected in camel yogurt at either temperature in the presence of LAB. The reduction of *E. coli* O157:H7 in the presence of LAB may be

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**Figure 3.** Behavior of *Listeria monocytogenes* in fermented camel milk incubated at 43°C in the absence (■) or presence (▲) of lactic acid bacteria for 5 h and stored at 4°C (A) or 10°C (B) for 14 d.
attributed to several factors, including the inhibitory effects of proteins present in camel milk, reduction of yogurt pH, influence of storage temperature, and the production of antimicrobial compounds besides acid by LAB. The inhibitory effects of proteins present in camel milk may also have reduced the viability of *Escherichia coli* O157:H7 in the absence of LAB in camel milk (at a pH value of 6.9). It has been reported that *E. coli* O157:H7 is able to grow in pasteurized cow milk at 12°C (Arias et al., 2001). However, camel milk contains significantly higher concentrations of whey proteins, including lysozyme, LF, and IgG than cow or buffalo milk (Elagamy, 2000). Furthermore, these proteins in camel milk are markedly more heat resistant than their

Figure 4. Behavior of *Escherichia coli* O157:H7 in fermented camel milk incubated at 43°C in the absence (■) or presence (▲) of lactic acid bacteria for 5 h and stored at 4°C (A) or 10°C (B) for 14 d.
counterparts in cow or buffalo milk, although the heat treatment may destroy part of the whey proteins in camel milk (Elagamy, 2000).

CONCLUSIONS

The viability of *L. monocytogenes* was not affected during fermentation of camel milk at 43°C for 5 h by LAB but it was significantly reduced during storage at 4 or 10°C. Although the viability of *E. coli* O157:H7 significantly increased during fermentation at 43°C, numbers were lower than the detection limit after 7 d of storage at 4 or 10°C in the presence of LAB. In the absence of LAB, *L. monocytogenes* numbers gradually increased during fermentation and storage of camel milk but were also inadequately controlled in the presence of LAB. The antimicrobial activity of proteins present in camel milk such as lysozyme, LF, and IgG and their ability to resist the pasteurization process may have contributed, in part, to the observed reduction in viability of *E. coli* O157:H7 and *L. monocytogenes*. In addition, numbers of LAB in camel yogurt remained ≥6.0 log$_{10}$ cfu/mL during fermentation and storage, which may have reduced the numbers of *E. coli* O157:H7 and *L. monocytogenes* by reduction of yogurt pH, production of antimicrobial bacteriocins, or both.

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