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Research article

The characterization of *Lactobacillus* strains in camel and bovine milk during fermentation: A comparison study

Omar A. Alhaj [a,*], Zeineb Jrad [b], Olfa Oussaief [b], Haitham A. Jahrami [c,d], Leena Ahmad [a], Mohammad A. Alshuniaber [e], Bhavbhuti M. Mehta [f]

[a] *Department of Nutrition, Faculty of Pharmacy and Medical Sciences, University of Petra, Amman, 11196, Jordan*
[b] *Livestock and Wildlife Laboratory, Arid Regions Institute (IRA) Médenine, University of Gabes, Gabes, 4100, Tunisia*
[c] *Government Hospitals, Manama, 329, Bahrain*
[d] *College of Medicine and Health Sciences, Arabian Gulf University, Manama, 329, Bahrain*
[e] *Department of Food Sciences and Nutrition, College of Food and Agriculture Sciences, King Saud University, Riyadh, 11451, Saudi Arabia*
[f] *Dairy Chemistry Department, SMC College of Dairy Science, Kamdhenu University, Anand, Gujarat, India*

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ABSTRACT

This study aims to compare the characterization of three *Lactobacillus* strains (*L. helveticus*, *L. acidophilus*, and *L. paracasei* subsp. *paracasei*) in camel milk and bovine milk during fermentation. Our finding showed that the average total viable counts of all three *Lactobacilli* strains in both milk types reached more than 7.0 log CFU/mL after 16 h of fermentation and continued to increase significantly ($p < 0.05$) as fermentation increased, which is according to the FAO and WHO, higher than the minimum recommended daily probiotic dose to provide the potential health benefits. The total count of *L. paracasei* subsp. *paracasei* was greater in fermented camel and bovine milk (8.76 and 8.98 log CFU/mL, respectively) compared to *L. helveticus*, and *L. acidophilus*. The *L. helveticus* exhibited the highest significant ($p < 0.05$) acidifying ability for both camel and bovine milk; on the other hand, *L. paracasei* subsp. *paracasei* revealed the highest significant ($p < 0.05$) pH in both milk. The *L. acidophilus* strain exhibited significantly ($p < 0.05$) the highest levels of free amino acids groups (FAAGs) among other tested strains in camel milk. It is concluded that the growth, viability, and proteolytic activity of three *Lactobacilli* strains were found to be mainly dependent on incubation time, strain, and type of milk.

1. Introduction

Camel milk contains the essential nutrients necessary for human health. The gross composition (fat, protein, lactose and ash) of camel milk is approximately comparable to that reported for bovine milk [1]. However, camel milk has varied calorie count by different species, country, season [2] and some compositional differences, including; higher levels of unsaturated fatty acids, iron, and vitamin C compared to bovine milk [3]. Camel milk is popular in many regions, especially in arid and desert countries, and is consumed as a fresh or fermented product due to its higher nutritive value and many health benefits [4] or even to contribute in solving severe

* Corresponding author.
E-mail addresses: omar.alhaj@uop.edu.jo (O.A. Alhaj), jradzeineb@yahoo.fr (Z. Jrad), olfa.loussaief@hotmail.fr (O. Oussaief), hjahrami@health.gov.bh (H.A. Jahrami), leena.ahmad@uop.edu.jo (L. Ahmad), malshuniaber@ksu.edu.sa (M.A. Alshuniaber), bhavbhuti5@yahoo.co.in (B.M. Mehta).

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acute malnutrition [5]. Moreover, fermented milk products have relatively higher shelf life as compared to that of milk. Interestingly, camel milk has been proven to possess medicinal properties, including anti-inflammatory, anti-cancer, anti-diabetic, anti-cholesterol, and antihypertensive potential [6]. Hence, camel milk products can be deliberated as one of the potential alternative source to bovine milk [7] and represent a major part of the diet, especially for people living in arid and urban areas such as Africa and Asia [8].

Raw camel milk was found to have natural antimicrobial compounds includes lactoperoxidase, lysozyme, lactoferrin, and immunoglobulins [9] which make it the most fermentation-resistant type of milk even in the worst hygiene conditions. In fact, fermentation is among the oldest methods of food preservation worldwide. Traditional fermented camel milk products are made in several countries with various denominations, like *Susac* in Kenya, *Gariss* in Sudan, *Shubat* in Kazakhstan [10]. These fermented products are found to have a positive health claim. The traditional method of fermentation (which occurs by allowing milk to ferment spontaneously in ambient temperature for one to two days until its autochthonous lactic acid bacteria (LAB) causes it to turn sour) involves complex microflora in the products that affect batch-to-batch variation [11]. The controlled fermentation using selected lactic acid bacteria (LAB) can lead to safer product with uniform quality.

LAB are known for their health claims and industrial potential. Several studies showed that selected LAB fermentation of camel and bovine milk increases its nutritional, sensory, and therapeutic properties [12–14]. Probiotic fermented milk is prepared via lactic acid fermentation using probiotic starter cultures. Probiotic LAB provides health benefits since they can avoid or reduce the symptoms of antibiotic-associated diarrhea, inflammatory bowel disease, constipation, hypertension, and diabetes. In addition, LAB releases several substances like organic acid and bioactive peptides during fermentation, which increase the product's shelf-life and health benefits [7, 15].

In fact, probiotics are added to dairy products, for instance, cheese and yogurt to enhance flavor and aroma and support human gut bacteria [16]. For example, *Lactobacillus* is a probiotic genus that produces anticarcinogenic and antibacterial compounds as well as organic acids for instance lactic acid, benzoic acid, and acetic acid. At the same time, bacteriocins, antimicrobial peptides that effectively suppress the growth of specific foodborne pathogens in addition to spoilage bacteria, are also defining properties of these bacteria. The selected three *Lactobacillus* strains (*L. helveticus*, *L. acidophilus*, and *L. paracasei* subsp. *paracasei*) were chosen in this study due to their commercial uses as health-promoting providers in fermented foods and frequent uses as dietary supplements. *Lactobacillus helveticus* is often used as a starting culture in numerous fermentations, particularly dairy product fermentation. *Lactobacillus* spp. are deliberated among the probiotic LAB strains. Several *Lactobacillus* strains are able to release encrypted bioactive peptides from primary structures of camel milk proteins [17]. *Lactobacillus helveticus* has strong proteolytic activity and is also an effective inhibitor of angiotensin-converting enzyme, which plays a role in hypertension relief [18,19]. Likewise, Solanki et al. [20] reported that antioxidant and ACE-inhibitory peptides could be released during fermentation of camel milk by *Lactobacillus helveticus* and *Lactobacillus acidophilus*, respectively. *Lactobacillus acidophilus* strain is a bacteriocin-producing, homo-fermentative, microaerophilic, gram-positive bacterium having rod morphology [21].

Furthermore, *in vitro* and *in vivo* investigations have found some *Lactobacillus* strains, for example, *L. paracasei* subsp. *paracasei* regulates molecules implicated in humoral and cell-mediated immune responses. Moreover, human studies have shown that fermented dairy products and traditional yogurt cultures contain *L. paracasei* subsp. *paracasei* play an essential role in regulating immunological function and immune responses [22]. In another study, Abdel-Hamid et al. [13] reported that the fermentation of camel milk by *Lactobacillus casei* resulted in the release of several bioactive peptides that provide health benefits, such as ACE-inhibitory, antioxidant, and anti-cancer peptides [23–25]. The potential health claims in milk were found to mainly depend on incubation time and strain [26]. The growth, viability, and proteolytic activity of three *Lactobacilli* strains in the current study were found to be mainly dependent on incubation time, strain and types of milk. This is because lactic acid bacteria required variable amounts of nutrition in particular amino acids during different growth stages, which depend on the liberation of free NH_3 groups that varied with the incubation conditions [27]. Therefore, the aim of this study was to investigate the characterization of three *Lactobacillus* strains (*L. helveticus, L. acidophilus and L. paracasei* subsp. *paracasei*) in camel milk during fermentation compared to bovine milk. On the other hand, before being transferred to an industrial scale, probiotic cultures of LAB should be tested for their suitability to obtain camel milk fermented products with acceptable sensory characteristics.

2. Materials and methods

2.1. Lactobacillus culture preparation

In this research, three *Lactobacilli* strains were cultured from freeze-dried strains, *Lactobacillus paracasei* subsp. *paracasei* (ATCC 334T) obtained from the American Type Culture Collection center (ATCC) located in Saint Cloud, Minnesota, USA. The strains *Lactobacillus helveticus* (LMG11445) and *Lactobacillus acidophilus* (LMG11430) were obtained from Belgian coordinated collections of microorganisms (BCCM-LMG). Each *Lactobacilli* strain was anaerobically activated individually for up to 24 h at 40 °C in sterile 10 mL MRS broth (Oxoid, Hampshire, England, UK) and repeated three times for each strain [4]. Thereafter, an active overnight culture (1 mL) of each strain was inoculated in 100 mL of sterile skim camel/bovine milk, to obtain 10^6-10^8 colony-forming units (CFU)/mL with a potential *Lactobacilli* strains, milk was incubated for 48 h at 40 °C for successive transfers.

2.2. Preparation of fermented camel and bovine milk

Whole camel and bovine fresh morning milk samples were obtained separately on May from five randomly healthy lactating female animals from private farms located in Saudi Arabia (central region). On the farm, animals were fed essentially the same diet consisting

of hay, and mixed grain concentrate, and fresh-cut alfalfa grass. Camel milk was collected from Majaheim breed (one humped), and bovine milk was collected from Holstein breed during the same stage of lactation (12–13 weeks). Fresh milk samples were kept in an icebox at 4 °C then directly delivered to the laboratory. The milk fat of both samples was separated using a separator (Friedberg, Germany). According to the modified procedure used by Alhaj et al. [28], Arnold steam steriliser method (1882) [29] was followed to sterilize skimmed milk samples at 85 °C for 30 min by exposing milk samples (bovine and camel) to steam for three successive days in an open valve autoclave apparatus, then milk (camel and bovine) was divided into equal portions of 500 mL for each sample and inoculated with 1.5 % of each *Lactobacilli* strain to prepare fermented camel and bovine milk. The fermentation lasted until the viable count of the *Lactobacilli* strain started to decline notably (after 104 h), then the following tests in the sections below were performed.

2.3. Total viable counts of fermented camel and bovine milk

The total viable counts for camel and bovine samples containing *L. helveticus*, *L. acidophilus,* and *L. paracasei* subsp. *paracasei*, were conducted for the entire fermentation period (104 h) at 40 °C using the pour plate technique following the procedure described by Alhaj et al. [26] and Donkor et al. [30]. The serial dilutions of camel and bovine samples in sterile 0.15 % (w/v) peptone water were carried out (Oxoid, Hampshire, England, UK). A 1 mL aliquot sample of camel and bovine milk samples was pour plated in triplicate using MRS agar medium (Oxoid, Hampshire, England, UK), then incubated in anaerobic jar at 40 °C for 72 h. As a result, the colony-forming units/mL as a measure of viable colony cells of each sample were counted. Moreover, the total bacterial counts of both samples (camel and bovine) were studied at 8 h intervals and represented as average of log CFU/mL.

2.4. Titratable acidity and pH determination

The titratable acidity and pH of unfermented and fermented milk samples were determined following the Association of Official Analytical Chemists International method [31] methods discussed by Abu-Taraboush et al. [32]. Acid development and pH of the samples were determined at 8 h intervals for 104 h in triplicate, and the average values of titratable acidity (TA) and pH samples were recorded, TA was reported as % lactic acid by weight using 0.11N NaOH. The pH is measured using pH meter.

2.5. Proteolytic activity of unfermented and fermented milk

Lactic acid bacteria produce extracellular proteinases to hydrolyze milk proteins; this leads to an increase in the amount of free amino acid groups (FAAGs), which was determined using the OPA method [33]. The proteolytic activity (degree of hydrolysis) of the unfermented and fermented camel and bovine milk samples was determined at 8 h intervals for 104 h using a UV–Vis spectrophotometer with an absorbance of 340 nm. The readings were expressed as the free amino acid group (FAAG) concentration (mM) in filtrate using the standard curve of Leu-Gly (Sigma-Aldrich, Saint Louis, Missouri, USA). The Phthaldialdehyde was obtained from Sigma-Aldrich, USA.

2.6. Statistical analysis

All experiments in this research were conducted in triplicates, then data were provided as means and ± standard deviation (SD). Using SPSS statistics 21 (SPSS Inc., Chicago, IL, USA), the One-way ANOVA test was used to analyze group differences, followed by Duncan's multiple range test. All data were assumed to be normally distributed, and the level of statistical significance was considered when results are $P < 0.05$ throughout.

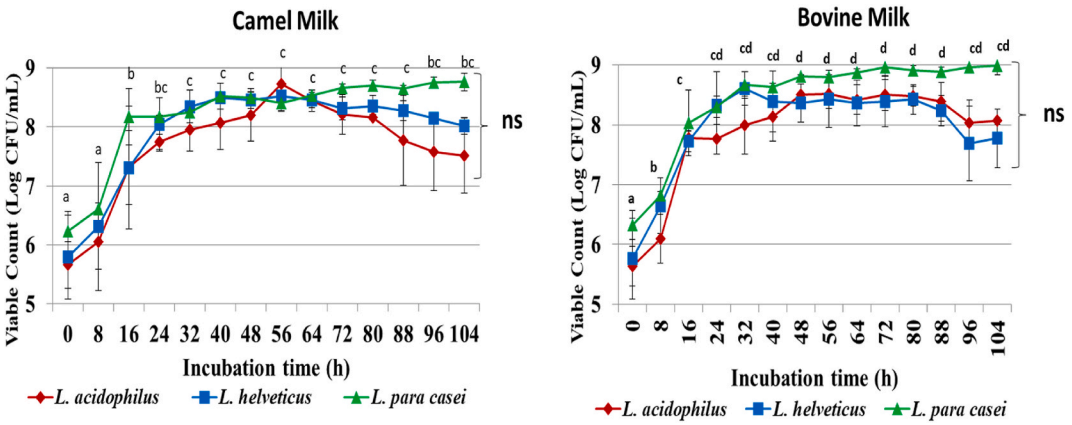

Fig. 1. Changes in total viable counts (Log CFU/mL) of *Lactobacilli* strains during fermentation of bovine and camel milk. Error bars represent SD. Values with different superscript letters are significantly different ($P > 0.05$) for a particular hour of fermentation. ns: non significantly different. Zero hour represents unfermented milk samples.

3. Results and discussion

3.1. Total viable counts of Lactobacillus strains during fermentation

The average bacterial counts (Log CFU/mL) of fermented camel milk samples compared to bovine milk with different *Lactobacilli* strains throughout the fermentation period are presented in Fig. 1. Our results showed that all three *Lactobacilli* strains can grow and survive in camel and bovine milk during the entire fermentation period of 104 h, confirming their potential as starter strains suitable to ferment camel milk. The LAB viability in studied milk is expected to be due to the high free amino acids (FAAs) in both raw camel and bovine milk [26]. According to Mehaia and Al-Kanhal [34], the high FAAs content correlates with greater bacterial growth and metabolism in different types of milk. As shown in Fig. 1, our finding showed that the average results of the total viable counts of all three *Lactobacilli* strains in both milk types have reached more than 7.0 log CFU/mL after 16 h of the fermentation period, which is according to the joint report guideline of FAO and WHO [35], higher than the minimum recommended daily probiotic dose to provide the potential health benefits. Then, the average total count increased to higher than 8.5 logs until the end of the fermentation period (104 h). Our results agree with Varga et al. [36], who reported that the probiotic population (*Bifidobacterium animalis* ssp. *lactis* BB-12, *Streptococcus thermophilus* CHCC 742/2130 and *Lactobacillus acidophilus* LA-5) sustained is more than 6.0 logs CFU/mL for fermented pasteurized camel and bovine milk during refrigerated storage. In another study [37], adding 1.0 % camel and bovine hydrolysates has shown to increase the total bacterial counts of yogurt culture after fermentation due to the presence of small peptides and free amino acids. Despite the reduction in pH values of fermented camel and bovine milk (Fig. 2), our finding showed that the pH generally remained within the ranges for the survivability (as low as of pH 3.1) of *Lactobacilli* strains especially with *L. helveticus*; this behavior agrees with that reported by Buchilina and Aryana [38], for the survivability of *bifidobacteria* strains in camel and bovine milk. In both fermented milk samples, our finding showed that the growth of *Lactobacillus* strains was significantly ($p < 0.05$) increased as the fermentation period increased, where growth remained increasing significantly ($p < 0.05$) for up to 24 h of fermentation. However, a decrease in *L. helveticus* and *L. acidophilus* viability was observed in both milk types after 88 h of the fermentation period and continued to the end of the fermentation period. This decrease in bacterial number could be attributed to the acid development caused by the large amounts of lactic acid, which inhibits bacterial growth due to post-acidification [39].

In this study, the viability of different *Lactobacilli* strains was found to vary with strain and milk types, but this difference was not statistically significant. Our finding showed that *L. paracasei* subsp. *paracasei* population was greater in both fermented camel and bovine milk (8.76 and 8.98 log CFU/mL, respectively) compared to other strains (*L. helveticus*, and *L. acidophilus*). Nonetheless, *L. helveticus* count was higher in fermented camel milk (8.01 log CFU/mL) than in fermented bovine milk (7.77 log CFU/mL). This could be related to the strain's proteolytic activity, which is more pronounced in camel milk than bovine milk, as reported by Ravaeschot et al. [40]. Thus, growth factors, for example, free NH_3 groups and peptides liberated during milk fermentation could play an essential role in keeping the viability of all culture strains. In fact, during the different stages of growth, lactic acid bacteria required variable amounts of nutrition in particular amino acids, which depend on the liberation of free NH_3 groups that varied with the incubation conditions [27]. The present results also agree with the findings by Mahmoudi et al. [41] for fermented camel milk and Hassan et al. [42] for fermented bovine milk with *L. helveticus* strain. The growth of *L. acidophilus* strain in both milk samples was relatively lower than other strains (*L. helveticus*, and *L. paracasei* subsp. *paracasei*). Stability of *L. paracasei* subsp. *paracasei* is depending on several factors including nutrient availability, metabolic activity, furthermore, interactions with other components in the milk such as mineral composition of milk. For this reason, many commercially available fermented milk products are produced with *Lactobacillus acidophilus* containing yogurt starters or produced from concentrated live *Lactobacillus acidophilus* cells cultivated and prepared in advance from broth [4].

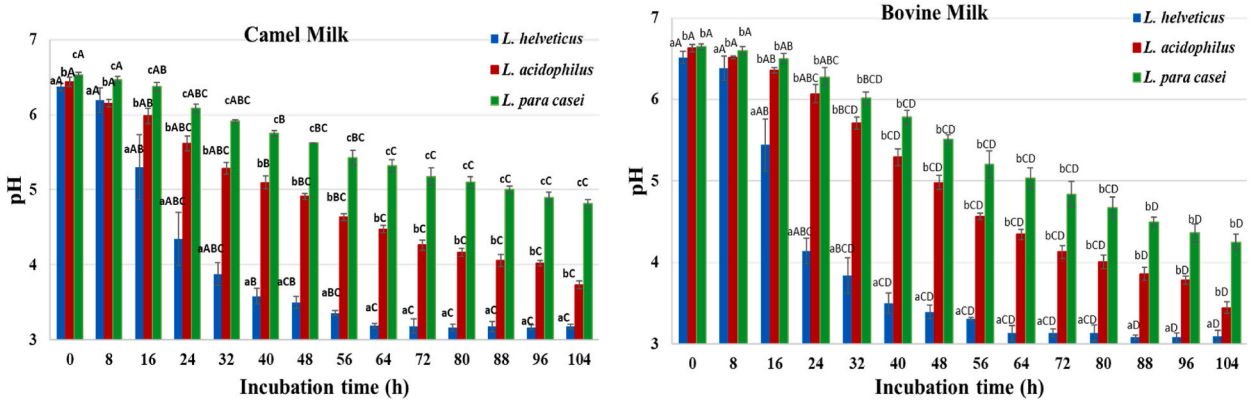

Fig. 2. Changes in pH values of fermented camel and cow milk by three *Lactobacilli* strains and during 104 h of fermentation period. Error bars represent SD. Mean values with different lowercase letters (a–c) were significantly different for each type of fermented milk with different *Lactobacilli* strains ($P < 0.05$); mean values with different uppercase letters (A–D) were significantly different for a particular hours of fermentation ($P < 0.05$). Zero hour represents unfermented milk samples.

3.2. Changes in pH and acidity of fermented camel and bovine milk

The changes in pH and titratable acidity of camel and bovine milk fermented with *Lactobacilli* strains are illustrated in Figs. 2 and 3, respectively. The pH values of fermented camel and bovine milk were affected by strain type and decreased significantly ($p < 0.05$) with the fermentation period. The *L. helveticus* strain decreased significantly the pH-values of fermented camel and bovine milk. This finding showed a favorable relationship between *lactobacilli* growth and pH lowering activity, meaning that *L. helveticus* with the highest rate growth can reduce apace the pH of the milk contrary to those with slower growth rate. As shown in Fig. 2, the initial pH of unfermented camel milk at 0 h was about 6.53, 6.44, and 6.37, then dropped to 4.81, 3.73, and 3.17 after 104 h of fermentation with *L. paracasei* subsp. *paracasei*, *L. acidophilus,* and *L. helveticus*, respectively. The final pH values of fermented bovine milk with the same strains were less (4.25, 3.44, and 3.09, respectively) compared to fermented camel milk. This could be attributed to the greater buffering capacity of camel milk caused by specific proteins and mineral composition in both milks [12,43]. Our findings are in contrast with Ayyash et al. [15]; this is due to the greater pH reduction in fermented camel milk as compared to bovine milk.

All *Lactobacilli* strains are able to acidify camel and bovine milk during 104 h of the fermentation period. As expected, the acidifying capacity of each strain can be explained by its ability to produce organic acids-that are desirable for flavor development. Our finding showed that *L. helveticus* strain has significantly ($p < 0.05$) the highest acidifying ability for both camel and bovine milk, which is in agreement with the results reported by Ref. [44]. On the other hand, *L. paracasei* subsp. *paracasei* revealed significantly ($p < 0.05$) the highest pH in both milk. Variations in the metabolic activities of the strains may be responsible for the difference in the acidifying ability between the used *Lactobacillus* spp. strains [11,45]. The decrease in pH values is mainly attributed to the production of lactic acid by the LAB strains. As illustrated in Fig. 3, at the end of fermentation period, the highest value in titratable acidity (%) was observed in camel (1.37 ± 1.10 %) and bovine milk (1.71 ± 1.21 %) fermented with *L. helveticus* strain, which is due to the capacity of this strain to breakdown lactose into lactic acid. In addition to lactose, *L. helveticus* strain can hydrolyze glucose and galactose via glycolysis pathway to produce mainly lactic acid [46]. It was noticed that there were no significant differences ($p < 0.05$) in the degree of acidity with the incubation time (0, 8, 16–104).

3.3. Proteolytic activity of unfermented and fermented camel and bovine milk

The results in Table 1 showed that the FAAGs content in fermented camel and bovine milk samples were higher than that of naturally occurring FAAGs noticed in unfermented milk (camel and bovine) samples. The current study showed that proteolytic activity is dependent on strains and milk type, this is similar to the results reported by Donkar et al. [30]. Moreover, camel and bovine milk fermented with *Lactobacillus* strains exhibited different proteolytic activity trends (Table 1). However, most of the liberated FAAGs from bovine milk proteins by three *Lactobacilli* strains occurred during the first 64 h of fermentation period, while liberated FAAGs from camel milk proteins occurred at different fermentation time depending on *Lactobacilli* strains. This is mainly related to the potential survivability of *Lactobacilli* strains in camel milk until the end of fermentation time, especially in relation to their internal and extracellular proteolytic enzymes, which produced significant levels of proteolysis activity. The activity of LAB was found to correlate substantially with higher concentrations of amino acids including tryptophan, valine, phenylalanine, leucine, and isoleucine [47]. The proteinases of Lactobacillus genera have the ability to release a wide range of oligopeptides, mostly contain four to eight amino acid residues [48]. As shown in Table 1, the proteolytic activity of the three strains increased with camel and bovine milk fermentation time, which is in agreement with the previous work of Alhaj et al. [26] and Donkor et al. [30]. Our finding also observed that along with the fermentation period, the FAAGs in fermented camel milk samples were significantly higher ($p < 0.05$) by all *Lactobacillus*

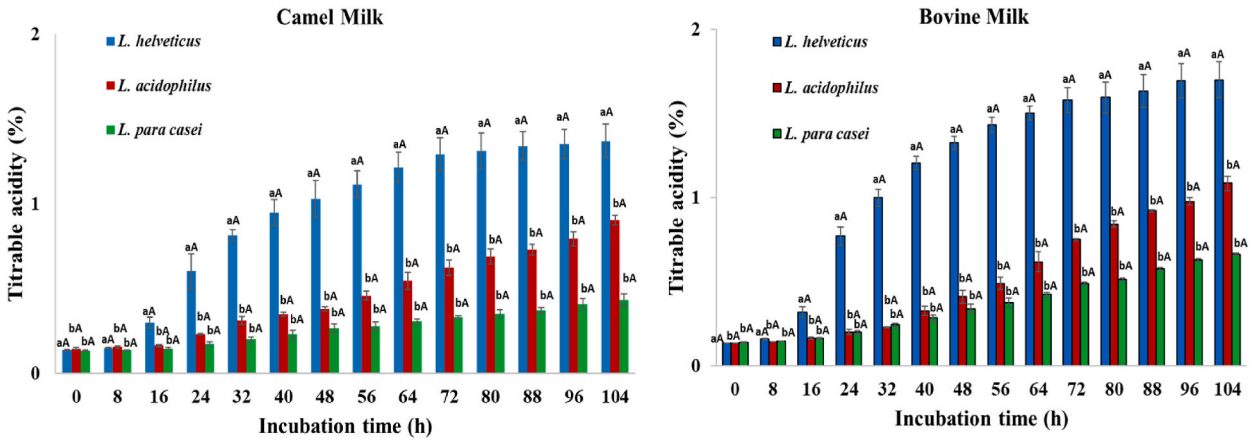

Fig. 3. Changes in titratable acidity during fermentation of camel and cow milk with different *Lactobacilli* strains. Error bars represent SD. Mean values with different lowercase letters (a–b) were significantly different for each type of fermented milk with different *Lactobacilli* strains ($P < 0.05$); mean values with the same uppercase letter (A) are non-significantly different for a particular hours of fermentation ($P > 0.05$). Zero hour represents unfermented milk samples.

Table 1
Proteolytic activity (changes in concentration of free amino acid group) of camel and bovine milk fermented with *Lactobacilli* strains using OPA method.

Fermentation period (h)	Bovine Milk Free amino acid group (m*M*)			Camel Milk Free amino acid group (m*M*)		
	L. helveticus	*L. acidophilus*	*L. paracasei* subsp. *paracasei*	*L. helveticus*	*L. acidophilus*	*L. paracasei* subsp. *paracasei*
0	0.200 ± 0.017^{aA}	0.162 ± 0.004^{aA}	0.179 ± 0.022^{bA}	0.294 ± 0.127^{aA}	0.231 ± 0.024^{bA}	0.281 ± 0.060^{cA}
8	0.204 ± 0.016^{aA}	0.165 ± 0.007^{aA}	0.184 ± 0.008^{bA}	0.320 ± 0.034^{aA}	0.247 ± 0.043^{bA}	0.285 ± 0.033^{cA}
16	0.339 ± 0.049^{aA}	0.180 ± 0.016^{aA}	0.207 ± 0.008^{bA}	0.578 ± 0.088^{aA}	0.443 ± 0.090^{bA}	0.318 ± 0.031^{cA}
24	0.684 ± 0.185^{aA}	0.252 ± 0.027^{aA}	0.225 ± 0.010^{bA}	0.959 ± 0.343^{aA}	1.611 ± 0.106^{bA}	0.340 ± 0.028^{cA}
32	0.904 ± 0.205^{aA}	0.251 ± 0.078^{aA}	0.252 ± 0.010^{bA}	1.169 ± 0.338^{aA}	1.831 ± 0.083^{bA}	0.370 ± 0.011^{cA}
40	0.935 ± 0.125^{aA}	0.347 ± 0.113^{aA}	0.259 ± 0.012^{bA}	1.388 ± 0.174^{aA}	1.930 ± 0.097^{bA}	0.397 ± 0.021^{cA}
48	1.008 ± 0.197^{aA}	0.470 ± 0.035^{aA}	0.275 ± 0.007^{bA}	1.654 ± 0.119^{aA}	1.998 ± 0.038^{bA}	0.418 ± 0.026^{cA}
56	1.039 ± 0.186^{aA}	0.635 ± 0.154^{aA}	0.284 ± 0.012^{bA}	1.676 ± 0.028^{aA}	2.227 ± 0.143^{bA}	0.433 ± 0.030^{cA}
64	1.019 ± 0.026^{aA}	1.042 ± 0.094^{aA}	0.308 ± 0.011^{bA}	1.696 ± 0.033^{aA}	2.298 ± 0.105^{bA}	0.441 ± 0.037^{cA}
72	1.110 ± 0.136^{aA}	1.161 ± 0.037^{aA}	0.336 ± 0.008^{bA}	1.716 ± 0.173^{aA}	2.366 ± 0.108^{bA}	0.460 ± 0.040^{cA}
80	1.215 ± 0.025^{aA}	1.248 ± 0.042^{aA}	0.339 ± 0.026^{bA}	1.732 ± 0.100^{aA}	2.377 ± 0.082^{bA}	0.463 ± 0.052^{cA}
88	1.184 ± 0.183^{aA}	1.242 ± 0.117^{aA}	0.367 ± 0.013^{bA}	1.717 ± 0.138^{aA}	2.592 ± 0.175^{bA}	0.475 ± 0.067^{cA}
96	1.087 ± 0.162^{aA}	1.345 ± 0.082^{aA}	0.417 ± 0.029^{bA}	1.703 ± 0.418^{aA}	2.741 ± 0.052^{bA}	0.526 ± 0.045^{cA}
104	0.918 ± 0.077^{aA}	1.464 ± 0.064^{aA}	0.457 ± 0.019^{bA}	1.596 ± 0.289^{aA}	2.828 ± 0.047^{bA}	0.614 ± 0.071^{cA}

Mean values in same row, in the same milk, with different lowercase superscripts differ significantly ($p < 0.05$). Mean values in the same column with different uppercase superscripts differ significantly ($p < 0.05$). Zero hour represents unfermented milk samples. Free amino acid group represents the concentration of Leu-Gly.

strains than in bovine milk. This is because camel milk proteins are more susceptible to proteolytic enzymes produced by the *Lactobacillus* strains compared to bovine milk [15]. Similar findings were also reported by Ayyash et al. [15], and Shori and Baba [49]. Interesting fact, peptides are the result of microbial proteolysis; a broad potent bioactive peptide can be found in fermented camel milk with the used *Lactobacillus* strains.

Moreover, our finding showed that *L. acidophilus* exhibited significantly ($p < 0.05$) the highest FAAGs expressed as a proteolytic activity level among other tested strains (*L. helveticus*, and *L. paracasei* subsp. *paracasei*) in camel milk, and this activity was found to be highest at the end of fermentation period (104 h). This may be attributed to the ability of *L. acidophilus* to grow and survive in the acidic medium as a natural acid-tolerant strain [50] for up to 56 h (Fig. 1), then decreased thereafter. Solanki and Hati [51] reported that the increase in proteolytic activity with various fermentation periods was directly associated with the nutritional requirements of lactic acid bacteria in terms of amino acids.

However, the results showed that bovine milk samples fermented with *L. helveticus* demonstrated stronger proteolysis for up to 56 h than other *Lactobacillus* strains due to the ability to hydrolyze α_{S1}-, k, β-caseins, and α-lactalbumin in milk and the release of several bioactive peptides [52]. In addition, fermentation times (0, 8, 16–104 h) exhibited no significant difference ($p > 0.05$) among OPA findings for both fermented milks. On one hand, this difference in the capacity of protein hydrolysis of strains may result from variations in their proteolytic systems. Solanki and Hati [51] reported the presence of the proteolytic system in lactic acid bacteria, which possess different peptidases, including X-prolyl-dipeptidyl aminopeptidase and proline-specific aminopeptidase (PepR) which hydrolyze milk proteins. On the other hand, these distinctions in the protein hydrolysis in both milks may be due to the differences in protein structure in camel and bovine milk as well as the differences among species. The results in Table 1 also showed that *L. paracasei* subsp. *paracasei* strain has the lowest FAAGs level expressed as proteolytic activity among the other tested *Lactobacillus* strains in camel and bovine milk.

4. Conclusion

It is concluded that all three *Lactobacilli* strains (*L. helveticus*, *L. acidophilus* and *L. paracasei* subsp. *paracasei*) can grow and survive in camel and bovine milk during the entire fermentation period of 104 h. Moreover, the growth, viability, and activity of three *Lactobacilli* strains were found to be strain, incubation time, and milk-dependent. The average of the total bacterial counts of all three *Lactobacilli*

strains in both fermented milks was found to be higher (>7.0 log CFU/mL) than the minimum recommended daily probiotic dose and continued to increase until the end of the fermentation period. This means all three *Lactobacilli* strains are suitable for fermentation in both bovine and camel milk. Our finding also observed that along with the fermentation period, the FAAGs in fermented camel milk samples were significantly higher in all *Lactobacillus* strains than in bovine milk. The findings of this study would support the launch of commercial dairy products containing excellent nutritional value and potential probiotic health claims. More rigorously human clinical trials are necessary to confirm the potential health claims found *in vitro* results. These trails should be aligned with *in vivo* studies, through the latter can be time-consuming, resource-intensive, and costly [19].

However, fermentation conditions must be optimized to obtain acceptable sensory evaluation and enhance product quality. The combination of a *Lactobacilli* strain/s with yogurt cultures would provide the manufacturers the advantage in choosing the best strain or combination for a particular dairy product. This approach would also help the selected strain/s for better rapid growth and/or fermentation conditions along with the increase of health claims of the final product. Nonetheless, this research has some limitations; this includes assessing the survival curve of the three strains in storage temperature. Furthermore, the organoleptic test is also recommended for future studies to confirm consumers' acceptability of the products. Moreover, the characterization of three *Lactobacillus* strains in Bactrian camel milk is recommended for investigation compared to dromedary milk and cow's milk.

CRediT authorship contribution statement

Omar A. Alhaj: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Zeineb Jrad:** Writing – review & editing, Writing – original draft, Software, Investigation. **Olfa Oussaief:** Writing – original draft. **Haitham A. Jahrami:** Writing – review & editing, Writing – original draft, Supervision, Software, Formal analysis. **Leena Ahmad:** Writing – original draft, Software, Resources. **Mohammad A. Alshuniaber:** Investigation, Funding acquisition. **Bhavbhuti M. Mehta:** Writing – review & editing, Writing – original draft.

Data and code availability statement

Data will be made available on request.

Declaration of competing interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We further confirm that any aspect of the work covered in this manuscript that has involved human patients has been conducted with the ethical approval of all relevant bodies.

We understand that Dr. Omar Alhaj is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

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