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Improving the Antioxidant Properties of Fermented Camel Milk Using Some Strains of *Lactobacillus*

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Abstract

This study aimed at improving the antioxidant capacity of fermented camel milk using some single strains of *Lactobacillus* (*Lb. helveticus* B-734, *Lb. casei* subsp. *casei* B-1922, *Lb. paracasei* subsp. *paracasei* B-4560, *Lb. rhamnosus* B-1445 or *Lb. rhamnosus* B-442), as well as evaluating the acceptability of the final products. The acidity, proteolysis degree, antioxidant activity, viscosity and organoleptic properties of fermented milk were assessed during 14 days of storage at 4˚C. Total phenolic content (TPC), DPPH radical scavenging activity, Ferrous ion chelating ability (FCA) and Ferric reducing antioxidant power (FRAP) assays were used to determine the antioxidant activity of fermented milks. The results indicated that fermented milks differed significantly ($P < 0.05$) in all studied parameters due to the type of starter culture used. During storage period, samples containing *Lb. helveticus* had the highest proteolysis degree, while samples with commercial starter culture (control) showed the lowest degree of proteolysis. Fermented milks containing *Lactobacillus* strains showed higher DPPH radical scavenging activity compared to those samples containing commercial starter culture. By the end of storage, there was a significant improvement ($P < 0.05$) in scavenging activity for all fermented milk samples. Regarding FCA, at the beginning of storage fermented milks containing *Lb. paracasei*, *Lb. rhamnosus* B-442 or commercial starter culture had the highest FCA values, while *Lb. rhamnosus* B-1445 samples recorded the highest value at the end of storage. *Lb. helveticus* samples had the highest TPC and FRAP values ($P < 0.05$) throughout the storage. There was a high significant correlation ($P < 0.0001$) between the proteolysis degree and the values of FRAP and TPC. Samples containing *Lb. rhamnosus*

B-442, *Lb. rhamnosus* B-1445 or commercial starter culture received the highest taste and overall acceptability scores while *Lb. helveticus* samples were the lowest. It is recommended to use *Lb. rhamnosus* B-442 and *Lb. rhamnosus* B-1445 for producing fermented camel milk with high antioxidant activity and acceptability.

Keywords

Camel Milk, Fermented Milk, Antioxidant Activity, *Lactobacillus*

1. Introduction

Camels (*Camelus dromedarius*) are very important to many pastoral communities in dry zones by providing milk, meat and transportation. The global camel population is estimated to be around 32.6 million, and most of these camels are found in African countries such as Somalia, Sudan, Chad, and Kenya [1]. Camel milk (CM) can be considered as one of the alternatives to Bovine milk (BM) with enhanced functions and better digestibility than BM in the human gastrointestinal system [2]. Camel milk not only provides the required nutrition for people, but also offers several therapeutic properties [3]. Camel milk has been known as a source for the production of dairy products with excellent therapeutic properties [4].

Fermented camel milk is proven to have many health benefits such as anti-inflammatory, antimicrobial, antioxidant, anticancer, hypocholesterolemic effect, anti-diarrhea activity, angiotensin I-converting enzyme (ACE) inhibitory activity [5] [6]. Thus, fermented camel milk has been proven as a therapeutic product [7]. In addition to providing energy and nutrients, fermented camel milk is an excellent source of bioactive peptides [8].

Bioactive peptides can be derived from enzymatic hydrolysis and/or microbial proteolytic activities during milk fermentation. These peptides have specific biological activities [9], such as antioxidant, antimicrobial, antihypertensive, ACE inhibitory, immunomodulant, and mineral binding [10] [11]. The antioxidant effect of peptides includes radical-scavenging (both hydrogen-donating capability and free radical quenching) activity, inhibition of lipid peroxidation, metal ion chelation, or all of these properties [12]. The mode of action of these peptides is due to the composition and amino acids sequence [13]. Antioxidant activity is known to be affected by the sequence and exposure of terminal amino acids of peptides after protein hydrolysis [14]. The peptides with antioxidant activity usually consist of 5 - 11 amino acids, including hydrophobic amino acids as proline, histidine, tyrosine and tryptophan [15].

Lactic acid bacteria (LAB) are known for many health benefits and industrial importance. In addition, LAB used as starters are primarily responsible for the generation of bioactive peptides during milk fermentation [6]. The proteolytic system of LAB involved in casein utilization provides cells with essential amino

acids during growth in milk. It is also of industrial importance due to its contribution to the development of the organoleptic properties of fermented milk product [16]. Also, proteolytic system of LAB is possessed of an extracellular located serine proteinase, a transport system specific for di-, tri-, and oligopeptides, and a multitude of intracellular peptidases. Proteinases of lactic acid bacteria may hydrolyze more than 40% of the peptide bonds of αs1- and β-caseins, producing oligopeptides of 4 to 40 amino acid residues [17] [16]. Naturally, camel milk is a blend of many bio-functional components. Camel milk contains all essential nutrients on which lactic acid bacteria can readily act and produce many bio-functional components which give health benefits to the consumer [18]. In addition, after fermentation of camel milk the concentrations of free amino acids, fatty acids and organic acid were increased. Many studies found that antioxidative peptides were derived during the fermentation of camel milk [2] [12] [19] [20]. Kansci *et al.* [21] found that, during the gastrointestinal digestion or fermentation of bovine milk β-Casein, radical scavenging bioactive peptides were produced. Moslehishad *et al.* [22] found that the antioxidant activity of camel milk fermented with *Lactobacillus rhamnosus* PTCC1637 was higher than fermented bovine milk. Also, Salami *et al.* [23] reported that, the antioxidant activity of camel β-CN was significant increased after enzymatic hydrolysis with chymotrypsin. The objectives of the current study were to investigate the effect of proteolytic activity of some *Lactobacillus* strains on the antioxidant capacity of fermented camel milk, and to evaluate the acceptability of the final products.

2. Materials and Methods

2.1. Materials

Camel milk was obtained from Camel Research Center, Marsa Matrouh, Egypt. Lyophilized *Lactobacillus* (*Lb.*) strains (*Lb. helveticus* B-734, *Lb. casei* subsp. *casei* B-1922, *Lb. paracasei* subsp. *paracasei* B-4560, *Lb. rhamnosus* B-1445 and *Lb. rhamnosus* B-442) were supplied by the Agriculture Research Service (ARS) Culture Collection, Norwegian Radio Relay League (NRRL) Peoria, USA. Commercially-available lyophilized culture (Express 0.2, DVS) was purchased from Chr. Hansen Laboratories, Copenhagen, Denmark. Modified starch ETENIA™ 457 was obtained from AVEBE, Veendam, Netherlands. Ferrous sulphate ($FeSO_4 \cdot 7H_2O$, 20.09% iron) was obtained from Fisons Laboratory Reagent, England. 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) was purchased from Sigma-Aldrich (Munich, Germany). Ferric chloride, potassium ferricyanide and gallic acid were obtained from Loba Chemie, Mumbai, India.

2.2. Methods

2.2.1. Cultures Preparation

Lyophilized bacterial strains were activated in 10 mL of de Man, Rogosa, Sharpe (MRS) broth at 37˚C for 24 hr. Then, 1 mL of each culture was added to 100 mL

of MRS broth and incubated at 37˚C for 24 hr. One mL of each culture was inoculated into 100 mL of autoclaved reconstituted skimmed milk powder (11%) and incubated at 37˚C overnight and used for preparation of fermented milks.

2.2.2. Preparation of Fermented Milk

Camel milk (total solids 12.37%, protein 3.21%, fat 4.1%, pH 6.54, and acidity 0.170%) was divided into six parts. Modified starch (0.5%; w/v) was added to each part of milk before heat treatment. Fermented milk was prepared according to Tamime and Robinson [24]. All milks were heated separately at 90˚C for 10 min and rapidly cooled to 42˚C. The first part was inoculation with 0.04% (w/v) of commercial starter culture alone (as control). Each of the other five parts of milk was inoculated with 3% (v/v) of single strain of *Lactobacillus* (*Lb. helveticus* B-734, *Lb. casei* subsp. *casei* B-1922, *Lb. paracasei* subsp. *paracasei* B-4560, *Lb. rhamnosus* B-1445 and *Lb. rhamnosus* B-442). Fermentation was carried out at 40˚C until the pH reached 4.69 with samples containing the commercial starter culture (control), *Lb. casei*, and *Lb. rhamnosus* B-442. While, the pH values for samples containing *Lb. helveticus*, *Lb. paracasei*, and *Lb. rhamnosus* B-1445 were 5.05, 4.82 and 4.46, respectively. Then, the fermented milks were stored at 4˚C ± 1˚C for 14 days.

2.2.3. Chemical Composition

Camel milk was analyzed for total solids (%), protein (%) and fat (%) using the AOAC procedures [25]. The pH of milk was measured using a digital pH meter (Martini, Italy). Titratable acidity (lactic acid %) of raw and fermented camel milks was evaluated by titration with 0.1 N NaOH in the presence of phenolphthalein as an indicator. All analyses were performed in triplicate.

2.2.4. Preparation of Water-Soluble Extracts from Fermented Milks

Water-soluble extracts were prepared in accordance with the method of Shori and Baba [26]. Fermented milk samples (10 g) were homogenized with 2.5 ml of distilled water then acidified to pH 4.0 with 0.1 M HCl, followed by holding at 45˚C for 10 min and centrifugation at 10,000 g for 10 min at 4˚C. The pH of supernatants was adjusted to pH 7.0 using 0.1 M NaOH and re-centrifuged (10,000 g, 10 min/4˚C) for further precipitation of proteins and salts. The supernatants were collected and kept at −20˚C for further analyses.

2.2.5. Determination of Proteolysis Degree

1) Cadmium–ninhydrin assay

Total free amino groups in water-soluble extract were assessed by the Cadmium–ninhydrin method described by Folkertsma and Fox [27]. The concentrations of total free amino groups were quantified against a standard curve of leucine (0.0 - 0.3 mM). Results were expressed as leucine equivalent in mM/mL extract. All determinations were performed in triplicate.

2) Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE, 12.5% T, was conducted under reducing conditions using the

discontinuous buffer system described by Laemmli [28]. SDS-PAGE was performed on fermented camel milk samples using a Mini-PROTEAN electrophoresis cell (Bio-Rad Laboratories, Hercules, CA, USA). Five grams of each sample were stirred with cold acetone (30 mL) for 10 min to get rid of the fat and left to dry at room temperature. The dried powdered fermented milk sample (20 mg) was mixed with 500 µL of sample buffer. Samples were denatured by boiling for 5 min, and then 5 µL of each sample was injected. The data were analyzed by Total Lab software (V1.11).

2.2.6. Determination of Antioxidant Activity

1) Determination of total phenolic content

Total phenolic contents (TPC) of water-soluble extracts were determined in triplicate using the method developed by Abirami *et al.* [29]. One and half milliliters of Folin-Ciocalteu's reagent (diluted 10 times) and 1.2 mL of Na_2CO_3 (7.5% w/v) were added to 300 µL of water-soluble extract. Mixtures were shaken and kept at room temperature for 30 min before measuring absorbance at 765 nm using a spectrophotometer (Pg T80+, England). TPC was expressed as mg gallic acid equivalents (GAE)/mL extract.

2) DPPH radical scavenging activity assay

Scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined in triplicate according to the procedure described by Lim and Quah [30]. Two milliliters of 0.15 mM DPPH dissolved in methanol were added to 1 mLof water-soluble extracts, mixed well and left in the dark for 30 min at room temperature. Absorbance (Abs) was measured at 517 nm against distilled water as a blank using a spectrophotometer (Pg T80+, England). Control was prepared by adding 2 mL of DPPH to 1 mL of methanol. The radical scavenging activity of extracts was calculated as follows

$$\text{Radical scavenging activity } \% = \left(1 - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}}\right) \times 100$$

3) Ferrous ion chelating assay

Ferrous ion chelating assay (FCA) was carried out as described by Chan *et al.* [31]. A freshly prepared ferrous sulphate hydrate ($FeSO_4 \cdot xH_2O$) solution (2 mM) and ferrozine solution (5 mM) were both diluted 20 times before using. Diluted $FeSO_4 \cdot xH_2O$ (1 mL) was mixed with samples of water-soluble extracts (1 mL). Diluted ferrozine (1 mL) was then added to the mixture followed by incubation for 10 min at room temperature. Absorbance (Abs) was measured at 562 nm against distilled water as blank using a spectrophotometer (Pg T80+, England). The absorbance of control was determined similarly by replacing the supernatant with distilled water. The ferrous ion chelating ability (FCA) of extracts was determined in triplicate and calculated using the equation:

$$\text{FCA } \% = \left(1 - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}}\right) \times 100$$

4) Ferric reducing antioxidant power

Ferric ion reducing antioxidant power (FRAP) was determined according to

Oyaizu [32]. One milliliter of extracts was added to 2.5 mL of phosphate buffer (0.1 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1% w/v). The mixture was then incubated in a water bath at 50˚C for 20 min followed by cooling to room temperature and adding 2.5 mL of trichloroacetic acid (10% w/v). The contents of the tubes were centrifuged at 10,000 × g at 4˚C for 10 min. Next, 2.5 mL of supernatant was removed from each tube, and mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride solution (0.1% w/v). The mixtures were allowed to stand for 30 min then absorbance was measured at 700 nm using UV/Visible spectrophotometer, Pharmacia-LKB-Ultrospec III (Pharmacia, USA). The assay was done in triplicate. The FRAP values, expressed in mg GAE/mL extract, were derived from a standard curve.

2.2.7. Determination of Viscosity

The viscosity of fermented camel milk was measured in triplicate at 10˚C ± 1˚C using oscillatory viscometer (VR 3000M YR Viscometers, Spain), using spindle 2 at speed of 60 rpm.

2.2.8. Sensory Evaluation

Fermented milks were evaluated for color, taste, consistency and overall acceptability. The evaluation was conducted by 9 panelists, with experience in sensory evaluation of dairy products, from the Department of Dairy Science and Technology, Faculty of Agriculture, Alexandria University. Samples were presented cooled in plastic cups for sensory evaluation after 1, 7, and 14 days of refrigerated storage. Water was provided between evaluations of samples for mouth rinsing. The evaluation was determined using a 9-point hedonic scale (1 dislike extremely, 9 like extremely).

2.2.9. Statistical Analysis

The data were analyzed by a general linear model procedure (GLM) using SAS statistical analysis software package (SAS Procedure Guide "Version 6.12 Ed." SAS Institute Inc., Cary, 2004). The statistical analysis was performed using one-way analysis of variance (ANOVA). Means were compared by Duncan's test at the significance level of $P \leq 0.05$. Pearson's correlation coefficient was used to calculate the correlation.

3. Results and Discussion

3.1. Acidity

The acidity in fermented camel milk is related to the metabolic activity of the LAB. The changes in acidity (% lactic acid) of fermented camel milks prepared using single strains of *lactobacillus* during cold storage (14 days/4˚C) are shown in **Figure 1**. On the 1st day of storage, lower acidity values were observed in the fermented milks containing *Lb. helveticus*, *Lb. rhamnosus* B-442 and *Lb. casei*, while, higher acidity values were found in *Lb. rhamnosus* B-1445, *Lb. paracasei* and commercial starter culture samples. On 7th day, samples containing *Lb. casei*

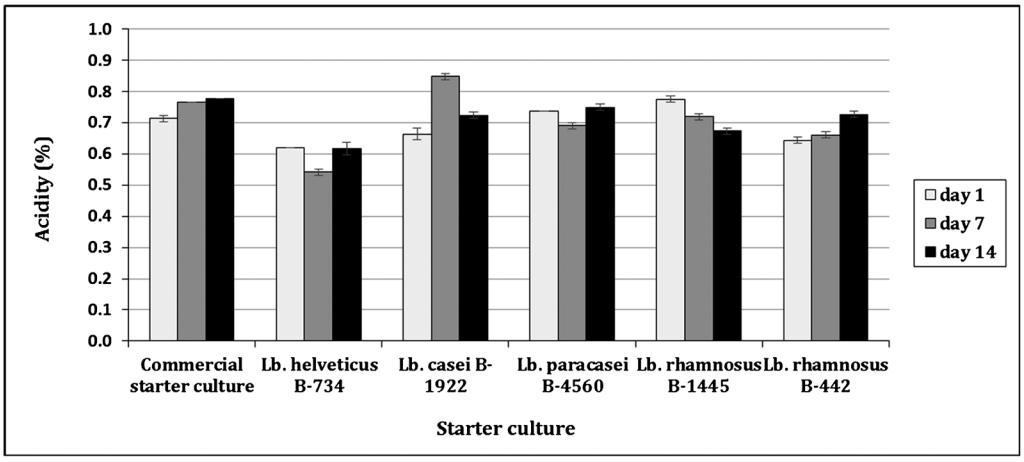

Figure 1. Changes in acidity (Mean values ± SD) of fermented camel milks prepared using single strains of *Lactobacillus* during cold storage.

or commercial starter culture had the highest acidity values, while the lowest acidity value was observed in *Lb. helveticus* samples. At the end of storage period (14 days), the acidity values of fermented milks were in the following order: commercial starter culture > *Lb. paracasei* = *Lb. casei* = *Lb. rhamnosus* B-442 > *Lb. rhamnosus* B-1445 > *Lb. helveticus*. From these results, it is clear that the fermented milk samples containing *Lb. helveticus* had the lowest acidity values during storage. Fluctuations in acidity values during storage of fermented milks may be explained by the relative changes in the formation of organic acids and the alkaline nature of milk protein breakdown products [33]. Al-Sheraji *et al.* [34] noted that the diversity in acidity development in camel milk-derived products is due to the differences in proteolysis. The level of lactic acid production depends on the amount of fermentable sugar and milk protein hydrolyzed by lactic acid bacteria [35]. De-Oliveira [36] reported that change in acidity may due to differential activity and microbial population during fermentation and storage.

3.2. Proteolysis Degree

3.2.1. Cadmium–Ninhydrin Assay

Cadmium-ninhydrin method was used to estimate proteolysis in fermented milk during a 14-day storage period. **Table 1** shows the proteolysis degree (mM leucine equivalent/mL extract) in fermented milks prepared using commercial starter culture and some single strains of *Lactobacillus*. The results revealed that there were significant differences ($P < 0.05$) in the degree of proteolysis among fermented milks, depending on the type of starter culture. During the storage period, fermented milks containing commercial starter culture had the lowest proteolysis degree, while samples with *Lb. helveticus* showed the highest degree of proteolysis ($P < 0.05$). Differences between treatments could be attributed to the variation between starter cultures in proteinase-endopeptidase activity [37]. Strains of *Lb. helveticus* are known to have one of the most complex proteolytic

Table 1. Proteolysis degree in fermented camel milk prepared with single strains of *Lactobacillus* during cold storage.

Starter culture	Total free amino groups (mM leucine equivalent/mL)		
	Day 1	Day 7	Day 14
Commercial starter culture	1.01 ± 0.03^{eB}	1.17 ± 0.06^{eA}	0.69 ± 0.02^{dC}
Lb. helveticus B-734	6.13 ± 0.11^{aA}	4.51 ± 0.10^{aB}	3.14 ± 0.17^{aC}
Lb. casei subsp. *casei* B-1922	1.26 ± 0.07^{dC}	3.66 ± 0.15^{cA}	2.26 ± 0.17^{cB}
Lb. paracasei subsp. *paracasei* B-4560	2.91 ± 0.02^{bB}	3.93 ± 0.08^{bA}	2.50 ± 0.06^{bC}
Lb. rhamnosus B-1445	2.93 ± 0.17^{bA}	2.32 ± 0.07^{dB}	2.12 ± 0.07^{cB}
Lb. rhamnosus B-442	2.45 ± 0.04^{cA}	2.22 ± 0.22^{dA}	0.89 ± 0.06^{Db}

Mean values (±SD) with different small letters within the same column are significantly different; means with different capital letters within the same row are significantly different ($P < 0.05$).

systems among lactic acid bacteria [38]. On the first day the proteolysis degree was in the following order: *Lb. helveticus* > *Lb. rhamnosus* B-1445 = *Lb. paracasei* > *Lb. rhamnosus* B-442 > *Lb. casei* > commercial starter culture. This arrangement differed on the 7th day and was as follows: *Lb. helveticus* > *Lb. paracasei* > *Lb. casei* > *Lb. rhamnosus* B-1445 = *Lb. rhamnosus* B-442 > commercial starter culture. This arrangement did not differ much on the 14th day. The results also showed a significant decrease ($P < 0.05$) in the proteolysis degree in fermented milk containing *Lb. helveticus* with increased storage time. Fermented milks containing *Lb. casei, Lb. paracasei* or commercial starter culture exhibited a maximum degree of proteolysis on the 7th day which decreased at the end of storage. The proteolysis degree of the samples prepared using *Lb. rhamnosus* B-1445 decreased on the 7th day, then stabilized until the end of the storage. As for fermented milk containing *Lb. rhamnosus* B-442, there were no significant differences between the 1st and 7th days in the degree of proteolysis, which decreased markedly ($P < 0.05$) at day 14.

3.2.2. SDS-Polyacrylamide Gel Electrophoresis (SDS–PAGE)

To determine the effect of *lactobacillus* strains on the degradation of camel milk proteins, samples of fermented milk at different times of storage were analyzed by SDS-PAGE (**Figure 2**). On the first day of storage, lower degree of proteolysis was observed in fermented milk containing commercial starter culture compared to the samples with single strains of *lactobacillus*. Meanwhile, *Lb. helveticus* showed higher proteolytic activity compared to other *lactobacillus* strains. On the 7th day the degree of proteolysis increased in most samples, and higher proteolysis was observed with *Lb. helveticus* and *Lb. casei* samples. The lowest proteolysis was observed with samples containing commercial starter culture. On the 14th day of storage the samples containing *Lb. helveticus* had the highest degree of proteolysis, while the lowest proteolysis was obtained with samples containing *Lb. casei* or *Lb. paracasei*. These results confirmed that the proteolytic

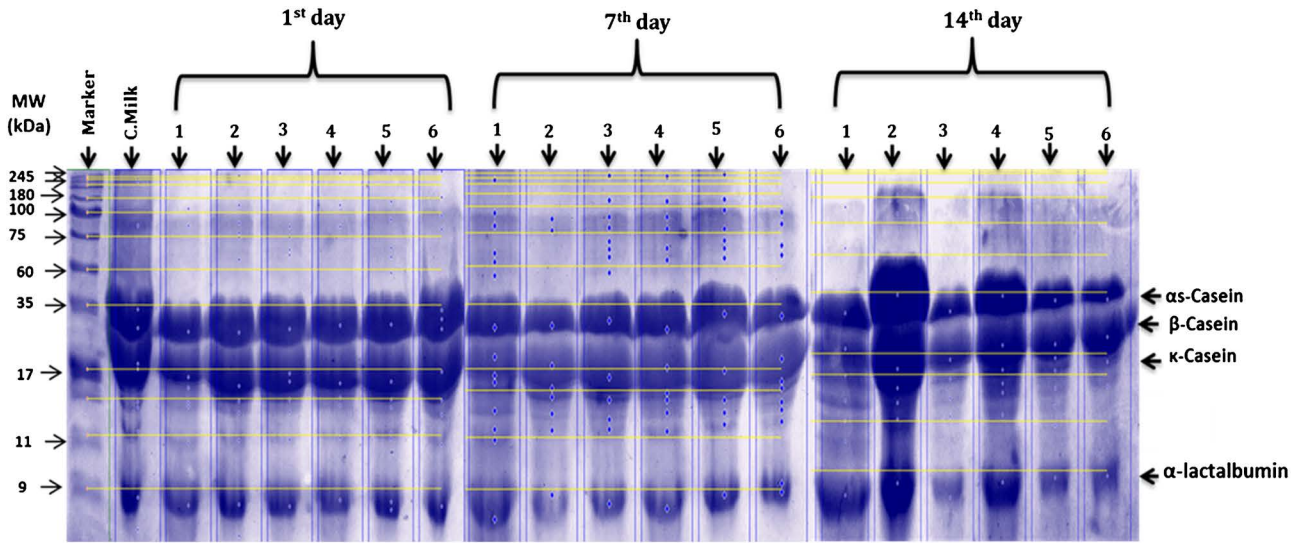

Figure 2. SDS-PAGE of fermented camel milk prepared using single strains of *Lactobacillus* during cold storage (14 days).C.Milk: Raw camel milk, 1: *Lb. helveticus* B-734 treatment, 2: *Lb. casei* subsp. *casei* B-1922 treatment, 3: *Lb. rhamnosus* B-1445 treatment, 4: *Lb. paracasei* subsp. *paracasei* B-4560 treatment, 5: *Lb. rhamnosus* B-442 treatment, 6: commercial starter culture treatment (control).

activity of most *lactobacillus* strains in this study increased with the increase in storage period and that the proteolytic activity of *Lb. helveticus* was the highest among the other *lactobacillus* strains. These results are consistent with those of El-Zahar *et al.* [39], who observed that all proteins gradually degraded during the cold storage of the yogurt. They also noted that αs_1-casein was higher degraded than β-casein during the cold storage. The proteolytic behavior of *lactobacillus* strains in this study in agree with that found by Li *et al.* [40], who reported that the intensity of κ-, β-, and α-casein bands in fermented milks were visually decreased during cold storage, meanwhile the electrophoretic bands referring to α-Lactalbumin and β-Lactoglobulin were unaffected in all treatments.

3.3. Antioxidant Activity

3.3.1. Total Phenolic Content

Data in **Figure 3** illustrate the effect of commercial starter culture and some strains of *Lactobacillus* on the total phenolic concentration of fermented camel milks during cold storage. The results showed that on the 1st day of storage the samples containing *Lb. helveticus* were the highest in TPC, while samples with commercial starter culture or *Lb. casei* were the lowest. Samples containing commercial starter culture, *Lb. casei* or *Lb. paracasei* showed an increase in TPC on day 7. Whereas, on day 14, the TPCs decreased significantly ($P < 0.05$) in all fermented milks except for samples containing *Lb. rhamnosus* B-1445. The order of TPC in fermented milk samples was as follows: *Lb. helveticus* > *Lb. paracasei* > *Lb. casei* > *Lb. rhamnosus* B-1445 > Lb. *rhamnosus* B-442 > commercial starter culture. The results also showed that TPC in fermented milk containing *Lb. helveticus* was the greatest ($P < 0.05$) throughout the storage compared to the other treatments. This result is related to the higher proteolytic activity of *Lb.*

helveticus. In this study, there was a highly significant correlation ($P < 0.0001$) between the TPC and proteolysis degree. The decrease in TPC for fermented milk after 7 days of storage is in agreement with the results of Shori [35], who found that the TPC in plan camel yogurt was significantly decreased on day 7, and increased on day 14 of storage. Moreover, the proteolysis of milk proteins during storage may result in the release of phenolic amino acids and non-phenolic compounds which may interfere during TPC determination [41].

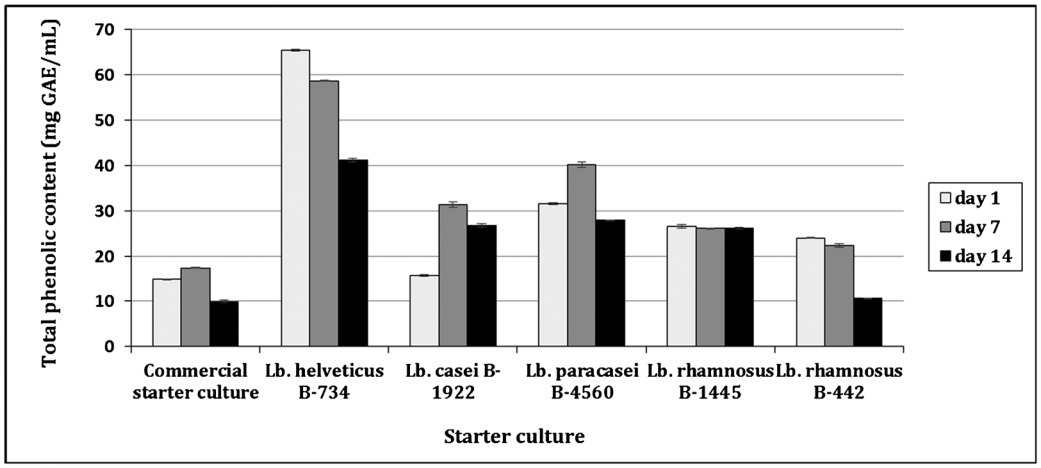

Figure 3. Changes in total phenolic content (Mean values ± SD) of fermented camel milks prepared using single strains of *Lactobacillus* during cold storage.

3.3.2. DPPH Radical Scavenging Activity

DPPH radical scavenging activity of fermented milks prepared using single strains of *Lactobacillus* as well as commercial starter culture was presented in **Figure 4**. As evident, there were significant differences ($P < 0.05$) in the scavenging activity values among the fermented milks. In addition, fermented milk containing commercial starter culture exhibited lower DPPH radical scavenging activity compared to other fermented milks during storage. On the 1st day, samples

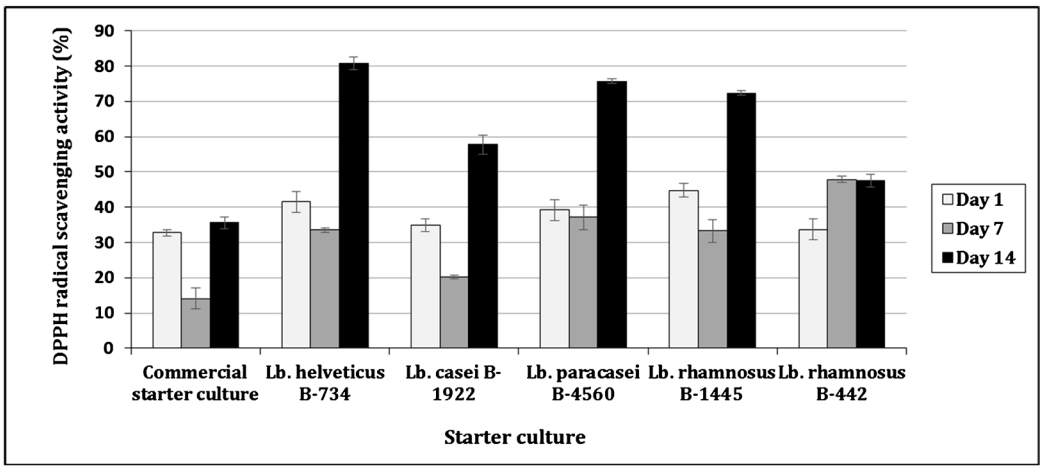

Figure 4. Changes in DPPH radical scavenging activity (Mean values ± SD) of fermented camel milks prepared using single strains of *Lactobacillus* during cold storage.

containing *Lb. rhamnosus* B-1445 or *Lb. helveticus* had the highest scavenging activity followed by samples with *Lb. paracasei* or *Lb. casei*, and then those containing *Lb. rhamnosus* B-442 or commercial starter. On the 7th day, the highest activity was for samples containing *Lb. rhamnosus* B-442 followed by those containing *Lb. paracasei*, *Lb. helveticus* or *Lb. rhamnosus* B-1445 and then *Lb. casei* and finally the samples containing the commercial starter culture. On the 14th day, the differences between samples were more pronounced compared to the 1st and 7th days of storage. The order of the samples in terms of activity was as follows: *Lb. helveticus* > *Lb. paracasei* > *Lb. rhamnosus* B-1445 > *Lb. casei* > *Lb. rhamnosus* B-442 > commercial starter. The results also revealed that the DPPH radical-scavenging activity for all fermented milks significantly decreased on day 7 of storage except for those samples containing *Lb. rhamnosus* B-442, which exhibited a significant increase ($P < 0.05$) in scavenging activity, while the scavenging activity of fermented milk containing *Lb. paracasei* was stable. At the end of storage period, there was a significant improvement ($P < 0.05$) in the scavenging activity for all fermented milk samples, particularly samples containing *Lb. helveticus*, *Lb. paracasei* and *Lb. rhamnosus* B-1445. In this study, no significant correlation ($P > 0.05$) was detected between the proteolysis degree and DPPH radical scavenging activity. This finding is consistent with the results of [42]. The absence of a relationship between the DPPH scavenging activity values and the degree of proteolysis indicates that the importance of the type and sequence position of functional amino acids in the peptides released for this activity [43] [44].

Overall, the antioxidant activity of fermented milk depends on various factors including the type and amount of the starter culture, the types of starter culture enzymes and the hydrolysis of proteins [45]. Further, the antioxidant properties of proteins and peptides count on many factors including the position of amino acids in their sequence as well as their physical structure, hydrophobicity and molecular weight [46].

3.3.3. Ferrous Ion Chelating Ability

Figure 5 presents the FCA of fermented milks prepared using single strains of *lactobacillus* during 14-day storage at 4˚C. The results showed that fermented milks differed significantly ($P < 0.05$) in FCA due to the type of starter culture. On the 1st day, samples containing *Lb. paracasei*, *Lb. rhamnosus* B-442 or commercial starter culture recorded the highest FCA, while fermented milk containing *Lb. helveticus* had the lowest FCA. After 7 days of storage, the FCA of samples containing *Lb. casei* was the highest compared to other treatments, while fermented milk with *Lb. helveticus* were still the lowest in chelating ability. The FCA of fermented milks was in the following order: samples containing *Lb. casei* were higher than those containing *Lb. paracasei* or commercial starter, followed by samples with *Lb. rhamnosus* B-1445 or *Lb. rhamnosus* B-442, and then fermented milks with *Lb. helveticus*. By the end of the storage period, the arrangement of samples differed completely in their ability to chelate iron. The FCA of

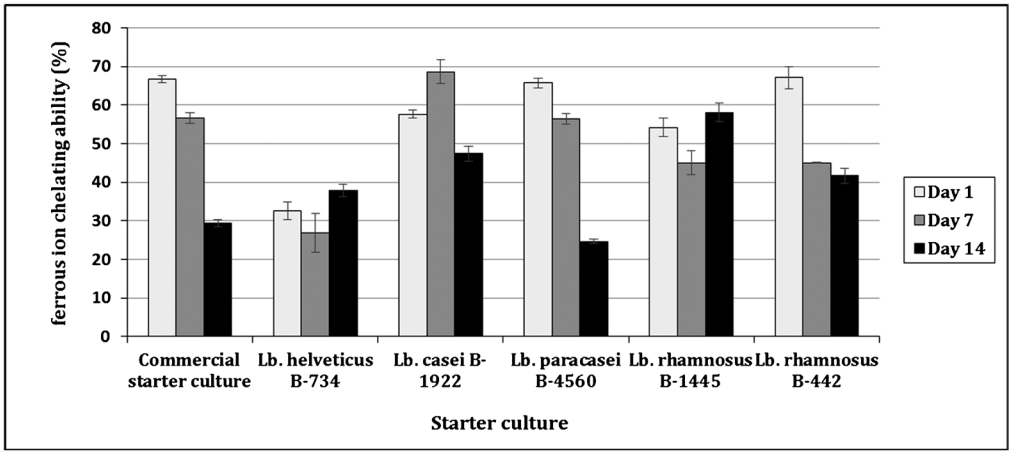

Figure 5. Changes in ferrous ion chelating ability (Mean values ± SD) of fermented camel milks prepared using single strains of *Lactobacillus* during cold storage.

fermented milks was in the following order: samples with *Lb. rhamnosus* B-1445 > *Lb. casei* > *Lb. rhamnosus* B-442 > *Lb. helveticus* > commercial starter culture > *Lb. paracasei*. The FCA of fermented milks could be attributed to the iron-binding peptides that are generated from milk proteins due to the proteolytic activity of the starter cultures. The hydrolysis of milk proteins by LAB to release peptides that have the potential to bind minerals has been reported [47] [48]. The functional groups of amino acid residues within derived peptides such as phosphate, carboxyl, hydroxyl and methyl as well as aromatic rings act as binding sites for transition metals ions [49]. In addition, The N- and C-terminals of peptides have been reported to contribute the metal chelating activity [50]. The variations in FCA between fermented milks during storage could be attributed to the structure of peptides that are generated as a result of the proteolytic activities of starter cultures [47]. In current study, no significant correlation ($P > 0.05$) was detected between the high degree of proteolysis and the FCA. A similar relationship between the hydrolysis degree and FCA was stated by [51].

The FCA decreased significantly ($P < 0.05$) for all fermented milks on the 7th day, with the exception of samples containing *Lb. paracasei*, where the ferrous chelating ability increased. On the 14th day of storage, a marked improvement in the FCA values was observed in fermented milks with *Lb. helveticus* and *Lb. rhamnosus* B-1445. While there was a continuous decrease in the FCA in the samples containing *Lb. casei*, *Lb. paracasei* and commercial starter culture. Regarding *Lb. rhamnosus* B-442 samples, the FCA was stable compared to day 7 of storage. A number of studies have found that the chelating capability of fermented milk samples increased with increased storage [45] [48].

3.3.4. Ferric Reducing Antioxidant Power

Data in **Figure 6** illustrate the FRAP values of fermented camel milks prepared using single strains of *lactobacillus* during cold storage. On the 1st day of storage, the greatest FRAP value (0.322 mg GAE/mL) was found with *Lb. helveticus* samples. While, on the 7th day the highest FRAP values (0.212, 0.211 and 0.206

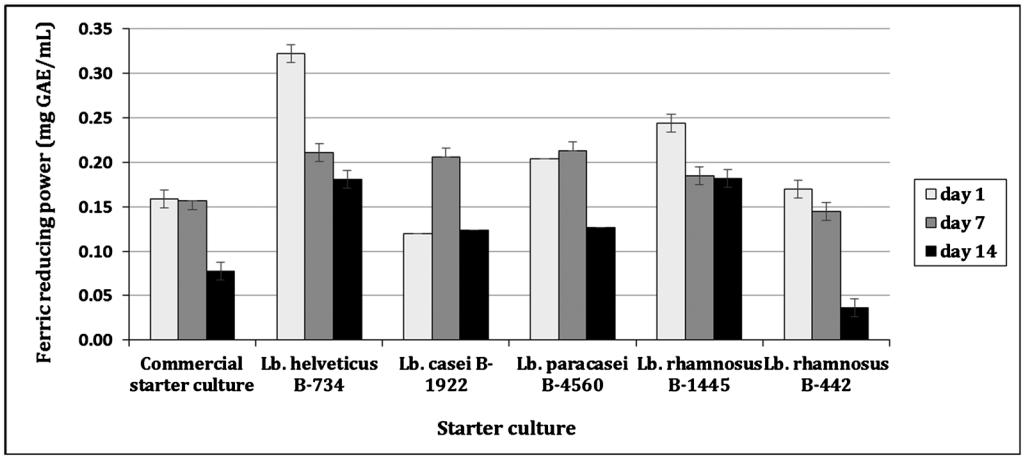

Figure 6. Changes in ferric reducing antioxidant power (Mean values ± SD) of fermented camel milks prepared using single strains of *Lactobacillus* during cold storage.

mg GAE/mL) were observed with the samples containing *Lb. paracasei*, *Lb. helveticus* and *Lb. casei*, respectively. On the 14th day the highest FRAP values (0.180 and 0.181 mg GAE/mL) were found with *Lb. helveticus* and *Lb. rhamnosus* B-1445 samples, respectively. On the other side, fermented milk samples containing *Lb. rhamnosus* B-442 or commercial starter culture showed the lowest FRAP values throughout storage.

The results also showed that on the 7th day of storage, fermented milks containing *Lb. casei* showed a significant increase ($P < 0.05$) in FRAP values. In addition, FRAP values did not change in samples containing *Lb. paracasei* or commercial starter culture, while there was a decrease in these values in samples with *Lb. helveticus, Lb. rhamnosus* B-442 or *Lb. rhamnosus* B-1445 compared to the FRAP values on the 1st day of storage. At the end of storage, all fermented milk samples showed a decrease in FRAP values. In this study, the correlation between the values of FRAP and proteolysis degree was highly significant ($P < 0.0001$). The change in the proteolytic activity of *Lactobacillus* strains during storage affected the release of peptides from milk protein and consequently impacted the FRAP values of fermented milks. It was reported that increased proteolysis in fermented milk caused increased generation of bioactive peptides, which ultimately resulted in increased antioxidant capacity [22]. Ayyash *et al.* [20] reported that the fermentation of camel milk with indigenous camel milk probiotic LAB strains (*Lb. reuteri*-KX881777, *Lb. plantarum*-KX881772, *Lb. plantarum*-KX881779) showed maximum antioxidant activity compared to bovine milk.

3.4. Viscosity

Figure 7 shows the changes in the viscosity of fermented camel milks prepared using commercial starter culture and single strains of *Lactobacillus* during storage. The type of starter culture and storage duration significantly ($P < 0.05$) affected the viscosity of fermented milk. All treatment showed a significant increase

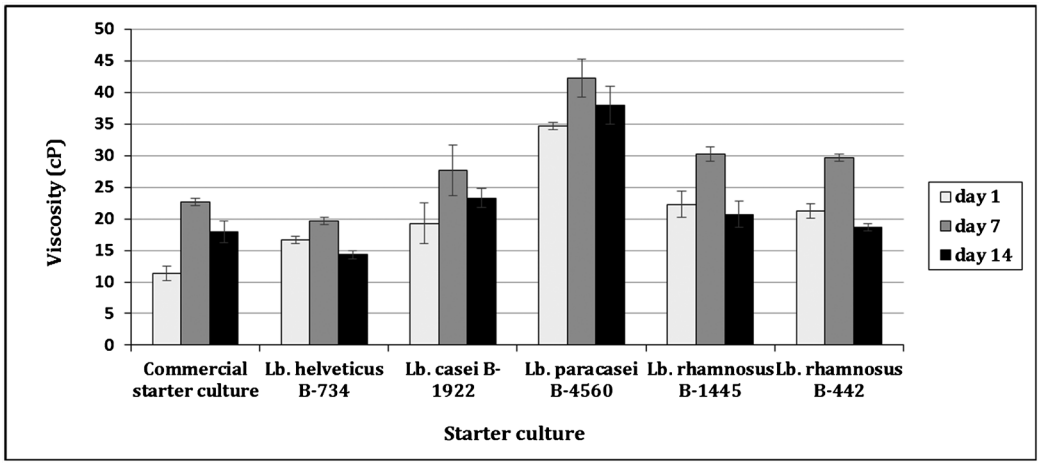

Figure 7. Changes in viscosity (Mean values ± SD) of fermented camel milks prepared using single strains of *Lactobacillus* during cold storage.

($P < 0.05$) in viscosity on the 7th day of storage. Whereas, at the end of storage (14th day) the viscosity were significantly decreased ($P < 0.05$) for all treatments. The highest viscosity was observed in *Lb. paracasei* samples during the storage times, where the viscosity values were 34.7, 42.3 and 38.0 cP on the 1st, 7th and 14th day, respectively. On the other hand, lowest viscosity values were recorded with samples prepared with *Lb. helveticus* or commercial starter culture throughout storage. The decrease in viscosity may be due to the degradation of protein during storage [52]. Fguiri *et al*, [53] reported that the exopolysaccharide synthesized by lactic acid bacteria can improve the texture and viscosity of the final product. Kavas [54] indicated that the viscosity of yogurt increased with increasing acidity during storage.

3.5. Sensory Evaluation

The sensory attributes scores of fermented milks prepared using single strains of *Lactobacillus* during 14 days of storage period are presented in **Table 2**. The findings revealed that fermented milk samples varied significantly ($P < 0.05$) in the mean values of taste scores. Fermented milks containing *Lb. rhamnosus* B442, *Lb. rhamnosus* B-1445 or commercial starter culture had the highest scores, while *Lb. helveticus* samples were the lowest. Additionally, the color scores for *Lb. helveticus* samples were the lowest, while the color scores of other samples were nearly similar. Previously, Moslehishad *et al.*, [22] found that fermented camel milks using *Lb. rhamnosus* PTCC 1637, *Lb. fermentum* PTCC 1638 or *Lb. plantarum* PTCC 1058, had high average scores for sensory quality compared to those samples prepared by other tested strains. Buffa *et al.* [55] reported that the redundant proteolysis that occurs by highly proteolytic strains can cause uncontrolled formation of bitter peptides in the final product. The consistency scores of all fermented milks did not differ significantly ($P > 0.05$), except for those samples with *Lb. helveticus* on the 1st day of storage, which obtained the lowest consistency scores ($P < 0.05$). In terms of overall acceptability,

Table 2. Sensory evaluation of fermented camel milks prepared using single strains of *Lactobacillus* during cold storage.

Starter culture	Sensory attributes			
	Color (9)	Taste (9)	Consistency (9)	Overall acceptability (9)
	Day 1			
Commercial starter	8.2 ± 0.45^{aA}	8.2 ± 0.84^{Aa}	8.0 ± 0.71^{aA}	8.0 ± 0.71^{aA}
Lb. helveticus B-734	6.7 ± 0.58^{bA}	5.0 ± 2.00^{bA}	6.3 ± 0.58^{bA}	5.3 ± 2.08^{cA}
Lb. casei subsp. *casei* B-1922	8.0 ± 0.00^{aA}	6.3 ± 0.76^{bA}	7.1 ± 0.69^{abA}	6.5 ± 0.76^{cbA}
Lb. paracasei subsp. *paracasei* B-4560	7.7 ± 0.49^{aA}	6.1 ± 1.46^{bA}	7.3 ± 0.76^{abA}	6.5 ± 1.61^{cbA}
Lb. rhamnosus B-1445	7.9 ± 0.38^{aA}	7.6 ± 0.79^{aA}	7.3 ± 0.49^{abA}	7.6 ± 0.53^{abA}
Lb. rhamnosus B-442	7.9 ± 0.38^{aA}	8.3 ± 0.49^{aA}	7.0 ± 1.00^{abA}	8.0 ± 0.58^{aAB}
	Day 7			
Commercial starter	8.2 ± 0.45^{aA}	7.4 ± 0.89^{abA}	8.2 ± 0.45^{aA}	8.0 ± 0.71^{abA}
Lb. helveticus B-734	7.4 ± 0.55^{bA}	2.0 ± 0.82^{dB}	7.2 ± 0.84^{aA}	2.4 ± 0.89^{dB}
Lb. casei subsp. *casei* B-1922	8.0 ± 0.00^{aA}	5.8 ± 2.28^{cbA}	7.0 ± 1.22^{aA}	6.2 ± 2.05^{cbA}
Lb. paracasei subsp. *paracasei* B-4560	8.2 ± 0.45^{aA}	4.2 ± 1.79^{cA}	7.6 ± 0.55^{aA}	4.8 ± 2.17^{cA}
Lb. rhamnosus B-1445	8.0 ± 0.00^{aA}	7.0 ± 1.41^{abA}	7.6 ± 0.89^{aA}	6.8 ± 1.79^{abA}
Lb. rhamnosus B-442	8.2 ± 0.45^{aA}	8.2 ± 0.45^{aA}	7.8 ± 0.84^{aA}	8.4 ± 0.55^{aA}
	Day 14			
Commercial starter	8.0 ± 0.82^{aA}	7.8 ± 0.96^{aA}	7.8 ± 0.96^{aA}	7.8 ± 0.96^{aA}
Lb. helveticus B-734	6.5 ± 1.73^{bA}	1.3 ± 0.50^{cB}	7.0 ± 1.83^{aA}	1.5 ± 0.58^{cB}
Lb. casei subsp. *casei* B-1922	8.0 ± 0.82^{aA}	5.3 ± 0.96^{bA}	7.5 ± 1.29^{aA}	5.5 ± 0.58^{bA}
Lb. paracasei subsp. *paracasei* B-4560	8.0 ± 0.82^{aA}	5.0 ± 2.94^{bA}	8.3 ± 0.96^{aA}	5.0 ± 2.94^{bA}
Lb. rhamnosus B-1445	8.0 ± 0.82^{aA}	7.3 ± 0.96^{abA}	8.3 ± 0.96^{aA}	7.5 ± 1.00^{Aa}
Lb. rhamnosus B-442	8.0 ± 0.82^{aA}	7.3 ± 1.26^{abA}	7.5 ± 1.00^{aA}	7.3 ± 1.26^{abB}

Mean values (± standard deviation) with different small letters within strains are significantly different at $P < 0.05$; means with different capital letters within days are significantly different at $P < 0.05$.

samples prepared with *Lb. rhamnosus* B-442, *Lb. rhamnosus* B-1445 or commercial starter culture obtained the highest scores, while fermented milks containing *Lb. helveticus* were the lowest in overall acceptance. Pearson's correlation data revealed that there was an inverse correlation between the proteolysis degree and the scores of color ($P = 0.02$), taste ($P < 0.0001$), and overall acceptability ($P = 0.0003$) of fermented milks. These results are in line with the findings of Amani *et al.* [56], who found that yogurts prepared using highly proteolytic strains had lower score of flavor, taste and overall acceptance than other samples produced using strains with low or medium proteolytic activity. However, these results disagree with the findings of Moslehishad *et al.*, [22], who stated that there is no specific relationship between the proteolytic activity of selected LAB and flavor defects or overall acceptance of fermented cow and camel milk. The

color and consistency scores were not affected by storage for all fermented milks. Also, the taste and overall acceptability scores remained stable during storage for all fermented milks except for samples containing *Lb. helveticus*, as these scores decreased significantly ($P < 0.05$) on the 14th day of storage.

4. Conclusion

The antioxidant activity is very important for human health. In this study the improvement of the antioxidant properties of fermented camel milk was investigated using some strains of *lactobacillus*. From the results it could be concluded that *Lb. rhamnosus* B-442 and *Lb. rhamnosus* B-1445 can be used to produce fermented camel milk with high antioxidant activity and acceptability.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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