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Antioxidant Properties of Fermented Camel Milk Prepared Using Different Microbial Cultures

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Abstract

This study investigated the effect of using different combinations of commercial starter culture and lactobacilli strains on the antioxidant properties of fermented camel milk for 14 days. The bacterial strains included *Lb. casei* subsp. *casei* B-1922, *Lb. paracasei* subsp. *paracasei* B-4560, *Lb. rhamnosus* B-442 and *Lb. rhamnosus* B-1445. The antioxidant activity of fermented milk was estimated using DPPH radical scavenging activity, ferrous ion chelating activity (FCA) and ferric reducing power assays. The total phenolic content (TPC), titratable acidity, proteolysis degree and sensory acceptability of samples were also evaluated. The results showed that all the studied parameters were affected by both the type of starter culture and storage. Samples containing the commercial starter and *Lb. rhamnosus* B-1445 had the highest DPPH radical scavenging activity and TPC throughout storage. Fermented milks prepared using the commercial starter and *Lb. rhamnosus* B-442 or *Lb. paracasei* retained their high FCA and reducing power throughout storage compared to the other samples. Fermented milk containing the commercial starter and *Lb. casei* showed the lowest antioxidant activity. The DPPH scavenging activity for all fermented milks decreased sharply during storage, while the change in FCA, reducing power and TPC differed among the samples during storage. The highest acidity was observed in the samples containing the commercial starter and *Lb. paracasei*, while fermented milk prepared using commercial starter and *Lb. rhamnosus* B-1445 had the lowest acidity. Samples containing the commercial starter and *Lb. casei* had the greatest proteolysis during the first week of storage, while samples containing the commercial starter and *Lb. rhamnosus* B-1445 had the highest proteolysis on day 14. Fermented milks with commercial starter and *Lb. rhamnosus* B-1445

or *Lb. paracasei* were the most acceptable products, while samples containing the commercial starter and *Lb. casei* were less acceptable.

Keywords

Antioxidant Activity, Fermented Camel Milk, Lactobacilli

1. Introduction

The antioxidant activity of dairy products is significant both for the shelf life and quality of the products and for protection against the excessive formation of oxygen free radicals within the human body [1]. Studies have shown that there is an inverse relationship between the consumption of natural antioxidant-rich foods and the incidence of certain types of cancers, diabetes, hypertension and cardiovascular diseases [1]. Dietary antioxidants are compounds that have the ability to scavenge free radicals and prevent their deleterious effects through several mechanisms [2].

Milk and dairy products possess antioxidant activity due to the presence of natural antioxidants such as caseins, whey proteins, oligosaccharides, vitamins A, E, and C, selenium, zinc, antioxidant enzymes, and bioactive peptides that are generated during milk fermentation and cheese ripening [2] [3]. In recent years, great attention has been paid to increase the antioxidant activity of milk and dairy products. Fortification of fermented milk with plant materials or their extracts rich in natural antioxidants, *i.e.* phenolics and other bioactive compounds has been studied to improve the antioxidant status of the product [2] [3]. The use of certain strains of lactic acid bacteria (LAB) in milk fermentation for the same purpose has also been investigated [4] [5].

The genus *Lactobacillus* constitutes a major group of LAB. Lactobacilli are bacteria of great industrial importance, as they are used in the production of fermented foods including dairy products, preservation and probiotic applications [6]. Lactobacilli can hydrolyze proteins in their environment to obtain the amino acids needed for their growth *via* their proteolytic system through the action of cell envelope proteinases [6]. It has been reported that fermentation of milk by *Lactobacillus* liberates a large number of peptides from milk proteins with diverse biological actions, including antioxidant activity [7]. The antioxidant activity of fermented milk is highly dependent on the strain or species of *Lactobacillus* used in the fermentation process [4] [5]. Therefore, the use of lactobacilli is considered as an effective strategy to produce fermented dairy products with a broad variety of antioxidant peptides.

Camel milk is well known for its therapeutic aspects that have been studied in humans, experimental animals and even at the cellular level [8] [9]. Besides, camel milk has a unique composition that distinguishes it from the milk of other ruminants [10].

Few studies have used single strains of the genus *Lactobacillus* to produce yogurt and fermented milks from camel milk with the aim of enhancing the antioxidant activity of the products [5] [11]. However, to our knowledge, there is no study on the effect of using starter culture combinations on the antioxidant activity of fermented milk made from camel milk. Therefore, this research aimed to investigate the effect of mixing commercial starter culture with strains of *Lactobacillus* on the antioxidant activity and total phenolic content of fermented camel milk. Also, the degree of proteolysis, titratable acidity and sensory properties of fermented milk were also evaluated. All studied parameters were estimated during 14 days of refrigerated storage.

2. Material and Methods

2.1. Materials

Camel milk was obtained from the Camel Research Center, Marsa Matrouh, Egypt. The strains of *Lactobacillus* (*Lb.*) were provided from the Agriculture Research Service (ARS) Culture Collection, Norwegian Radio Relay League (NRRL) Peoria, USA. The strains included *Lb. casei* subsp. *casei* B-1922, *Lb. paracasei* subsp. *paracasei* B-4560, *Lb. rhamnosus* B-442 and *Lb. rhamnosus* B-1445. Commercially available lyophilized culture (*Streptococcus thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*) (Express 0.2, DVS) was procured from Chr. Hansen Laboratories, Copenhagen, Denmark. Modified starch ETENIA™ 457 was supplied by AVEBE, Veendam, Netherlands.

2.2. Methods

2.2.1. Preparation of Cultures

Each strain of *Lactobacillus* was activated twice in de Man-Rogosa-Sharpe (MRS) broth at 37˚C for 24 hr. Next, one mL of each culture was inoculated into 100 mL of sterilized skim milk (11%) and incubated at 37˚C for 24 hr for use in the preparation of fermented milks.

2.2.2. Preparation of Fermented Milk

Fermented milk was prepared by the method of Tamime and Robinson [12]. Camel milk with 12.4% total solids, 3.2% protein, 4.1% fat, 0.17% acidity and pH 6.54 was divided into four batches. Each batch of milk was warmed to 40˚C and then modified starch as a thickening agent (0.5%, w/v) was added to it followed by heating to 90˚C for 10 min. The batches were cooled to 37˚C, and each batch of milk was inoculated with an individual strain (1.5%, v/v) and then incubated for 1.5 hr. Each of the four batches was then heated again to 40˚C and inoculated with a commercial starter culture (0.02%, w/v), poured into plastic containers, and incubated at 40˚C. When the pH value decreased to 4.6, all samples were transferred to a refrigerator and stored at 4˚C ± 1˚C for 14 days. Samples were analyzed after 1, 7 and 14 days of storage in triplicate. The four microbial cultures used in camel milk fermentation were: 1) commercial starter culture and *Lb. casei* B-1922; 2) commercial starter culture and *Lb. paracasei* B-4560; 3)

commercial starter culture and *Lb. rhamnosus* B-442; 4) commercial starter culture and *Lb. rhamnosus* B-1445.

2.2.3. Chemical Composition of Milk

Chemical composition of camel milk (fat, protein and total solids %) was estimated in triplicate using the AOAC procedures [13].

2.2.4. pH and Titratable Acidity

The pH values of milk and fermented milk samples were measured using a digital pH meter (Martini, Italy). The titratable acidity (expressed as lactic acid %) was determined in triplicate by titration of samples with 0.1N NaOH using phenolphthalein as an indicator [13].

2.2.5. Preparation of Water-Soluble Extracts

The water-soluble extracts were prepared from fermented milks according to the method of Shori and Baba [14]. Ten grams of fermented milk samples were mixed with 2.5 mL of distilled water and acidified to pH 4.0 with 0.1 M HCl, then kept at 45˚C for 10 min and centrifuged at 10,000 ×*g* for 10 min at 4˚C. The pH of the supernatants was adjusted to 7.0 using 0.1 M NaOH and re-centrifuged. The supernatants were kept at −20˚C until analyses were performed.

2.2.6. Proteolysis Degree

1) Cadmium–ninhydrin assay

Free amino groups in water-soluble extracts were determined by the cadmium–ninhydrin assay [15]. Results were expressed as mmol leucine equivalent per mL of extract.

2) Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), 12.5% T, was carried out using the discontinuous buffer system described by Laemmli [16]. Five grams of each sample were stirred with 30 mL of cold acetone for 10 min to remove the fat and left to dry at room temperature. The dry powder of fermented milk (20 mg) was mixed with 500 µL of the sample buffer. Samples were boiled for five min, and then five µL of each sample was injected.

2.2.7. Antioxidant Activity

1) DPPH radical scavenging activity assay

The radical scavenging activity of the water-soluble extracts was determined with 1, 1-diphenyl-2-picrylhydrazyl (DPPH) according to the procedure described by Lim and Quah [17]. The radical scavenging activity of extracts was calculated as follows:

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

2) Ferrous ion chelating activity assay

The ferrous ion chelating activity (FCA) of the water-soluble extracts was performed as described by Chan *et al.* [18]. The FCA of extracts was calculated

as follows:

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

3) Ferric reducing power assay

The ferric reducing power of water-soluble extracts was estimated according to Oyaizu [19]. The reducing power values were expressed as mg gallic acid equivalents (GAE) per mL of extract.

2.2.8. Total Phenolic Content

The total phenolic content (TPC) of water-soluble extracts was determined with accordance to the Folin–Ciocalteu method used by Chan *et al.* [18]. The TPC was expressed as mg GAE per mL of extract.

2.2.9. Sensory Evaluation

The sensory attributes of fermented milks were evaluated by five trained members of the Department of Dairy Science and Technology, Faculty of Agriculture, Alexandria University. Samples were codified and presented cooled in plastic cups to the panelists, who were asked to rinse their mouths with water between each sample test. Fermented milks were evaluated for color, taste, consistency and overall acceptability on a nine-point hedonic scale (0 = dislike extremely; 9 = like extremely).

2.2.10. 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 $p < 0.05$. Pearson's correlation coefficient (r) was used to calculate the correlation.

3. Results and Discussion

LAB are used to ferment a wide range of food products. In the early days the most important feature of these organisms was the conversion of available sugar into lactic acid to achieve the preservation of the fermented product. In the modern fermentation processes of the dairy industry, other aspects such as flavor development and increased antioxidant activity of products for instance have become just as important as preservation. To obtain fermented dairy products with all these features, different microbial cultures were developed in this study.

3.1. Proteolysis Degree

3.1.1. Cadmium–Ninhydrin Assay

Table 1 shows the degree of proteolysis (free amino groups) in fermented camel milks prepared using combinations of commercial starter culture and strains of *Lactobacillus* during 14 days of cold storage. The results showed that on the 1st day of storage, the degree of proteolysis in fermented milk containing the

Table 1. Proteolysis degree in fermented camel milks prepared using combinations of commercial starter culture and strains of *Lactobacillus* during storage.

Starter culture	Free amino groups (mmol leucine equivalent/mL of extract)		
	Day 1	Day 7	Day 14
Commercial starter culture + *Lb. casei* B-1922	1.97 ± 0.05^{aA}	1.86 ± 0.05^{aB}	1.17 ± 0.02^{bC}
Commercial starter culture + *Lb. paracasei* B-4560	0.93 ± 0.04^{dC}	1.21 ± 0.07^{bA}	1.06 ± 0.08^{cB}
Commercial starter culture + *Lb. rhamnosus* B-442	1.08 ± 0.00^{cA}	1.14 ± 0.07^{bA}	1.07 ± 0.05^{cA}
Commercial starter culture + *Lb. rhamnosus* B-1445	1.15 ± 0.03^{bB}	1.15 ± 0.04^{bB}	1.31 ± 0.02^{aA}

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

commercial starter and *Lb. casei* was the greatest ($p < 0.05$) compared to the other three treatments. Although there were significant differences ($p < 0.05$) among the other three treatments in the degree of proteolysis, the differences were slight. On the 7th day, the highest degree of proteolysis ($p < 0.05$) was also recorded with fermented milk containing commercial starter and *Lb. casei*, while there were no significant differences among the other three treatments in the degree of proteolysis. On the 14th day, samples containing the commercial starter and *Lb. rhamnosus* B-1445 had the highest degree of proteolysis followed by samples with commercial starter and *Lb. casei* and then samples with commercial starter and *Lb. rhamnosus* B-442. Samples containing commercial starter and *Lb. paracasei* were not significantly different from samples containing commercial starter and *Lb. rhamnosus* B-442 in the degree of proteolysis. The differences among fermented milks in the degree of proteolysis could be attributed to the differences among strains in proteinase-endopeptidase activity [20] [21]. Peptides resulting from the hydrolysis of milk proteins by LAB are essential for the sustainable growth of these bacteria in milk [22].

Changes in the degree of proteolysis in fermented milks during storage varied according to the type of starter culture used. Regarding samples with commercial starter and *Lb. rhamnosus* B-1445, the values of proteolysis degree remained constant throughout the first week of storage, followed by a significant increase ($p < 0.05$) in these values on the 14th day of storage. Samples containing commercial starter and *Lb. rhamnosus* B-442 showed stability in the degree of proteolysis throughout the storage period. Proteolysis was significantly increased ($p < 0.05$) on day 7 for samples containing commercial starter and *Lb. paracasei*, followed by a decline on day 14. Fermented milks containing commercial starter and *Lb. casei* showed a continuous decrease ($p < 0.05$) in the degree of proteoly-

sis with increasing storage time.

3.1.2. SDS-PAGE Electrophoresis

SDS-PAGE shows the proteolytic activity of different microbial cultures used in this study on camel milk proteins (Figure 1). A low degree of proteolysis in fermented milk samples could be seen in SDS-PAGE. This could be due to the low differences in the proteolytic system of the lactobacilli strains used in this study. The proteolytic systems of lactobacilli strains have endopeptidase and aminopeptidase enzymes to hydrolyze high molecular weight (Mw) peptides into small Mw peptides and amino acids [23]. However, SDS-PAGE does not allow the visualization of peptides in the 10 kDa and 1 kDa range [24], so the small Mw peptides in that range and amino acids produced by LAB could not be seen on SDS-PAGE. The obtained results from SDS-PAGE and cadmium-ninhydrin assay confirm the early publications, as during milk fermentation only 1% - 2% of milk proteins undergo proteolysis and the principal substrate is casein but limited degradation of whey proteins may also occur [25] [26]. The effect of this proteolysis is that fermented milks have a higher content of peptides and free amino acids [27].

3.2. Titratable Acidity

Titratable acidity of fermented camel milks during storage is presented in Table 2. The results showed that throughout storage the acidity values of fermented milks were in the normal range, between 0.720 and 0.838 [28]. On the first day of storage, the acidity of fermented milks did not differ significantly ($p > 0.05$) except for samples made using the commercial starter and *Lb. rhamnosus* B-442, which showed slightly higher acidity ($p < 0.05$) than the other treatments. On

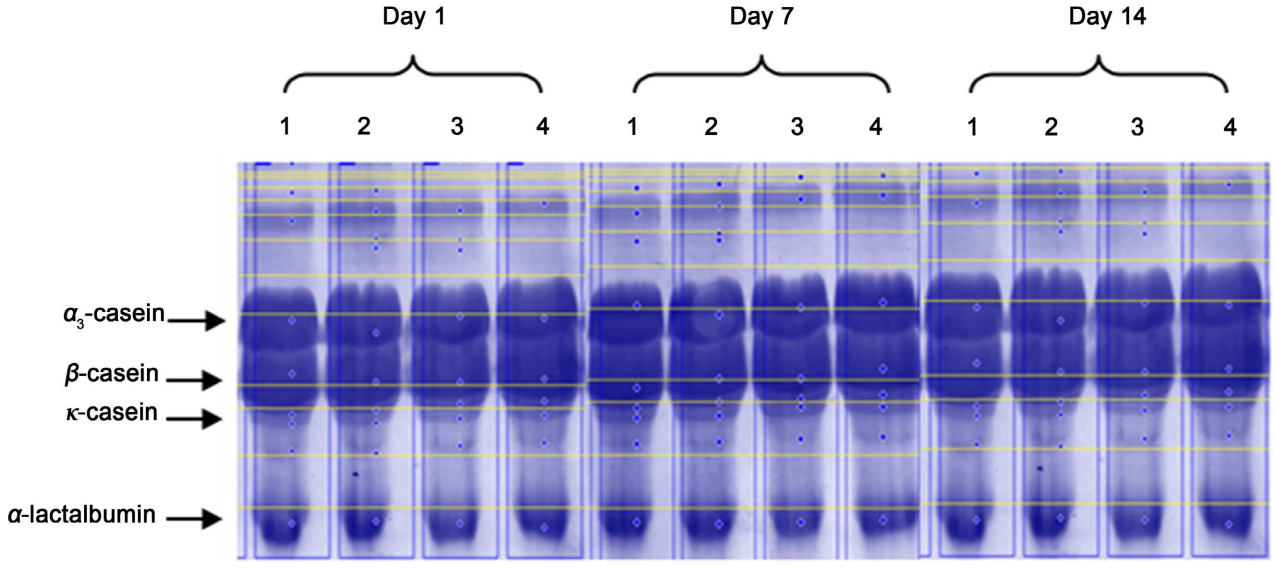

Figure 1. SDS-PAGE of fermented camel milk prepared using combinations of commercial starter culture and strains of *Lactobacillus* during cold storage. (1) Commercial starter culture and *Lb. casei* B-1922; (2) commercial starter culture and *Lb. paracasei* B-4560; (3) commercial starter culture and *Lb. rhamnosus* B-442; (4) commercial starter culture and *Lb. rhamnosus* B-1445.

Table 2. Titratable acidity of fermented camel milk prepared using combinations of commercial starter culture and strains of *Lactobacillus* during period.

Starter culture	Titratable acidity (% lactic acid)		
	Day 1	Day 7	Day 14
Commercial starter culture + *Lb. casei* B-1922	0.748 ± 0.00^{abC}	0.778 ± 0.00^{bB}	0.813 ± 0.00^{bA}
Commercial starter culture + *Lb. paracasei* B-4560	0.724 ± 0.01^{bC}	0.807 ± 0.00^{aB}	0.838 ± 0.01^{aA}
Commercial starter culture + *Lb. rhamnosus* B-442	0.760 ± 0.02^{aB}	0.793 ± 0.01^{abA}	0.787 ± 0.01^{cAB}
Commercial starter culture + *Lb. rhamnosus* B-1445	0.720 ± 0.01^{bB}	0.741 ± 0.01^{cAB}	0.753 ± 0.01^{dA}

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

the 7th and 14th days of storage, the effect of starter culture on the acidity of fermented milk was more pronounced ($p < 0.05$). The highest acidity was observed in the samples made with the commercial starter and *Lb. paracasei*. While fermented milk prepared using commercial starter and *Lb. rhamnosus* B-1445 had the lowest acidity values. The same results were reported by Li *et al.* [21], who found that the pH values of fermented milk were significantly affected by the different strain combinations during storage except for the first day of storage.

Changes in titratable acidity of fermented milks during storage are shown in Table 2. During storage, there was a significant increase ($p < 0.05$) in the acidity of all treatments. After 14 days of storage, the acidity of fermented milk samples reached a maximum value. However, the increase in acidity of samples containing the commercial starter and *Lb. paracasei* or *Lb. casei* was higher and faster than samples with the commercial starter and *Lb. rhamnosus* B-1445 or *Lb. rhamnosus* B-442. A similar trend was reported by Tian *et al.* [29], who found that yogurt fermented by traditional starter and *Lb. casei* LC2W CGMCC NO.0828 exhibited a great increase in titratable acidity and was markedly higher than samples fermented by *Lb. rhamnosus* GG ATCC 53103 during storage. The differences among fermented milks in titratable acidity during storage could be attributed to the efficiency of starter cultures to ferment lactose into lactic acid [11]. Previously, Tian *et al.* [29] reported that the differences between yogurts in acidity during refrigerated storage are due to variation in the post-acidifying activity of the starter cultures. Other studies attributed differences in titratable acidity of fermented milks to the proteolytic activity of the starter cultures, as fermented milks made by starter cultures with higher proteolytic activity exhibited lower ability to produce acid during fermentation and storage [30]. In the current study, there was a negative but non-significant correlation ($p > 0.05$)

between the titratable acidity and the degree of proteolysis. This result agrees with that obtained by Abd El-Fattah *et al.* [7].

3.3. Antioxidant Activity

The antioxidant activity of fermented milks was evaluated by various assays with different mechanisms to elucidate the antioxidant capabilities of fermented milks.

3.3.1. DPPH Radical Scavenging Activity

The DPPH radical scavenging method is based on electron donation of antioxidants to neutralize DPPH radicals. The DPPH radical scavenging activity of fermented camel milks during storage is shown in Table 3. After one day of storage, all treatments showed high radical scavenging activity without any significant differences among them except for samples fermented by the commercial starter and *Lb. casei*, which showed lower scavenging activity value ($p < 0.05$). At the end of storage, the differences in radical scavenging activity among samples were more remarkable ($p < 0.05$) compared to the 1st and 7th day of storage. The highest radical scavenging activity was observed in samples with commercial starter and *Lb. rhamnosus* B-1445 ($p < 0.05$), while samples prepared using a

Table 3. Antioxidant activity and total phenolic content of fermented camel milks prepared using combinations of commercial starter culture and strains of *Lactobacillus* during storage.

Starter culture	DPPH radical scavenging activity (%)			Ferrous ion chelating activity (%)			Ferric reducing power (mg GAE/mL of extract)			Total phenolic content (mg GAE/mL of extract)		
	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
Commercial starter culture + *Lb. casei* B-1922	66.8 ± 3.99^{bA}	27.2 ± 0.55^{bB}	24.4 ± 1.44^{cB}	67.7 ± 0.70^{aA}	60.0 ± 0.88^{bB}	34.1 ± 0.36^{cC}	0.208 ± 0.02^{cB}	0.249 ± 0.01^{aA}	0.103 ± 0.01^{bC}	11.2 ± 0.60^{cB}	12.8 ± 0.10^{cA}	6.3 ± 0.41^{cC}
Commercial starter culture + *Lb. paracasei* B-4560	87.2 ± 2.39^{aA}	23.9 ± 1.34^{bB}	20.9 ± 1.87^{dB}	57.6 ± 1.22^{bA}	57.2 ± 1.36^{bA}	61.3 ± 2.44^{aA}	0.300 ± 0.01^{abA}	0.259 ± 0.02^{aB}	0.251 ± 0.01^{aB}	12.1 ± 0.69^{bcB}	13.8 ± 0.52^{bA}	14.8 ± 0.07^{aA}
Commercial starter culture + *Lb. rhamnosus* B-442	84.8 ± 0.90^{aA}	38.5 ± 0.11^{aB}	29.7 ± 0.59^{bC}	51.1 ± 4.79^{cB}	72.5 ± 0.12^{aA}	65.1 ± 3.16^{aA}	0.336 ± 0.03^{aA}	0.274 ± 0.03^{aB}	0.258 ± 0.02^{aB}	12.9 ± 0.19^{abA}	13.2 ± 0.25^{bcA}	13.3 ± 0.83^{bA}
Commercial starter culture + *Lb. rhamnosus* B-1445	83.6 ± 4.68^{aA}	36.1 ± 3.92^{aB}	34.1 ± 0.64^{aB}	48.8 ± 1.49^{cB}	57.2 ± 4.69^{bA}	41.6 ± 0.70^{bC}	0.276 ± 0.01^{bA}	0.197 ± 0.02^{bB}	0.270 ± 0.02^{aA}	13.5 ± 0.42^{aB}	15.2 ± 0.76^{aA}	15.5 ± 0.15^{aA}

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

mixture of commercial starter and *Lb. casei* or *Lb. paracasei* had the lowest values. These results are consistent with those of El-Sayed *et al.* [5], who reported that the DPPH radical scavenging activity of fermented milk is strain-dependent. Other studies have indicated that the antioxidant activity of fermented milk depends on many factors including the types of starter culture enzymes and the hydrolysis of proteins [31]. In the current study, the differences in the radical scavenging activity among fermented milks could be attributed to the type of peptides released rather than the degree of proteolysis. The results showed that there was no significant correlation ($p > 0.05$) between the degree of proteolysis and DPPH radical scavenging activity of fermented milk. This result is consistent with that of El-Sayed *et al.* [5].

The obtained results clearly showed that the radical scavenging activity of fermented milks was significantly ($p < 0.05$) affected by the storage period. The highest values of radical scavenging activity were achieved after one day of storage for all treatments. These values decreased dramatically ($p < 0.05$) in all fermented milks on the 7th day of storage. At the end of storage, the radical scavenging activity remained stable in all fermented milks as compared to day 7 of storage except for samples containing the commercial starter and *Lb. rhamnosus* B-442 where a decrease in scavenging activity values occurred. A similar trend of DPPH scavenging activity during storage has been reported by Li *et al.* [21] and Kumar and Kumar [32]. The decrease in radical scavenging activity with increasing storage time is likely due to the cleavage in regions of peptides that have antioxidant activity during proteolysis [33].

3.3.2. Ferrous Ion Chelating Activity

Chelation of transition metals, such as iron, is considered one of the mechanisms of antioxidant activity to prevent lipid peroxidation [34]. Ferrous ion chelating activity (FCA) of fermented camel milks during storage is presented in **Table 3**. The results showed that the FCA varied significantly ($p < 0.05$) among samples due to the type of starter culture. At the beginning of storage, the samples containing commercial starter and *Lb. casei* had the greatest value of FCA ($p < 0.05$). On contrary, fermented milks made with mixture of commercial starter and *Lb. rhamnosus* B-442 or B-1445 had the lowest value of FCA without any significant differences between them. On day 7 of storage, the highest value of FCA was observed in fermented milk containing commercial starter culture and *Lb. rhamnosus* B-442, while the other treatments had the lowest chelating ability without any significant differences among them. After 14 days of storage, fermented milks containing the commercial starter and *Lb. rhamnosus* B-442 or *Lb. paracasei* recorded the highest FCA, while samples with the commercial starter and *Lb. casei* had the lowest value.

The changes in FCA of fermented milks during storage are shown in **Table 3**. During storage, there were different trends regarding changes in the ability of fermented milks to chelate ferrous ions. A significant improvement ($p < 0.05$) in the FCA value of samples containing the commercial starter and *Lb. rhamnosus*

B-442 was observed after 7 days of storage, and this value did not differ significantly at day 14 of storage. Similarly, the FCA of the samples containing the commercial starter and *Lb. rhamnosus* B-1445 increased significantly ($p < 0.05$) on the 7th day of storage, but decreased with increasing storage. No significant changes ($p > 0.05$) were noticed in the FCA values of the samples containing the commercial starter and *Lb. paracasei* throughout storage. There was a persistent decrease ($p < 0.05$) in FCA values for samples containing the commercial starter and *Lb. casei* with increasing storage period. The differences in FCA values among fermented milks as well as changes in FCA during storage could be attributed to the type and sequence of amino acids in the iron-binding peptides released due to the proteolytic activity of the starter cultures [5] [7]. The functional groups of amino acid residues within peptides such as phosphate, sulfhydryl, carboxyl, hydroxyl and aromatic rings act as major binding sites for transition metals ions [35]. Besides, the N- and C-terminals of peptides contribute to the metal chelating activity [36]. In the current study, no significant relationship ($p > 0.05$) was found between the concentration of peptides and the chelating activity of fermented milk. A similar correlation between the proteolysis degree and chelating activity was reported [5]. The lack of a direct relationship between the FCA and the degree of proteolysis could underline the importance of the peptide structure for metal chelation [35].

3.3.3. Ferric Reducing Power

The reducing power assay measures the reducing capacity of antioxidants by donating an electron. The reducing power values of fermented camel milks during cold storage were presented in **Table 3**. The results showed that reducing power of fermented milks were significantly ($p < 0.05$) influenced by the type of microbial culture.The differences among samples in reducing power were more noticeable on the 1st day than on the 7th and 14th days of storage. On the first day of storage, the highest reducing power values were observed in samples prepared with the commercial starter and *Lb. rhamnosus* B-442 or *Lb. paracasei*, followed by samples with the commercial starter and *Lb. rhamnosus* B-1445 and then samples containing the commercial starter and *Lb. casei*. On days 7 and 14 of storage, there were no significant differences among the fermented milks in reducing power values, except for those containing the commercial starter and *Lb. rhamnosus* B-1445 on day 7 and those containing the commercial starter and *Lb. casei* on day 14 of storage as they recorded the lowest reducing power values compared to the other samples.

The results also showed that fermented milks differed in terms of the change in reducing power during storage. The values of reducing power increased significantly ($p < 0.05$) in samples containing the commercial starter and *Lb. casei* on day 7 of storage, followed by a sharp decrease in these values with prolonged storage. On the contrary, reducing power values decreased significantly ($p < 0.05$) in samples containing the commercial starter and *Lb. rhamnosus* B-1445 after 7 days of storage, followed by a significant ($p < 0.05$) increase in these val-

ues at the end of storage. Likewise, fermented milks with the commercial starter and *Lb. rhamnosus* B-442 or *Lb. paracasei* showed significant ($p < 0.05$) decrease in reducing power values on day 7; these values did not change significantly by the end of the storage period. Other studies observed a continuous decrease in the reducing power of probiotic yogurts supplemented with fruits over a 15-day storage period [32]. Conversely, Sah *et al.* [37] found an increase in the reducing power of yogurt during storage. The variations among fermented milks in reducing power during storage could be due to their phenolic content. The obtained results showed a significant positive correlation between the reducing power and total phenolic content ($r = 0.63$, $P = 0.029$). This result is in agreement with the findings of Madhu *et al.* [38]. The differences among samples in reducing power during storage may also be attributed to the reductants formed by starter cultures such as organic acids [39]. In addition, peptides released during fermentation and storage that have the ability to donate hydrogen, may also be involved in the reducing ability [39].

3.4. Total Phenolic Content

The total phenolic content (TPC) of fermented camel milks during 14 days of storage is presented in **Table 3**. During the entire storage period, all samples showed varying values of TPC. Fermented milk containing the commercial starter and *Lb. rhamnosus* B-1445 had the highest TPC throughout the storage period ($p < 0.05$), while samples containing the commercial starter and *Lb. casei* had the lowest TPC. Samples with commercial starter and *Lb. rhamnosus* B-442 or *Lb. paracasei* had moderate TPC values. The differences in TPC among treatments may be attributable to the hydrolysis of milk proteins by starter cultures, which may release active compounds during fermentation and storage such as amino acids with phenolic side chains [40]. Further, the presence of some microbial metabolites such as organic acids, certain peptides and free amino acids could interfere in phenolics estimation [41].

Storage period also had a significant ($p < 0.05$) impact on TPC of fermented milks except for samples containing commercial starter and *Lb.* rhamnosus B-442 as there was no significant change in TPC during storage. The values of TPC increased significantly ($p < 0.05$) on day 7, followed by a non-significant increase on day 14 of storage in samples containing commercial starter and *Lb. paracasei* or *Lb. rhamnosus* B-1445. In the latter two samples, TPC increased significantly ($p < 0.05$) by the end of storage compared to the first day. This increase could be related to the metabolic activities of the starter cultures, which can degrade complex phenolic compounds in milk into free phenolics [42]. The TPC value of fermented milk with the commercial starter and *Lb. casei* increased significantly ($p < 0.05$) on the 7th day, however, this value decreased drastically ($p < 0.05$) on the 14th day of storage. The decrease in TPC could be attributed to the degradation of phenolic structures by enzymatic activity of starter culture during storage [43]. The results of Shori [44] revealed that the TPC in plain-camel

yogurt decreased on day 7, and increased on day 14 of storage. Some studies reported an increase in the TPC of plain yogurt throughout storage [40], while others observed a decrease [38].

3.5. Sensory Evaluation

The organoleptic evaluation of fermented camel milks during 14 days of cold storage is shown in **Table 4**. From the results, it is clear that on the first day all samples were acceptable and no significant differences ($p > 0.05$) were observed among them in terms of color, taste, consistency and overall acceptability scores. Similarly, at day 7 the type of starter culture did not significantly affect the sensory attributes except for consistency. The highest consistency scores ($p < 0.05$) were recorded with samples containing the commercial starter and *Lb. rhamnosus* B-1445, while samples containing the starter culture and *Lb. paracasei* scored the lowest. In general, among all samples, fermented milk with commercial starter and *Lb. rhamnosus*B-1445 was the most acceptable product. On the 14th day, there were no significant differences ($p > 0.05$) among treatments in terms of color. Concerning taste and consistency, all treatments had similar scores, which were significantly ($p < 0.05$) higher than those of samples containing the

Table 4. Sensory evaluation of fermented camel milks prepared using combinations of commercial starter culture and strains of *Lactobacillus* during cold storage.

Starter culture	Sensory attributes			
	Color (9)	Taste (9)	Consistency (9)	Overall acceptability (9)
		Day 1		
Commercial starter culture + *Lb. casei* B-1922	8.2 ± 1.10^{aA}	7.0 ± 1.22^{aA}	7.0 ± 1.00^{aA}	7.2 ± 0.84^{aA}
Commercial starter culture + *Lb. paracasei* B-4560	8.2 ± 1.10^{aA}	6.8 ± 1.10^{aA}	7.2 ± 1.30^{aA}	7.2 ± 1.09^{aA}
Commercial starter culture + *Lb. rhamnosus* B-442	8.0 ± 1.41^{aA}	6.8 ± 0.84^{aA}	7.4 ± 0.89^{aA}	7.0 ± 1.00^{aA}
Commercial starter culture + *Lb. rhamnosus* B-1445	8.0 ± 1.41^{aA}	7.4 ± 1.52^{aA}	7.4 ± 1.67^{aA}	7.4 ± 1.52^{aA}
		Day 7		
Commercial starter culture + *Lb. casei* B-1922	8.2 ± 0.50^{aA}	7.3 ± 0.50^{aA}	7.8 ± 0.96^{abA}	7.8 ± 0.96^{aA}
Commercial starter culture + *Lb. paracasei* B-4560	8.5 ± 0.58^{aA}	7.0 ± 1.83^{aA}	7.0 ± 1.41^{bA}	7.5 ± 1.73^{aA}
Commercial starter culture + *Lb. rhamnosus* B-442	7.8 ± 1.26^{aA}	6.8 ± 1.26^{aA}	7.3 ± 0.96^{abA}	6.8 ± 1.89^{aA}
Commercial starter culture + *Lb. rhamnosus* B-1445	8.5 ± 0.58^{aA}	8.0 ± 1.15^{aA}	8.8 ± 0.50^{aA}	8.5 ± 1.00^{aA}
		Day 14		
Commercial starter culture + *Lb. casei* B-1922	8.5 ± 0.71^{aA}	6.0 ± 0.00^{bA}	6.0 ± 0.00^{bA}	6.5 ± 0.71^{bA}
Commercial starter culture + *Lb. paracasei* B-4560	9.0 ± 0.00^{aA}	7.5 ± 0.71^{aA}	7.5 ± 0.71^{aA}	8.0 ± 0.00^{aA}
Commercial starter culture + *Lb. rhamnosus* B-442	8.5 ± 0.71^{aA}	7.5 ± 0.71^{aA}	7.5 ± 0.71^{aA}	7.5 ± 0.71^{abA}
Commercial starter culture + *Lb. rhamnosus* B-1445	9.0 ± 0.00^{aA}	8.0 ± 0.00^{aA}	8.0 ± 0.00^{aA}	8.0 ± 0.00^{aA}

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

commercial starter and *Lb. casei*. Regarding overall acceptability, fermented milks containing the commercial starter and *Lb. rhamnosus*B-1445 or *Lb. paracasei* had the best scores, followed by samples containing commercial starter and *Lb. rhamnosus* B-442 and then samples containing commercial starter and *Lb. casei*. Earlier, Moslehishad *et al.* [45] observed that fermented camel milks prepared with *Lb. rhamnosus* PTCC 1637, *Lb. fermentum* PTCC 1638 or *Lb. plantarum* PTCC 1058 had higher organoleptic quality scores compared to samples prepared using other tested strains. Recently, fermented camel milks containing *Lb. rhamnosus* B-1445 or *Lb. rhamnosus* B-442 received the highest taste and overall acceptability scores compared to other treatments [5]. The results of Pearson's correlation showed that there was no relationship ($p > 0.05$) between the degree of proteolysis in fermented milks and the studied sensory characteristics. These results are in agreement with those of Moslehishad *et al.* [45]. On the other hand, storage time had no significant effect ($p > 0.05$) on all studied sensory attributes. These results are consistent with those of El-Sayed *et al.* [5].

4. Conclusion

Fermented camel milks showed different antioxidative mechanisms depending on the type of starter culture and storage. All fermented milks except those containing the commercial starter and *Lb. casei* had high antioxidant activity and high organoleptic quality. Fermented milks containing commercial starter and *Lb. rhamnosus* B-1445 or *Lb. paracasei* were the most acceptable products.

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Conflicts of Interest

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

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