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The Impact of Using some Adjunct Cultures on the Quality of Fermented Camel Milk Fortified with Iron

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ABSTRACT

Lipid oxidation is one of the key determinants of the quality of iron-fortified dairy products. In this study, using starter cultures possessing a high ability to release metal-binding peptides during fermentation and storage was proposed to address the quality problems related to fortification of fermented milk with iron. For this purpose, a combination of commercial yogurt starter (CS) with *Lactobacillus plantarum* B-4496 (LP) or *Lactobacillus paracasei* subsp. *paracasei* B-4560 (LPC) was used to ferment camel milk, which was fortified with ferrous sulphate at a level of 40 mg iron/kg milk. Fermented milk was stored at 4±1°C for 14 days. Proteolysis degree and thiobarbituric acid (TBA) value were evaluated for all treatments, while iron chelating activity (ICA) and fatty acids composition were determined for the iron-free treatments. The results revealed that starter culture combinations had a significant influence ($p<0.05$) on all studied parameters. Samples containing CS-LPC showed the highest proteolysis degree, ICA and TBA values as compared to CS or CS-LP samples. The presence of iron significantly increased TBA value of fermented milk. Proteolysis degree, ICA and TBA values increased significantly in all treatments with increasing storage time except for the TBA values of iron-free samples and iron-fortified samples containing CS that remained stable during storage. Differences in fatty acids composition were observed among fermented milks and ranged from little to remarkable. The high ICA, particularly in CS-LPC treatment did not prevent the development of lipid oxidation during storage. This study suggests that the ability to chelate iron in fermented milk is likely to be less important than the effect related to the fatty acids composition. Therefore, evaluating the ability of starter cultures to alter the fatty acids composition of dairy products is critical to determine their suitability for use in iron-fortified milks.

Keywords: iron-fortified fermented camel milk, iron chelating activity, adjunct cultures, thiobarbituric acid, fatty acids composition.

INTRODUCTION

Anemia is a serious global public health problem that affects a large number of children and women. More than 30% of the world's population suffers from anemia (WHO, 2016). The most common cause of anemia is iron deficiency, which is usually caused by insufficient dietary intake of iron, poor iron absorption, or both (Gaucheron, 2000). Iron fortification of foods has been reported to be the most cost-effective long-term strategy to address iron deficiency (Fiedler and Macdonald, 2009).

Fermented milk is an important component of the diet worldwide in addition to being a product with distinctive nutritional and therapeutic value (Khan *et al*., 2019). Iron fortification of fermented milk seems to be an effective approach to improve dietary iron intake (Nkhata *et. al*., 2015). Besides, fermented milk has no effect on iron absorption from the diet (Galan *et al*., 1991 and Tidehag *et al*., 1995). Moreover, fermentation of milk using lactic acid bacteria (LAB) caused a marked enhancement in iron and zinc availability from iron-fortified fermented milks (Drago and Valencia, 2002).

Iron is a difficult micronutrient to add to foods due to its potentially negative effect on organoleptic properties (Allen *et al*., 2006). Iron fortification may cause metallic taste, undesirable color changes and unacceptable flavor as a result of the oxidation of fat (Mehansho, 2006). There is a variety of iron compounds used to fortify food, including ferrous sulfate. This compound is cheap and has high bioavailability, but due to its high reactivity, it produces objectionable changes in vehicle-foods reducing their quality and shelf life (Jackson and Lee, 1992). On the other hand, there are iron compounds that produce good-quality product but have limited bioavailability (Allen *et al*., 2006). Some attempts have been made to reduce the harmful effect of iron fortification on the quality of fermented milk. Fortification of yogurt with iron chelated to casein or whey protein (Hekmat and McMahon, 1997) as well as microencapsulation of ferrous sulphate (Nkhata *et al*., 2015 and Sadiq and Doosh, 2019) have been reported as effective techniques to minimize the undesirable changes related to iron fortification.

LAB is a heterogeneous group of bacteria that play an important role in a variety of fermentation processes. LAB can produce a vast number of metabolites responsible for the organoleptic properties, microbial safety, and increased shelf life of the final product (Rodgers, 2008). They are also known for their potential health and nutritional benefits (Nagpal *et al*., 2012). Among LAB, *Lactobacillus* strains have been extensively exploited for their probiotic properties (Jonkers *et al*., 2012). Also, they are being increasingly marketed as adjunct cultures in fermented milk products to give additional properties such as improving the products' flavor, texture or visual appearance (Marinaki *et al*., 2016). Additionally, fermentation with LAB is an inexpensive and effective method for improving the oxidative

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stability of fermented milk. Several studies reported that fermentation of milk by LAB releases a large number of peptides with different biological activities including the capability to chelate iron (Yilmaz-Ersan *et al*., 2016 and Abd El-Fattah *et al*., 2017). The ability of these peptides to chelate iron is attributed to the side-chain of amino acid residues within peptides sequences such as cysteine, histidine, serine, aspartate and glutamate, which possess strong affinities for iron (Walters *et al*., 2018). Chelation of transition metals is considered one of the mechanisms of antioxidant activity to prevent catalysis of hydroperoxide decomposition and lipid peroxidation (Gupta, 2015). In addition, the metal-chelating capacity of peptides can facilitate the absorption of chelated iron and calcium in the human digestive tract (Walters *et al* 2018).

In view of the foregoing, this study assumes that the use of starter cultures possessing a high ability to release metal-binding peptides during fermentation and storage could be beneficial in preventing or reducing lipid oxidation in iron-fortified fermented camel milk during storage. Thus, the harmful effect of iron on product quality could be avoided, besides taking advantage of desirable effects and health benefits of these cultures. For this purpose, two starter culture combinations, previously examined for ICA, were used to ferment camel milk. Fermented milk was fortified with ferrous sulphate at a level of 40 mg iron/ kg milk. Proteolysis degree and thiobarbituric acid (TBA) value were estimated for all treatments. The ICA and fatty acids composition were determined for the iron-free treatments. To our best knowledge, no studies have been addressed on this research point.

MATERIALS AND METHODS

Materials

Camel milk was supplied from Camel Research Center, Marsa Matrouh, Egypt. Lyophilized bacterial cultures namely; *Lactobacillus plantarum* B-4496 and *Lactobacillus paracasei* subsp. *paracasei* B-4560 were kindly provided by the Agriculture Research Service (ARS) Culture Collection, Norwegian Radio Relay League (NRRL) Peoria, USA. Commercial yogurt starter culture (YFL812), which contained *Streptococcus thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, was purchased from Chr. Hansen Laboratories (Copenhagen, Denmark) in direct vat set form. Ferrous sulphate ($FeSO_4.7H_2O$, 20.09% iron) was obtained from Fisons Laboratory Reagent, England.

Methods

Cultures preparation

Bacterial strains, which previously activated and preserved in glycerol stock solution were cultured twice in de Man, Rogosa, Sharpe (MRS) broth at 37°C for 16-18 h prior to use. Bacterial cells were harvested by centrifugation at 2100 × *g* for 15 min, washed twice with 0.85% sterile saline solution and resuspended in 11% (w/v) autoclaved reconstituted cow skim milk (121°C/10 min) before being used**.**

Preparation of fermented milk

Camel milk (pH 6.56±0.0, acidity 0.15±0.01%, moisture 11.3±0.04%, fat 3.4±0.06%, protein 2.6±0.15%) was divided into six portions. Three portions were used to prepare fermented milks without iron fortification. The other three portions were used to produce iron-fortified fermented milks. Ferrous sulphate was added to milk at a concentration of 40 mg iron/ kg milk. Fermented milk was prepared according to Tamime and Robinson (2007). All milks were heated separately at 85°C for 20 min and cooled to 42°C. Fermented milks were made by inoculating milk with 0.02% (w/v) of commercial yogurt starter alone or in combination with *Lactobacillus plantarum* or *Lactobacillus paracasei* subsp. *paracasei* at a level of 7 log cfu/mL. Fermentation was carried out at 42°C until pH 4.7 was reached. Then, fermented milks were stored refrigerated at 4±1°C for 14 days. Treatments were coded as follows: CS: unfortified fermented milk with commercial yogurt starter alone; CS-LP: unfortified fermented milk with CS and *Lactobacillus plantarum*; CS-LPC: unfortified fermented milk with CS and *Lactobacillus paracasei* subsp. *paracasei;* CS-Fe: iron-fortified fermented milk with CS; CS-LP-Fe: iron-fortified fermented milk with CS and LP; CS-LPC-Fe: iron-fortified fermented milk with CS and LPC.

Analyses

Analyses of fermented milk were carried out after 7 days (to give time for the iron to fully interact with milk component) and 14 days of cold storage.

Titratable acidity and pH

The titratable acidity of milk (expressed as lactic acid %) was determined by titration with 0.1 N NaOH using phenolphthalein as an indicator (Ling, 1963). The pH of the milk was measured before and during fermentation using a digital pH meter (Martini, Italy). All analyses were conducted in triplicate.

Chemical composition of milk

Chemical composition of milk (fat, protein and total solids %) was estimated in triplicate using the AOAC procedures (AOAC, 2005).

Preparation of water-soluble extract from fermented milk

Water-soluble extract (WSE) was prepared in accordance with the method of Shori and Baba (2013). Fermented milk (10 g) was 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. Then, the homogenate was centrifuged at 10,000×g for 10 min at 4°C. The pH of supernatant was adjusted to pH 7.0 using 0.1 M NaOH and recentrifuged for further precipitation of proteins and salts. The supernatant was collected and kept at -20°C for further analyses.

Determination of proteolysis degree

Proteolysis degree was assessed by the cadmium–ninhydrin method described by Folkertsma and Fox (1992). The Cd-ninhydrin reagent was prepared by dissolving 0.8 g of ninhydrin in a mixture of 80 mL 99.5% ethanol and 10 mL acetic acid, followed by the addition of 1 g $CdCl_2$ dissolved in 1 mL of distilled water. Briefly, 100 µL of WSE was diluted up to 1 mL with distilled water, and then mixed with 2 mL of Cd–ninhydrin reagent. The mixture was heated at 84°C for 5 min and then cooled to room temperature. The absorbance was measured at 507 nm against blank (1 mL of distilled water with 2 mL of Cd-ninhydrin reagent) using UV/Visible spectrophotometer (Pharmacia-LK-Ultrospec III, Pharmacia, USA). The concentrations of free amino acids 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 carried out in triplicate.

Iron chelating activity

Iron chelating activity (ICA) was carried out as described by Chan *et. al*., (2007). Freshly prepared solution of ferrous sulphate (2 mM) and ferrozine solution (5 mM) were diluted 20 times. Diluted ferrous sulphate (1 mL) was mixed with 1 mL of WSE, followed by 1 mL of diluted ferrozine. The

mixture was incubated for 10 min at room temperature. Absorbance (Abs) was measured at 562 nm against distilled water as blank. The absorbance of control was determined by replacing the WSE with distilled water. The ICA of extracts was calculated using the equation:

Iron chelating activity (%) = (1-Abs $_{sample}$/Abs$_{control}$) × 100

Thiobarbituric acid assay

Thiobarbituric acid (TBA) method for monitoring lipid oxidation in foods was carried out as described by Hekmat and Mcmahon (1997). The test was performed in triplicate. One gram of fermented milk was weighed into a glass screw-top test tube; distilled water was used instead of sample as a blank. Next, 9 mL of 15% (w/v) trichloroacetic acid and 0.375% (w/v) TBA in 0.25N HCl solution was added, mixed well, and heated in a boiling water bath for 15 min. Tubes were then cooled to room temperature and centrifuged at 8000X*g* for 15 min at 20°C. Absorbance was measured in the supernatant at 535 nm against the blank (distilled water instead of sample).

Fatty acids composition

Fat extraction and derivatization

Fat was extracted from milk and fermented milk using a modified Bligh and Dyer chloroform-methanol extraction method (Tapia *et. al*., 2019). Fatty acids methyl esters were prepared using sulphuric-methanol as described by Radwan (1978).

Gas chromatography-mass spectrometry

The Gas chromatography-mass spectrometry (GC-MS) analysis was carried out using a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TR-5 MS column (30 m × 0.32 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 60°C for 1 min; rising at 4°C/min to 240°C and held for 1 min. The injector and detector were held at 210°C. Diluted samples (1:10 hexane, v/v) of 1µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. The identification of the chemical constituents of the essential oil was de-convoluted using AMDIS software (www.amdis.net) and identified by its retention indices (relative to n-alkanes C8-C22), mass spectrum matching to authentic standards (when available)., Wiley spectral library collection and NSIT library database. The percentage of individual fatty acids was calculated from the ratio of their peak area to the total peak area of identified acids×100.

Statistical analysis

Data were evaluated statistically using analysis of variance (ANOVA). Means were compared by Duncan's test at the significance level of $p < 0.05$. All the statistical analysis was done using SAS statistical analysis software package (SAS Procedure Guide 'Version 6.12 Ed.' SAS Institute Inc., Cary, 2004).

RESULTS AND DISCUSSION

Proteolysis degree

Table (1) presents the proteolysis degree in unfortified and iron-fortified fermented camel milk prepared with different combinations of *Lactobacillus* strains with CS and stored at 4°C for 14 days. As evident, there was a significant variation ($p<0.05$) in the proteolysis degree among all fermented milks. The proteolysis degree ranged from 1.08 to 1.80 mM leucine equivalent/mL. In iron-fortified fermented milk, the proteolysis degree in the samples containing CS-LPC was significantly higher ($p<0.05$) than those samples containing CS or CS-LP. The differences between treatments could be due to the variation between the starter culture combinations in proteinase-endopeptidase activity (Sasaki *et. al*., 1995). The iron-free samples had nearly the same behavior as the iron-fortified fermented milks in terms of proteolysis degree. Except on day 7, as the CS-LPC treatment showed the highest proteolysis degree, followed by the CS samples, while CS-LP treatment exhibited the lowest degree of proteolysis ($p<0.05$).

Table 1. The proteolysis degree in iron-fortified and unfortified fermented camel milk prepared with combinations of starter cultures during cold storage.

Treatment	Total free amino acids (mM leucine equivalent/mL)	
	Day 7	Day 14
CS	1.18±0.02^{cB}	1.32±0.07^{cdA}
CS-LP	1.08±0.03^{dB}	1.23±0.06^{dA}
CS-LPC	1.34±0.02^{bB}	1.66±0.09^{bA}
CS-Fe	1.16±0.05^{cB}	1.39±0.05^{cA}
CS-LP-Fe	1.16±0.02^{cB}	1.42±0.05^{cA}
CS-LPC-Fe	1.40±0.03^{aB}	1.80±0.02^{aA}

CS: Commercial starter culture without iron; CS-LP: CS + *L. plantarum* without iron; CS-LPC: CS + *L. paracasei* subsp. *paracasei* without iron; CS-Fe: CS with iron; CS-LP-Fe: CS + *L. plantarum* with iron; CS-LPC-Fe: CS + *L. paracasei* subsp. *paracasei* with iron. 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 results also showed that there was a significant increase ($p<0.05$) in the proteolysis degree in all fermented milks with increasing storage time. Studies have revealed that proteases and peptidases released from LAB are active during fermentation and post-storage in fermented dairy products (Li *et. al*., 2019). In addition, Iron fortification did not affect the degree of proteolysis in the samples containing CS, while it significantly improved ($p<0.05$) the degree of proteolysis in samples containing CS-LP or CS-LPC compared to the iron-free samples.

Iron chelating activity

Figure (1) illustrates the iron chelating activity (ICA) of unfortified fermented milks prepared using combinations of starter cultures on the 7th and 14th day of storage. As shown, all treatments showed high ability to chelate iron. The ICA of fermented milk could be attributed to the iron-binding peptides which are released from milk proteins due to the proteolytic activity of the starter cultures (Abubakr *et. al*., 2012; Yilmaz-Ersan *et. al*., 2016 and Abd El-Fattah *et. al*., 2017). The functional groups of amino acid residues within peptides such as phosphate, carboxyl, hydroxyl, sulfhydryl and methyl as well as aromatic rings act as principal sites for the binding of transition metals (Walters *et. al*., 2018). In addition, the effect of antioxidants found naturally in milk such as caseins and whey proteins (Khan *et. al*., 2019) cannot be ignored. This ability increased significantly ($p<0.05$) with increasing storage time. In another study, the chelating capability of kefir samples increased with increased storage, the maximum value was reached after 21 days of storage (Yilmaz-Ersan *et. al*., 2016).

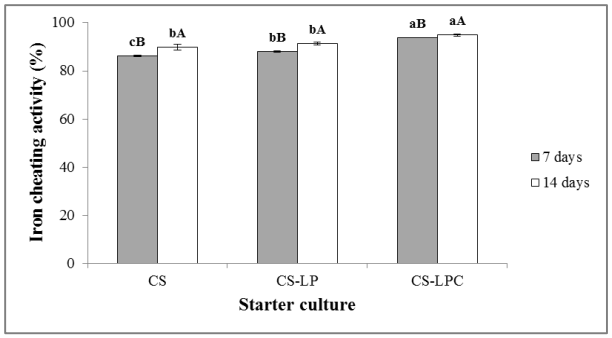

Fig. 1. Iron chelating activity of unfortified fermented camel milks prepared using combinations of starter cultures during cold storage.

CS: Commercial starter culture without iron; CS-LP: CS + *L. plantarum* without iron; CS-LPC: CS + *L. paracasei* subsp. *paracasei* without iron. Mean values (± standard deviation) with different small letters (effect of starter culture) are significantly different; means with different capital letters (effect of storage) are significantly different (p <0.05).

During storage, the starter culture combinations had a significant impact ($p < 0.05$) on the ICA of fermented milk. On the 7th day of storage, the CS-LPC treatment had the highest ICA (93.7%), followed by CS-PL, which exhibited moderate chelation (88.0%), while CS treatment showed the lowest ICA (86.2%). After 14 days of storage, this arrangement differed somewhat as the chelating activity of CS-LPC treatment was still the highest in chelating ability, while, no significant ($p>0.05$) difference was noticed between CS-LP and CS samples for chelating iron. Differences in ICA among fermented milks could be attributed to the degree of proteolysis as well as the type and sequence of amino acids in peptides released by starter cultures during fermentation (Abd El-Fattah et. al., 2017) and storage. Previously, Kunji et. al., (1996) stated that LAB showed specificity towards particular cleavage sites or sequences during proteolysis. Recently, Walters et. al., (2018) have reported that the structure of peptides determines the degree of chelation.

TBA value

Lipid oxidation is one of the key determinants of the quality of iron-fortified dairy products. TBA assay is a method for the determination of malondialdehyde (MDA), a secondary product of lipid oxidation resulting from breakdown of hydroperoxide. Table (2) shows the effect of iron fortification and starter culture combinations on the TBA values, measured as absorbance at 535 nm, of fermented camel milk during the storage. It is evident that the type of starter culture had a significant effect ($p<0.05$) on the TBA values in either iron-fortified or unfortified fermented milks.

Regarding iron-free treatments, samples containing CS-LPC showed the highest TBA values ($p<0.05$) throughout the storage period as compared to CS or CS-LP samples. Whereas, there were no significant differences ($p>0.05$) between samples containing CS and those containing CS-LP. Storage time had no significant impact ($p>0.05$) on the TBA value for these treatments. Jackson and Penumetcha (2019) have reported that lipid oxidation is not a concern in yogurt due to the low pH, low storage temperature, and low oxygen exposure; however, enrichment of yogurt with iron or ω-3 fatty acids could shift this balance toward lipid oxidation. This is indeed what happened in iron-fortified fermented milks, as the presence of iron significantly increased ($p<0.05$) the TBA values of these samples compared to those free from iron. These results are in agreement with those reported by Dabour *et. al.*, (2019) and Nkhata *et. al.*, (2015). On the 7th day, samples containing CS-LPC were the highest in the TBA value, followed by the samples prepared using CS or CS-LP. It was also observed that there were no significant differences in TBA values between CS and CS-LP samples. By the end of the 2nd week, the samples differed significantly among themselves in TBA values. The highest value of TBA was recorded in the samples containing CS-LPC followed by those containing CS-LP followed by the samples with CS.

Table 2. Changes in thiobarbituric acid value in iron-fortified and unfortified fermented camel milk with combinations of starter cultures during cold storage.

Treatment	TBA value (Absorbance, 535 nm)	
	Day 7	Day 14
CS	0.151 ± 0.004^{dA}	0.141 ± 0.002^{eA}
CS-LP	0.120 ± 0.01^{dA}	0.134 ± 0.02^{eA}
CS-LPC	0.191 ± 0.01^{cA}	0.183 ± 0.01^{dA}
CS-Fe	0.298 ± 0.02^{bA}	0.293 ± 0.01^{cA}
CS-LP-Fe	0.266 ± 0.01^{bB}	0.339 ± 0.02^{bA}
CS-LPC-Fe	0.559 ± 0.04^{aB}	0.942 ± 0.01^{aA}

CS: Commercial starter culture without iron; CS-LP: CS + *L. plantarum* without iron; CS-LPC: CS + *L. paracasei* subsp. *paracasei* without iron; CS-Fe: CS with iron; CS-LP-Fe: CS + *L. plantarum* with iron; CS-LPC-Fe: CS + *L. paracasei* subsp. *paracasei* with iron. 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).

During storage, the TBA value for iron-fortified samples containing CS was stable, while the storage time had a significant influence ($p<0.05$) on the TBA values of other two treatments. By the end of the storage, the TBA value increased dramatically in the samples containing CS-LPC, while the increase in TBA value was lower for the samples containing CS-LP. The increase in the TBA values during storage may be attributed to increased MDA formation due to continued oxidation. Previous studies have reported that iron-fortified samples showed a continuous increase in TBA value during storage (Dabour *et. al.*, 2019 and Nkhata *et. al.*, 2015).

In this study, despite the high ICA, particularly in the samples containing CS-LPC, it did not prevent the development of lipid oxidation during storage. It is known that the fatty acids composition of food is one of the main factors affecting lipid oxidation. It is therefore possible that as a result of the activity of starter cultures during fermentation and storage, specific fatty acids were formed that promoted the lipid oxidation, particularly in the iron-fortified fermented milks. Many studies revealed that lactic starters had the ability to alter the composition of fatty acids of fermented milk products (Abdelrahman, 2019 and Jrad *et. al.*, 2020). To verify this explanation, the fatty acids composition of unfortified fermented milks was determined on day 14 of storage as the effect of starter culture type and storage on the TBA value of the iron-fortified samples was more pronounced.

Fatty acids composition

Data in Table (3) presents the fatty acids composition of milk and unfortified fermented milks prepared with CS, CS-LP and CS-LPC after 14 days of storage. As shown, camel milk fat contained high content of long-chain fatty acids, the predominant fatty acids were C14:0, C16:0, C16:1, C18:0 and C18:1. The major polyunsaturated fatty acid was linoleic acid. These findings are in accordance with findings reported by Gorban and Izzeldin (2001) and Konuspayeva *et. al.*, (2008). The results also showed that the total saturated and unsaturated fatty acids in camel milk were 62.23 and 37.76%, respectively.

Previously, Gorban and Izzeldin (2001) found that the total content of saturated and unsaturated fatty acids of camel milk was 66.1 and 30.5%, respectively. The data in Table (3) are close to those mentioned in the literature, with some differences due to several factors, including animal species, seasons and regions (Konuspayeva *et. al*., 2008), management system and lactation stage (Chamekh *et. al*., 2020).

Table 3. Fatty acids composition (%) of camel milk and unfortified fermented milk with starter cultures after 14 days of cold storage.

Fatty acids	Milk	Treatment		
		CS	CS-LP	CS-LPC
Saturated fatty acids				
Capric acid, C10:0	0.23	0.21	0.21	0.41
Lauric acid, C12:0	1.06	0.93	0.95	1.53
Tridecanoic acid, C13:0	0.33	ND	ND	0.30
Myristic acid, C14:0	14.59	14.49	14.59	13.79
Pentadecanoic acid, C15:0	3.46	2.48	2.25	0.22
Palmitic acid, C16:0	22.99	23.30	23.64	24.47
Heptadecanoic acid, C17:0	3.30	3.53	3.00	1.67
Stearic acid, C18:0	16.27	16.57	16.34	13.57
Nonadecanoic acid, C19:0	ND	0.15	ND	ND
Arachidic acid, C20:0	ND	ND	0.24	ND
Unsaturated fatty acids				
Myristoleic acid, C14:1 *cis* 9	1.21	1.20	1.20	1.15
Myristelaidic acid, C14:1 *trans* 9	ND	ND	ND	0.33
Hexadecenoic acid, C16:1	11.34	11.40	10.95	12.07
trans-palmitoleic acid, C16:1 *trans* 7	ND	ND	0.54	ND
Octadecenoic acid, C18:1	21.33	21.79	21.92	21.00
Elaidic acid, C18:1 trans 9	ND	ND	ND	3.33
Linoleic acid, C18: 2 *cis* 9,12	3.57	3.30	3.52	5.15
8,11-Octadecadienoic acid, C18: 2	ND	0.28	0.19	ND
cis-11-Eicosenoic acid, C20:1	0.31	0.38	0.29	ND
Arachidonic acid, C20:4 *cis* 5, 8, 11, 14	ND	ND	0.15	0.64
Erucic acid, C22:1 cis 13	ND	ND	ND	0.30
Total saturated fatty acids	62.23	61.66	61.22	55.96
Total unsaturated fatty acids	37.76	38.35	38.76	43.97

CS: Commercial starter culture without iron; CS-LP: CS + *L. plantarum* without iron; CS-LPC: CS + *L. paracasei* subsp. *paracasei* without iron. ND: Not detected

The results also showed a change in the fatty acids composition of fermented milk compared to milk. This change ranged from very slight to considerable, depending on the type of starter culture. This could be attributed to the different enzymatic activities of the starter cultures during fermentation and/or storage. LAB were found to have lipolytic activity, which varies in degree from strain to strain (Carini *et. al*., 1972). Pecová *et. al*., (2019) have reported no significant changes observed in the fatty acids composition of yogurt produced using yogurt starter culture in comparison with milk. A study by Alm (1982) revealed that fermentation of milk resulted in relatively small changes in fat composition. While, other studies have found changes in the fatty acids composition of fermented milks compared to the milk (Ahmed *et. al*., 2016a; Abdelrahman 2019 and Jrad *et. al*., 2020).

In all fermented milks, palmitic, stearic and myristic acids were the major saturated fatty acids. The fatty acid C18:1 followed by C16:1 was the major monounsaturated fatty acid. The major polyunsaturated fatty acid was linoleic acid. These results are in agreement with those reported by Abdelrahman (2019). There was a little change in the composition of saturated and unsaturated fatty acids in fermented milks containing CS or CS-LP compared to the milk. But what distinguished the sample containing CS-LP from the one containing CS was the presence of two unsaturated fatty acids, *trans*-palmitoleic acid and arachidonic acid, which were not identified in milk. Although these two acids are present in low concentrations, they may be responsible for the little increase in TBA values of iron-fortified fermented milk containing CS-LP.

On the other hand, fermentation of milk using CS-LPC resulted in a remarkable change in the fatty acids profile. There was an increase in the total unsaturated fatty acids and a decrease in total saturated fatty acids compared to the other two treatments. Similar results were observed in dromedary yogurt obtained by a co-fermentation process with autochthonous bacteria (*Enterococcus faecium* and *Streptococcus macedonicus*) in comparison with control made using commercial yogurt cultures (Jrad *et. al*., 2020). Concerning unsaturated fatty acids, Myristelaidic acid (C14:1 *trans* 9), Elaidic acid (C18:1 *trans* 9) and Erucic acid (C22:1 *cis* 13) were detected in elevated concentrations in the samples containing CS-LPC and were not identified in milk or in the other two fermented milks. Previously, a varied content of C18:1 and C18:2 *trans* isomers have been recognized in stored yogurt made from cow, goat or sheep milk (Paszczyk *et. al*., 2020). Moreover, the linoleic acid (C18:2, ω6) was higher in the samples containing CS-LPC (approx. one and a half times) compared to the milk and other fermented milks. This finding is compatible with the result reported by Jrad *et. al*., (2020) who found that linoleic acid was higher in the co-fermentation process with autochthonous bacteria. However, Abdelrahman (2019) reported that linoleic acid was increased in fermented camel milk, either by *Lactobacillus acidophilus* or commercial yogurt cultures. In addition, arachidonic acid (C20: 4, ω6) was 4.3 folds higher in the samples with CS-LPC than those containing CS-LP but not detected in milk or fermented milk prepared using CS. The presence of *trans* and polyunsaturated fatty acids at these concentrations in fermented milk containing CS-LPC could be the reason for the higher TBA value in these samples than the fermented milks containing CS or CS-LP. The fatty acids composition is known to impact oxidative stability (Havemose *et al*., 2004). Unsaturated fatty acids, mainly ω-9, ω-6, and ω-3 series, are highly sensitive to oxidation (Ahmed *et al*., 2016b). The risk of oxidation increases with increasing the degree of unsaturation (Belanche *et. al*., 2017). Besides, the presence of iron triggered the lipid oxidation (Amaral *et. al*., 2018).

Control of iron is important in iron-fortified products. Iron stimulates lipid oxidation by generating reactive oxygen species, which negatively impact the quality and shelf life of food and food products. In this study the use of starter cultures with high ability to produce iron-chelating peptides was proposed to address the quality problems related to iron fortification of fermented milk. Where, metal-chelating property can sequester and stabilize iron, thus inhibiting or limiting lipid oxidation during storage. In current study, the results of TBA were unexpected because the high chelating activity in the samples containing CS-LP or CS-LPC did not prevent the development of lipid oxidation. Fermentation of camel milk using combinations of commercial starter culture and *Lactobacillus* strains, particularly L. *paracasei* subsp. *paracasei* resulted in a change in the proportion of individual fatty acids. This was coupled with a change in the molecular fatty acids configuration, resulting in variations in the proportion of fatty acid isomers. Perhaps these changes made lipid extremely sensitive to oxidation. Besides, the difference in the ICA values between treatments was not very large. Therefore, the ability to chelate iron in fermented milks is likely to be less important than the effect related to the fatty acids composition. Therefore, evaluating the extent to which starter cultures are able to alter the fatty acids composition in dairy products is critical to determining their

suitability for use in the manufacture of iron-fortified fermented dairy products.

CONCLUSION

From the results obtained, it can be concluded that the presence of iron did not adversely affect the proteolytic activity of starter cultures. However, it significantly increased TBA value of fermented milk. The high ICA, particularly in CS-LPC sample did not prevent the development of lipid oxidation during storage. Lactic starters had the ability to alter the composition of fatty acids of fermented milk. Fatty acids composition of food is one the major factors affecting lipid oxidation. Adjunct cultures must be selected carefully before manufacturing iron-fortified fermented milk to avoid the harmful effect of iron on the product quality.

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تأثير استخدام بعض البادئات المساعدة على جودة لبن الإبل المختمر المدعم بالحديد

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قسم تربية الحيوان ، شعبة الإنتاج الحيواني والدواجن ، مركز بحوث الصحراء ، المطرية ، القاهرة ، ج.م.ع

اكسدة الدهون هي أحدى المحددات الرئيسية لجودة منتجات الألبان المدعمة بالحديد. في هذه الدراسة تم اقتراح استخدام مزارع بادئات ذات قدرة عالية على إطلاق ببتيدات خالبة للحديد أثناء التخمر والتخزين كوسيلة لمعالجة المشاكل المتعلقة بجودة اللبن المختمر المدعم بالحديد. لهذا الغرض، تم استخدام مزيج من مزرعة البادئ التجارية المستخدمة فى تصنيع اليوجورت (CS) مع *L. plantarum* B-4496 (LP) أو مع *L. paracasei* subsp. *paracasei* B-4560 (LPC) لتخمير لبن الإبل. تم تدعيم اللبن بكبريتات الحديدوز بمستوى 40 مجم حديد/ كجم لبن. تم تخزين اللبن المختمر على 4 ± 1 °م لمدة 14 يومًا. تم تقدير درجة تحلل البروتين و قيمة حمض الثيوباربيتوريك (TBA) لجميع المعاملات بينما تم تقدير النشاط الخالب الحديد (ICA) ، وتركيب الأحماض الدهنية للمعاملات الغير مدعمة بالحديد. أظهرت النتائج أن مزرعة البادئ كان لها تأثيرا معنويا ($p<0.05$) على جميع الصفات المدروسة. أظهرت العينات التي تحتوي على CS-LPC أعلى درجة تحلل البروتين ، ICA و TBA مقارنةً بالعينات المحتوية على CS أو CS-LP . أدى التدعيم بالحديد إلى زيادة معنوية في قيمة TBA للبن المختمر. زادت درجة تحلل البروتين و ICA و TBA بشكل ملحوظ في جميع المعاملات مع زيادة وقت التخزين باستثناء قيم TBAللعينات الخالية من الحديد والعينات المدعمة بالحديد المحتوية على CS والتي ظلت مستقرة طوال التخزين. لوحظت اختلافات في تركيب الأحماض الدهنية بين الألبان المختمرة والتى تراوحت من البسيطة الى الكبيرة. لم يمنع ارتفاع ال ICA ، خاصة في العينات المحتوية CS-LPC ، من تطور أكسدة الدهون أثناء التخزين. تشير هذه الدراسة إلى أنه من المحتمل ان تكون القدرة على خلب الحديد في اللبن المختمر أقل أهمية من التأثير المرتبط بتركيب الأحماض الدهنية. لذلك، فإن تقييم مدى قدرة مزارع البادئات على تغيير تركيب الأحماض الدهنية للمنتجات اللبنية يعد أمرا بالغ الأهمية لتحديد مدى ملاءمتها للاستخدام في تصنيع الألبان المختمرة المدعمة بالحديد. بصفة عامة، يجب إختيار مزارع البادئات بعناية فائقة قبل استخدامها فى تصنيع المنتج اللبنى المختمر المدعم بالحديد لتجنب التأثير الضار للحديد على جودة المنتج.