DOI : 10.5958/2277-8934.2023.00039.5 *Vol 30 No 3, p 311-321*

DEVELOPMENT AND FUNCTIONAL APPRAISAL OF FERMENTED CAMEL MILK BEVERAGE

Hiral Kumari Patel, Sreeja V. and Kiran S.
Dairy Microbiology Department, SMC College of Dairy Science, Kamdhenu University, Anand, Gujarat, India, 388110

ABSTRACT

In spite of the potential health benefits the camel milk possess, there is a scarcity of fermented camel milk products in India. This research explored fermentation as a means to develop a fermented camel milk beverage with acceptable sensory and enhanced health benefits. Lactic strains used in the study included *Streptococcus thermophilus* MTCC 5460 (MD2), *Lacticaseibacillus rhamnosus* MTCC 5462 (I4), *Lactiplantibacillus plantarum* M11, *Lactobacillus helveticus* MTCC 5463 (V3) and *Lacticaseibacillus casei* NK9. Each lactic strains were studied for their growth behaviour in camel milk which was found insignificant among strains. The strains in different combinations (A=MD2+I4, B=MD2+M11, C=MD2+V3 and D=MD2+I4) were used to form starter cultures A, B, C and D for beverage preparation. The sensory acceptability and lactic count of beverage B was significantly ($p<0.05$) high, hence it was selected. To enhance the flavour of the beverage, cumin and black salt were added in different combinations on w/w basis (B1=0.25 cumin+0.3 black salt, B2= 0.5 cumin+0.4 black salt, B3=0.75 cumin+0.5 black salt, B4=0.3 cumin+0.4 black salt, B5= 0.5 cumin+0.4 black salt) in which combination B2 showed significantly higher ($p<0.05$) overall acceptability. Shelf life of fermented camel milk beverage was evaluated at 7±1°C. pH of the beverage decreased significantly ($p<0.05$) from 4.70 to 4.12 with significant ($p<0.05$) decrease in scores of flavour (8.25 to 5.57), body and texture (8.50 to 8.14) and overall acceptability (8.34 to 5.23) throughout the storage period. Overall acceptability score decreased to <6.0 on 18th day of storage. Beverage had a shelf life of 15 days. The percentage values of biofunctional attributes viz., ACE inhibition, α-amylase inhibition, α-glucosidase inhibition, antioxidant activity and proteolytic activity (mg/mL of histidine) of the fresh beverage was 49.86, 55.90, 35.96, 21.87 and 7.80, respectively which increased significantly ($p<0.05$) to 58.69, 58.23, 38.88, 28.50 and 8.27, respectively at the end of shelf life.

Key words: Biofunctional attributes, camel milk, fermented beverage, growth and acidification profile, shelf life, *streptococcus thermophilus* MTCC 5460

Camel milk is considered as a medicinal food owing to its strong immune-modulatory, antioxidative (Habib *et al,* 2013), antibacterial (Mojtahedi *et al,* 2018), antiviral, antifungal, anti-hepatitis, hypoglycemic and anti-cancerous activities (Gizachew *et al,* 2014; Kaskous, 2016; Jilo and Tegegne, 2016). But camel milk possesses a typical sensory characteristic contributed by its components and salty flavour which makes it less desirable for direct consumption by the consumer. Fermentation of camel milk may lead to a product with acceptable sensory attributes and enhanced nutritional and biofunctional activities.

Fermented camel milk products namely Gariss, Suusac and Shubat are traditionally consumed in countries such as Sudan, Kenya and Central Asia-particularly in Kazakhstan, Uzbekistan and Turkmenistan, respectively (Farah *et al,* 1990; Abdelgadir *et al,* 1998). Very scanty literature is available regarding fermentation of camel milk by lactic cultures. Additionally, in contrast to the milk from other dairy species, the viscosity of the product made from camel milk remains same during the fermentation process owing to the protein composition (Jumah *et al,* 2001) as well as the naturally occurring antimicrobial compounds present in camel milk (Attia *et al,* 2001). Fermented beverage type products therefore seems to have great promise.

In India, fermented camel milk products are not available in the market. This research explored fermentation as a means to develop a fermented camel milk beverage with improved sensory and functional attributes.

Materials and Methods

Lactic strains

Lactic Acid Bacteria (LAB) strains used in the study *viz. Streptococcus thermophilus* MTCC 5460 (MD2), *Lacticaseibacillus rhamnosus* MTCC 5462 (I4), *Lactiplantibacillus plantarum* M11 (M11), *Lactobacillus helveticus* MTCC 5463 (V3) and *Lacticaseibacillus casei* NK9 (NK9) were obtained from Dairy Microbiology

SEND REPRINT REQUEST TO HIRAL KUMARI PATEL email: hiralpatel7380@gmail.com

Department, SMC College of Dairy Science, Kamdhenu University, Anand, Gujarat, India. The strains were activated in sterilised reconstituted skim milk (12% T.S.) at 37±1ºC and stored at 5±2ºC. The strains were given three successive transfers prior to their use in reconstituted skim milk to ensure its activity during the course of study.

Camel milk

Camel milk was procured from Gujarat Cooperative Milk Marketing Federation Ltd., Anand, Gujarat, India. Cumin and black salt were purchased from local market, Anand, Gujarat, India. The roasted cumin powder was strained through wire mesh to obtain fine powder.

Evaluation of growth and acidification profile of Lactic Acid Bacteria (LAB) strains in camel milk

Each lactic strains were inoculated in the heated (90°C / 10 min), cooled (37°C) milk @ 2% and incubated at 37±1°C. During incubation, samples were drawn at interval of 0, 6, 12, 18, 24, 30, 36, 42 and 48 h and analysed for pH, titratable acidity and lactic count.

Estimation of Titratable acidity and pH

Titratable acidity (TA) was determined by method mentioned in FSSAI (2015). The product pH was determined using a pH meter (Chemi Line, Ahmedabad, India, Benchtop Meter with probe and stand). The time required to reach pH 4.7 was considered the fermentation time and expressed as hours.

Microbiological analysis

Microbiological analysis was performed as described in Chaudhary and Sreeja (2020). Briefly, serial dilutions of the samples were prepared in phosphate buffer and from selected dilution, 1 mL was transferred to petri dishes in duplicates. Then, 15-20 mL of sterile agar (de Man, Rogosa and Sharpe Agar for Lactobacilli and M17 for Streptococcal count) was poured in petri plates and mixed properly. Once the agar got solidified, second layer of respective agar was poured and allowed to solidify. Incubation was carried out at 37±1°C for 48-72 h. The typical colonies were counted and the count was expressed as log CFU/mL (IS: 1479-3, 1977).

Selection of starter culture for preparation of fermented camel milk beverage

Lactic strains were used in four combinations (A: MD2+I4, B: MD2+M11, C: MD2+V3 and D: MD2+NK9) in order to prepare the starter cultures. Fermented camel milk beverage was prepared according to the flow chart shown in Fig 1. The best starter culture was selected on the basis of sensory attributes and lactic count of the fermented beverage. This selected starter culture was used in the further study.

Vacreated Camel milk
(Vacreation at 70°C and 250 mm Hg vacuum)
↓
Heat treatment (90°C/10 min)
↓
Cooled to 40°C
↓
Inoculation with starter culture @ 2% (1:1)
↓
Filled in sterilised glass bottles
↓
Incubation at 37±1°C till pH 4.7
↓
Cooling and Storage (7±1°C)

Fig 1. Method for preparation of fermented camel milk beverage.

Sensory evaluation

Sensory evaluation of the beverages were carried out by expert judges (n=10) using 9-point Hedonic scale.

Viscosity

Viscosity of the beverage was measured at 25°C using Brookfield viscometer (model LVDV-E Viscometer, Brookfield) with a constant shear rate using spindle No. 62 at 12 RPM. Viscosity was measured in terms of centipoise (cP).

Optimisation of level of cumin and black salt for the beverage

Fermented camel milk was incorporated with different levels (w/w) of cumin and black salt. Different combinations included B1=0.25 cumin and 0.3 black salt, B2= 0.5 cumin and 0.4 black salt, B3=0.75 cumin and 0.5 black salt, B4=0.3 cumin and 0.4 black salt and B5= 0.5 cumin and 0.4 black salt on w/w basis. The level of cumin and black salt was optimised based on the sensory score obtained on 9-point Hedonic scale. Highest scoring sample was taken as the optimised product.

Compositional Analysis

Camel milk and fermented camel milk beverage were analysed for its Total solids, fat, protein, lactose, salt, vitamin C and ash). Total solid, fat and lactose content were estimated by Gravimetric, Gerber and Lane Eynon methods, respectively as described in the BIS Handbook (IS: SP-18, Part-XI, 1981). Protein content was estimated by following macro-Kjeldahl method as described in AOAC (2010). Ash content of the sample was estimated using the procedure in BIS handbook (IS: SP 18: Part XI, 1981). Mohr's method was used to estimate salt content (FSSAI, 2015). Vitamin C content was evaluated using 2,6-dichlorophenol indophenol method (IS: 5838, 1970).

Evaluation of shelf stability of fermented camel milk beverage

To determine the shelf life of fermented camel milk beverage, the beverage was packaged in sterilised glass bottles and stored at refrigerated condition (7±1°C) and was analysed for sensory evaluation attributes, titratable acidity, pH, lactic count at interval of 3 days till the end of its shelf life (sensory score <6 on 9-point hedonic scale).

Evaluation of Biofunctional attributes of fermented camel milk beverage

Fermented camel milk beverage was evaluated *in vitro* for its ACE inhibitory activity, antidiabetic activity, antimicrobial activity, antioxidant activity and proteolytic activity. Unfermented milk was used as control.

ACE-inhibitory activity, antidiabetic activity (measured as α-Amylase inhibitory activity and α-Glucosidase inhibitory activity), antimicrobial activity and antioxidant activity of samples was determined as described in Chaudhary and Sreeja (2020). Antimicrobial activity was evaluated against *Bacillus cereus* MTCC 1272, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* MTCC 1687, *Salmonella typhimurium* ATCC 14028 and *Staphylococcus aureus* MTCC 737. Antioxidant activity was evaluated by 2, 2'-Azino-bis 3- ethylbenzothaizoline-6-sulfonic acid (ABTS) Assay. Proteolytic activity was evaluated using o-phthalaldehyde (OPA) method (Thakkar *et al,* 2018).

Statistical analysis

The data related to composition of milk and beverage were analysed using two sample t-test. The data related to Optimisation of starter culture, flavour, shelf life, biofunctional attributes, growth behavior and antimicrobial activity were analysed using Completely Randomised Design (CRD) and Factorial CRD.

Results and Discussion

Growth and acidification profile of LAB strains in camel milk

The comparative changes in pH and titratable acidity of camel milk fermented by different strains are shown in Fig 2 and 3, respectively. Decrease in pH of camel milk was found to be similar in all the cultures (Fig 2). Titratable acidity of the camel milk increased significantly ($p<0.05$) throughout the incubation of 48 hours. All cultures had shown similar increase in acidity up to 18 hours of incubation. After that, M11 and NK9 showed slow increase in acidity while MD2, V3 and I4 showed fast increase in acidity and then reached maximum at 48th hour of incubation (Fig 3).

Different cultures exhibited almost similar growth behaviour in camel milk (Fig 4). From 0 to 6 hours, all strains exhibited relatively slower growth. After that, the strains showed log phase up to 24 hours of incubation. V3 showed significantly ($p<0.05$) low microbial count than all other cultures. Stationary growth of cultures was observed from 24 to 36 hours, and after that the count decreased till the end of incubation.

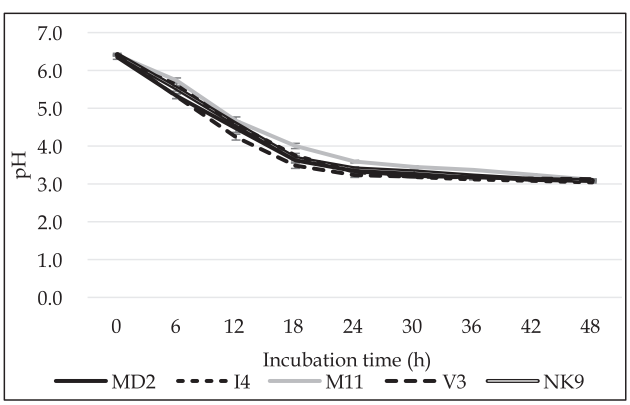

Fig 2. Comparative changes in pH of camel milk inoculated with different cultures.

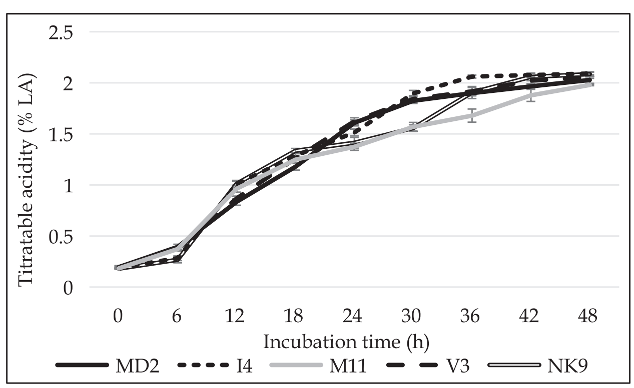

Fig 3. Comparative changes in titratable acidity of camel milk inoculated with different cultures.

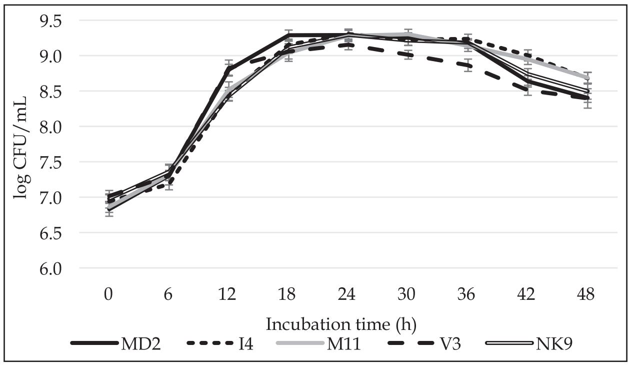

Fig 4. Comparative changes in lactic count of camel milk inoculated with different cultures.

Abu-Taraboush *et al* (1998) studied the growth characteristics of *Bifidobacterium breve* NCFB 2258, *Bifidobacterium bifidum* NCFB 2715, *Bifidobacterium longum* ATCC 15707 and *Bifidobacterium angulatum* ATCC 27535 in camel milk and bovine milk. They observed rapid growth of Bifidobacteria in both the milks. They also reported that decrease in pH during first 10 hours of incubation was minimum, but after that pH decreased significantly up to 36 hours of incubation. We have also observed that starter strains initially grew slowly but after 6 hours of incubation, they grew rapidly in camel milk.

Screening of starter culture for preparation of fermented camel milk beverage

Effect of starter cultures on the fermentation time

The fermentation time taken by different starter cultures to reach pH 4.7 was observed to be significantly ($p<0.05$) different (Table 1). Starter culture B took minimum time (9.54 h) to reach pH 4.7, which was followed by C (9.86 h) and A (10.10 h), respectively. D took the maximum time (10.73 h) to reach pH 4.7. Fermentation ability of the starter cultures was reported to be slower in camel milk in comparison to that of bovine, goat as well as sheep milk (El Zubeir *et al,* 2012; Berhe *et al,* 2018). Camel milk was reported to inhibit the growth of microorganisms owing to the presence of antibacterial and antiviral protective enzymes and proteins like lactoferrin, lactoperoxidase, lysozymes, immunoglobulins (Ig) and peptidoglycan recognition protein (El Sayed *et al,* 1992; Mojtahedi *et al,* 2018). This characteristic of camel milk has been proved a constraint in the manufacture of fermented milk products like yoghurt.

El Zubeir *et al* (2012) studied the processing properties of yoghurt made from nonbovine milk such as camel, goat and sheep milks and found that camel milk took maximum incubation time among all. Berhe *et al* (2018) compared the acidification activities of commercial starter culture in bovine and camel milk. Higher acidification activity was observed in bovine milk as compared to camel milk at their corresponding incubation temperature. Time taken by starter culture to reach pH 4.6 in case of camel milk was found to be significantly higher (1:15 to 4:10 h) than that in bovine milk.

Effect of starter cultures on the lactic count

Lactic count (Lactobacilli and Streptococcal count) of camel milk beverage fermented by different starter cultures is depicted in Table 1. Lactobacilli counts were highest in beverage B (9.89) and D (9.87) and were found to be at par with each other, which is followed by beverage A (9.57) and C (9.32), respectively. Similarly, Streptococcal counts (log CFU/mL) were highest in beverage B (9.85) and D (9.71) and were found to be at par with each other, which is followed by beverage A (9.58) and C (9.29), respectively. Beverages B and D had the highest Lactobacilli as well as Streptococcal counts and were found to be at par with each other.

Rahman *et al* (2009) studied the viable starter culture counts of camel milk fermented by selected bacterial starter cultures. After the fermentation of 6 hours, viable starter culture count of camel milk inoculated with St. *thermophilus* 37, Lb. *delbrueckii* sp. *bulgaricus* CH2, Lc. *lactis*, Lb. *acidophilus* and mixed yoghurt culture in log10CFU/mL were 7.61, 8.03, 6.71, 7.52 and 8.2, respectively. Varga *et al* (2014) evaluated the viability of cultures in honey

Table 1. Effect of different starter cultures on fermentation time, lactic count and viscosity of fermented camel milk beverages.

Beverages	Average fermentation time (h) to reach pH 4.7 at 37°C	Lactobacilli count (log CFU/mL)	Streptococcal count (log CFU/mL)	Viscosity (cP) at 25°C
A	10.10^{b}±0.12	9.57^{b}±0.11	9.58^{b}±0.13	15.13^{b}±1.78
B	9.54^{d}±0.09	9.89^{a}±0.16	9.85^{a}±0.14	34.38^{a}±2.07
C	9.86^{c}±0.07	9.32^{c}±0.12	9.29^{c}±0.10	17.63^{b}±1.95
D	10.73^{a}±0.09	9.87^{a}±0.12	$9.71a^{b}$±0.14	32.50^{a}±1.77

A = MD2+I4, B = MD2+M11, C= MD2+V3, D= MD2+NK9. Each observation is mean of four replications. Values with different superscripts in the column differ significantly ($p<0.05$).

enriched fermented camel milk. Starter culture used were consists of Lb. *acidophilus* LA-5, *Bifidobacterium animalis* spp. *lactis* BB-12 and *Streptococcus thermophilus* CHCC 742/2130. After the fermentation of milk till pH 4.7, Lb. acidophilus LA-5 counts were 7.11 and 7.58 log^{10}CFU/mL, *Bifidobacterium animalis* spp. *lactis* BB-12 counts were 7.38 and 7.36 log^{10}CFU/mL and *Streptococcus thermophilus* CHCC 742/2130 counts were 9.03 and 9.01 log10CFU/mL in control and honey enriched fermented camel milk, respectively.

Effect of starter cultures on the viscosity

Beverages B (34.38 cP) and D (32.50 cP) were reported to have significantly ($p<0.05$) higher viscosity as compared to that of beverages A (15.13 cP) and C (17.63 cP) as shown in Table 1. The effect of starter cultures on viscosity of a camel milk beverage has been least reported. However, the studies depicting the effect of incorporation of additives on the viscosity of fermented camel milk has been reported. Ibrahim and Khalifa (2015a) reported that fortification of fermented camel milk with dietary fibre had viscosity of 16.54 mPas and this viscosity was found to be increasing with increase in rate of addition of dietary fibre. EI-Deeb *et al* (2017) prepared the flavoured fermented camel milk with the addition of cinnamon and doum palm water extract. Cinnamon extract was added at the rate of 1%, 2% and 3% while doum palm extract was added at the rate of 5%, 7 % and 9 %. Viscosity of fermented camel milk was found to be 21 mPas and it was found to increase with the addition of cinnamon extract up to 2% while addition of cinnamon extract at the rate of 3% and addition of doum palm were found to decrease the viscosity. Shahein *et al* (2022) evaluated the effect of addition of date syrup on physicochemical properties of fermented camel milk. Viscosity of fermented camel milk was found to be 30 ± 4.84 cP and it was found a increased significantly with the increase in rate of addition of date syrup.

Effect of starter cultures on the sensory attributes

The beverages prepared using all starter cultures were found to be significantly ($p<0.05$) different from each other in terms of flavour, body and texture and overall acceptability (Table 2). Beverage B was found to be superior in flavour (7.77), body and texture (8.29) and overall acceptability (8.26). However, all the beverages were found to be at par with each other in terms of acidity and colour and appearance.

Rahman *et al* (2009) evaluated the sensory attributes of camel milk fermented by selected bacterial starter cultures. Starter cultures used in the study were *Streptococcus thermophilus* 37, *Lactobacillus delbrueckii* sp. *bulgaricus* CH2, *Lactococcus lactis, Lactobacillus acidophilus* and mixed yoghurt culture (*St. thermophilus* and *Lb. bulgaricus* in 1:1 ratio). Sensory scores indicated that camel milk fermented by mixed yoghurt culture was the most acceptable in all

Table 2. Effect of starter cultures on the sensory attributes of fermented camel milk beverages.

Beverages	Sensory scores (9-point Hedonic scale)			
	Flavour	Body and Texture	Colour and appearance	Overall acceptability
A	$6.89^{d}\pm0.04$	$7.75^{b}\pm0.11$	7.78 ± 0.15	$7.13^{c}\pm0.15$
B	$7.77^{a}\pm0.13$	$8.29^{a}\pm0.08$	7.89 ± 0.15	$8.26^{a}\pm0.11$
C	$7.25^{c}\pm0.06$	$7.76^{b}\pm0.11$	7.83 ± 0.09	$7.31^{c}\pm0.16$
D	$7.47^{b}\pm0.06$	$8.07^{a}\pm0.17$	7.86 ± 0.13	$7.68^{b}\pm0.10$

A = MD2+I4, B = MD2+M11, C= MD2+V3, D= MD2+NK9. Each observation is mean of four replications. Values with different superscripts in the column differ significantly ($p<0.05$).

Table 3. Effect of cumin and black salt addition on sensory attributes of fermented camel milk beverages.

Beverages	Sensory scores (9-point Hedonic scale)			
	Flavour	Body and Texture	Colour and appearance	Overall acceptability
B1	$7.38^{b}\pm0.17$	7.59 ± 0.11	$7.47^{bc}\pm0.14$	$7.29^{b}\pm0.09$
B2	$8.11^{a}\pm0.11$	7.86 ± 0.08	$8.06^{a}\pm0.14$	$8.06^{a}\pm0.15$
B3	$7.67^{b}\pm0.17$	7.68 ± 0.20	$7.29^{c}\pm0.09$	$7.58^{b}\pm0.20$
B4	$7.46^{b}\pm0.23$	7.80 ± 0.12	$7.61^{bc}\pm0.15$	$7.32^{b}\pm0.11$
B5	$7.41^{b}\pm0.15$	7.52 ± 0.18	$7.73^{b}\pm0.13$	$7.38^{b}\pm0.17$

B1 = 0.25 w/w cumin and 0.4 w/w black salt, B2 = 0.5 w/w cumin and 0.4 w/w black salt, B3 = 0.75 w/w cumin and 0.4 w/w black salt, B4 = 0.5 w/w cumin and 0.3 w/w black salt, B5 = 0.5 w/w cumin and 0.5 w/w black salt. Each observation is mean of three replications. Values with different superscripts in the columns differ significantly ($p<0.05$).

attributes, which is followed by camel milk fermented by *Lb. acidophilus, Lb. bulgaricus, St. thermophilus* and *Lc. lactis*, respectively. Ibrahim (2015) studied the effect of using EPS producing starter cultures on sensory attributes of fermented camel milk. He reported that yoghurt made with EPS producing starter culture were preferred for body and texture, colour and appearance and overall acceptability while yoghurt made with the use of non-EPS producing starter cultures was preferred for flavour. This was because yoghurt made with EPS producing starter cultures were reported to have significantly lower acetaldehyde content.

From the above observations, beverage B prepared using starter culture comprising of MD2 and M11 was found to be the most acceptable in terms of sensory attributes and lactic count.

Optimisation of level of cumin and black salt for the beverage

In order to improve the flavour of the beverage, we incorporated cumin and black salt into it and various rates on sensory score of fermented camel milk beverage is shown in Table 3. Combination B2 scored highest marks for flavour (8.11), colour and appearance (8.06) as well as overall acceptability (8.06). Body and texture as well as acidity were found to be similar in all combinations. Thus, combination B2 (0.5 w/w cumin and 0.4 w/w black salt) was selected. Studies depicting the use of cumin and black salt as flavouring for fermented camel beverage were not seen previously.

EI-Deeb *et al* (2017) studied the effect of addition of water extract of *Cinnamomum verum* (cinnamon) and *Hyphaene thebaica* (doum palm) in fermented camel milk. Sucrose solution @ 6% was added to milk before heating. Cinnamon extract was added at the rate of 1%, 2% and 3% while doum palm extract was added at the rate of 5%, 7% and 9%. Sensory evaluation has shown that addition of cinnamon extract at the rate of 1 and 2% and addition of doum palm extract at the rate of 5% significantly improved the total scores. Addition of cinnamon extract at the rate of 3 % and addition of doum palm extract at the rate of 7 and 9 % resulted in significantly fewer total scores in sensory evaluation.

Shahein *et al* (2022) investigated the effect of incorporation of date syrup on sensory attributes of fermented camel milk. Date syrup was added at the rate of 6% and 8% in camel milk before pasteurisation. They found that fermented camel milk added with 8% date syrup had significantly high scores for all sensory attributes, i.e. flavour, consistency, appearance and total score which is followed by fermented camel milk added with 6% date syrup.

Compositional Analysis of beverage

The composition of camel milk and fermented camel milk beverage is shown in Table 4. Total solids, ash and chloride content increased significantly ($p<0.05$) in fermented camel milk beverage than that in camel milk. This was due to addition of cumin and black salt in the beverage. Fat and protein content of camel milk and fermented camel milk were found to be similar. However, lactose and vitamin C content of camel milk were found to be higher in camel milk as compared to that in fermented camel milk. Lactose was utilised by starter culture during fermentation as an energy source which resulted in decrease in lactose in fermented camel milk beverage. However, vitamin C is heat sensitive and thus, it might get destroyed during the heat treatment given to milk which resulted in decrease in vitamin C content of fermented camel milk beverage.

Our results were in agreement with that of Magdi *et al* (2010) who found a significant decrease in the vitamin C content in the fermented camel milk in comparison to that in camel milk. They also found a significant reduction in lactose content in fermented camel milk than that in camel milk. Yoganandi *et al* (2014) evaluated composition of camel milk from Kutch region of Gujarat, India. Average total solids, fat, protein, lactose, ash and chloride content reported by them were 9.95, 2.90, 2.66, 3.77, 0.84 and 0.25%, respectively. These results were found to be similar to our results.

Determination of shelf stability of beverage

To evaluate the shelf life of fermented camel milk beverage, it was filled in sterilised glass bottles

Table 4. Composition of camel milk and fermented camel milk beverage.

Parameter	Total Solids (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)	Chloride content (%)	Vitamin C (mg/100mL)
Camel milk	9.34±0.03	2.83b±0.04	2.39b±0.02	3.51±0.01	0.749± 0.005	0.201±0.002	1.31±0.04
Fermented camel milk beverage	10.43±0.04	2.88a±0.04	2.42a±0.03	3.02±0.02	0.873±0.004	0.248±0.006	0.46±0.01

Each observation is mean of three replications.

and stored at refrigeration temperature (7±1°C). Beverage was evaluated for pH, titratable acidity, lactic count, sensory evaluation and biofunctional attributes.

Effect of storage period on pH and titratable acidity

The changes in pH and titratable acidity (% LA) of fermented camel milk beverage during storage are shown in Table 5. pH was found decreased significantly ($p<0.05$) due to slow and continuous lactic acid production by starter cultures used in beverage. Fresh beverage had an average pH of 4.70 which decreased to 4.12 on 18th day of storage. Titratable acidity of the fresh beverage was 0.71 % LA which increased significantly ($p<0.05$) throughout the storage period and reached 1.25 % LA on 18th day of storage. Ibrahim and Khalifa (2015a) studied the changes in pH and titratable acidity of fermented camel milk yoghurt fortified with fibre. They found significant reduction in pH and significant increase in titratable acidity of all yoghurts during the storage of 21 days. El-Deeb *et al* (2017) studied the changes in pH and titratable acidity of fermented camel milk flavoured with cinnamon and doum palm extracts. They found significant decrease in pH and simultaneously significant increase in titratable acidity of all fermented camel milks during the storage of 21 days. However, increase in acidity of flavoured fermented camel milk was less than that in control (fermented camel milk).

Effect of storage period on Lactobacilli and Streptococcal count (Table 5)

Both counts increased significantly ($p<0.05$) initially up to 6 days and then significant decrease was found ($p<0.05$) throughout the storage period. Hassan *et al* (2007) reported an increase in the lactobacilli counts of Gariss during first 6 days of storage and a subsequent significant decrease in the counts throughout the storage period of 10 days.

Ibrahim and Khalifa (2015a) studied the starter culture count of fermented camel milk yoghurt fortified with orange fibre and date fibre individually. Rate of addition of fibre were 1.5, 3 and 4.5%. *Streptococcus thermophilus* counts were found to be increased significantly up to 7 days of storage and later decrease a significantly up to 21 days of storage was seen. However, increase in *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis* counts were observed up to 14 days of storage, and later a decrease significantly up to 21 days. All the counts were found to be increased with increase in

Table 5. Changes in pH, titratable acidity and lactic count of fermented camel milk beverage during storage.

Storage period (in Days)	pH	Titratable acidity (% Lactic Acid)	Lactobacilli count (log CFU/mL)	Streptococcal count (log CFU/mL)
0	4.70^{a}±0.01	0.71^{g}±0.01	9.67^{cd}±0.10	9.51^{d}±0.11
3	4.59^{b}±0.02	0.83^{f}±0.02	10.11^{ab}±0.17	9.99^{b}±0.13
6	4.54^{c}±0.02	0.94^{e}±0.01	10.25^{a}±0.14	10.29^{a}±0.09
9	4.43^{d}±0.01	0.99^{d}±0.01	10.14^{ab}±0.11	10.03^{b}±0.11
12	4.33^{e}±0.01	1.05^{c}±0.02	9.87^{bc}±0.09	9.76^{c}±0.10
15	4.25^{f}±0.02	1.11^{b}±0.01	9.53^{d}±0.14	9.57^{cd}±0.10
18	4.12^{g}±0.01	1.25^{a}±0.01	9.15^{e}±0.12	9.18^{e}±0.07

Each observation is mean of three replications. Values with different superscripts in the columns differ significantly ($p<0.05$).

Table 6. Changes in the sensory attributes of fermented camel milk beverage during storage.

Storage period (Days)	Sensory scores (9-point Hedonic scale)				
	Flavour	Body and Texture	Acidity	Colour and appearance	Overall acceptability
0	8.25^{a}±0.10	8.50^{a}±0.11	8.40^{a}±0.05	8.50±0.12	8.34^{a}±0.07
3	7.96^{b}±0.07	8.38^{ab}±0.08	7.99^{b}±0.07	8.50±0.10	7.84^{b}±0.11
6	7.59^{c}±0.11	8.35^{ab}±0.07	7.54^{c}±0.07	8.46±0.08	7.58^{c}±0.11
9	7.25^{d}±0.08	8.34^{ab}±0.05	7.29^{d}±0.10	8.44±0.10	7.31^{d}±0.09
12	6.80^{e}±0.18	8.27^{bc}±0.09	6.74^{e}±0.17	8.37±0.06	7.04^{e}±0.13
15	6.53^{f}±0.11	8.24^{bc}±0.08	6.52^{f}±0.13	8.33±0.06	6.76^{f}±0.15
18	5.57^{g}±0.14	8.14^{c}±0.11	6.20^{g}±0.06	8.30±0.05	5.23^{g}±0.16

Each observation is mean of three replications. Values with different superscripts in the columns differ significantly ($p<0.05$).

rate of addition of date fibre and these were further higher in orange fibre. Ibrahim (2015) prepared camel milk yoghurt made with EPS producing and non-EPS producing starter cultures. *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* were found to be increased up to 14 days of storage and later, the counts decreased up to 21 days of storage. All these studies supported our findings that culture count were found to be increased in initial storage days and later, these were found to be decreased till the end of storage study.

Effect of storage period on sensory attributes (Table 6)

Sensory scores of fermented camel milk beverage during shelf life decreased significantly ($p<0.05$) to 5.57, 8.14, 6.20 and 5.23 on 18th day of storage. However, the change in the colour and appearance score was not significant. Judges reported a slight bitter off-flavour which may be due to proteolytic activity. On the basis of sensory score, fermented camel milk beverage was rejected on 18th day of storage. The camel milk beverage had a shelf life of 15 days. Ibtisam and Marowa (2009) reported that Gariss (fermented camel milk) prepared from pasteurised milk had a shelf life of 17 days at 8°C. Ibrahim and Khalifa (2015b) reported sensory scores of camel milk yoghurt added with stabilisers decreased significantly during the 21 days of storage. El-Deeb *et al* (2017) studied the sensory attributes of fermented camel milk flavoured with cinnamon and doum palm extract. Flavour, body and texture, acidity, appearance and total score of all fermented milk were found to be decreased significantly throughout the storage of 21 days.

Evaluation of the biofunctional attributes of fermented camel milk beverage (Table 7)

ACE inhibitory activity of fresh fermented camel milk beverage was 49.86% which increased significantly ($p<0.05$) to 59.56% on 18th day of storage. This increase in ACE inhibitory activity might be due to proteolytic activity leading to peptides possessing such activity. High proline content in camel milk is another reason responsible for higher ACE inhibitory activity in fermented camel milk (El-Salam and El-Shibiny, 2013).

The α-amylase inhibition activity of fermented camel milk beverage increased throughout storage period. Fresh beverage had inhibitory activity of 55.90% which increased to 58.69% on 18th day of storage. However, the inhibition activity of beverage on days 3, 6 and 9 were found at par with each other. α-glucosidase inhibition activity of fresh beverage was 35.96% which increased significantly ($p<0.05$) to 39.15% on 18th day of storage. α-glucosidase inhibition activity was highest on 9th day of storage and after that, change in the activity was not significant.

Fresh beverage had antioxidant activity of 21.87% which increased significantly ($p<0.05$) to 29.52% on 18th day of storage. Higher proteolytic activity resulted in productions of functional peptides which are responsible for high antioxidant activity. Further, presence of several amino acids in peptides can improve antioxidant properties (Aluko, 2012).

Ayyash *et al* (2018a) evaluated the biofunctional attributes of fermented camel milk and compared it with fermented bovine milk. *Lactococcus lactis* KX881782, one of the probiotics isolated from camel milk, was compared with probiotic strain *Lb. acidophilus* DSM9126. Camel milk fermented by *Lactococcus lactis* KX881782 had significantly higher ACE inhibitory activity than that in bovine milk throughout the storage. Further, the ACE inhibitory activity increased significantly throughout the storage. α-amylase inhibition activity of fermented camel milk was found to be increased significantly throughout the storage of 21 days while that of change in fermented bovine milk was found to be

Table 7. Changes in the biofunctional attributes of fermented camel milk beverage during storage.

Storage period (Days)	ACE inhibitory activity (%)	α-amylase inhibition activity (%)	α-Glucosidase inhibition activity (%)	Antioxidant activity (%)	Proteolytic activity (mg/mL)
0	49.86^{g}±0.45	55.90^{f}±0.35	35.96^{d}±0.37	21.87^{g}±0.23	7.80^{f}±0.03
3	52.68^{f}±0.34	56.51^{e}±0.15	37.21^{c}±0.33	23.72^{f}±0.51	7.88^{e}±0.03
6	54.28^{e}±0.48	56.94^{de}±0.20	38.70^{b}±0.25	25.57^{e}±0.31	7.97^{d}±0.04
9	56.55^{d}±0.40	57.12^{d}±0.15	39.28^{a}±0.21	26.39^{d}±0.19	8.08^{c}±0.03
12	57.86^{c}±0.24	57.63^{c}±0.21	39.11^{ab}±0.11	27.45^{c}±0.24	8.15^{c}±0.02
15	58.69^{b}±0.40	58.23^{b}±0.12	38.88^{ab}±0.13	28.50^{b}±0.28	8.27^{b}±0.03
18	59.56^{a}±0.21	58.69^{a}±0.14	39.15^{ab}±0.28	29.52^{a}±0.25	8.35^{a}±0.03

Each observation is mean of three replications. Values with different superscripts in the column differ significantly ($p<0.05$).

nonsignificant. Camel milk fermented by *Lactococcus lactis* KX881782 had shown higher α-amylase inhibition activity than that in bovine milk. However, they could not observe any significant changes in the α-glucosidase inhibition activity during storage. Proteolytic activity was found to be increased significantly with the increase in storage period. Camel milk fermented by *Lactococcus lactis* KX881782 had higher antioxidant activity than that in camel milk fermented by *Lb. acidophilus* DSM9126. However, change in antioxidant activity throughout the storage was found to be nonsignificant except in bovine milk fermented by *Lb. acidophilus* DSM9126, in which it was found to be increased during storage. Ayyash *et al* (2018b) compared the biofunctinal attributes of camel milk and bovine milk both of which were fermented by probiotic strains. Probiotic strains used in the study were *Lb. plantarum* DSM2648, *Lb. reuteri* KX881777, Lb. *plantarum* KX881772 and *Lb. plantarum* KX881779. They observed significantly higher ACE inhibitory activity in fermented camel milk than that in fermented bovine milk except in case of *Lb. plantarum* DSM2648, in which fermented bovine milk possessed significantly higher ACE inhibitory activity than that in camel milk. They also observed that ACE inhibitory activity of all fermented milk increased significantly during the storage of 21 days. All fermented milks were reported to have α-amylase inhibition of more than 34%, except camel milk fermented by *Lb. plantarum* KX881772. Further, camel milk fermented by *Lb. plantarum* KX881772 was reported to have significantly lower α-amylase inhibition activity than that in bovine milk fermented by same strain. Except bovine milk fermented by *Lb. plantarum* KX881779, α-amylase inhibition activity of all were found to be increased significantly throughout the storage of 21 days. They observed that α-glucosidase inhibition activity and proteolytic activity was increased significantly during the storage of 21 days. Antioxidant activity of camel milk fermented by all strains were reported to be significantly higher than that in fermented bovine milk. Further, antioxidant activity was reported to increased significantly throughout the storage of 21 days. Camel milk fermented by *Lb. reuteri* KX881777 and *Lb. plantarum* KX881779 shown higher antioxidant activity than camel milk fermented by *Lb. plantarum* DSM2648 and *Lb. plantarum* KX881772. Lafta *et al* (2014) evaluated the antimicrobial activity of fermented camel milk. Starter cultures used in the study were *Lb. delbrueckii subsp. bulgaricus* and *St. thermophilus*, individually and mixed culture. Zone of inhibition (mm) of camel milk fermented by *Lb. delbrueckii subsp. bulgaricus* against *P. aeruginosa*, *E. coli* and *S. aureus* were 30.1, 29.8 and 23.5 mm, respectively while that of camel milk fermented by *St. thermophilus* against the same were 27.2, 25.4 and 22.1 mm, respectively. Zone of inhibition of camel milk fermented by mixed culture against *P. aeruginosa, E. coli* and *S. aureus* were 32.4, 30.2 and 25.5 mm, respectively.

Conclusion

Type of starter culture has a significant impact on the quality attributes of fermented camel milk beverage, hence, proper selection of starter culture is an important step. As per our results, *Streptococcus thermophilus* MTCC 5460 + *Lactiplantibacillus plantarum* M11 can be successfully employed for preparation of fermented camel milk beverage. Sensory attributes of fermented camel milk beverage can be further improved by addition of cumin and black salt. The developed beverage had a shelf life of 15 days at refrigeration temperature. The beverage had promising biofunctional attributes. The study concluded that camel milk can be successfully processed to fermented camel milk beverage having acceptable sensory attributes as well as enhanced biofunctional activities.

Conflict of interest

The authors declare no competing interests.

References

Abdelgadir WS, Ahmed TK and Dirar HA. The traditional fermented milk products of the Sudan. International Journal of Food Microbiology. 1998; 44(1-2):1-13. https://doi.org/10.1016/s0168-1605(98)00090-7

Abu-Taraboush HM, Al-Dagal MM and Al-Royli MA. Growth, viability and proteolytic activity of Bifidobacteria in whole camel milk. Journal of Dairy Science. 1998; 81(2):354-361. https://doi.org/10.3168/jds.S0022-0302(98)75584-5

Aluko RE. Bioactive peptides. Functional Foods and Nutraceuticals. 2012; pp 37-61. https://doi.org/10.1007/978-1-4614-3480-1_3

AOAC. Official Methods of Analysis of Association of Official Analytical Chemists. 2010. 18th Edition, Washington, DC.

Attia H, Kherouatou N and Dhouib A. Dromedary milk lactic acid fermentation: microbiological and rheological characteristics. Journal of Industrial Microbiology and Biotechnology. 2001; 26:263-270. https://doi.org/10.1038/sj.jim.7000111

Ayyash M, Al-Dhaheri AS, Al Mahadin S, Kizhakkayil J and Abushelaibi A. *In vitro* investigation of anticancer, antihypertensive, antidiabetic and antioxidant activities of camel milk fermented with camel milk probiotic: A comparative study with fermented bovine milk. Journal of Dairy Science. 2018a; 101(2):900-911. https://doi.org/10.3168/jds.2017-13400

Ayyash M, Al-Nuaimi AK, Al-Mahadin S and Liu SQ. *In vitro* investigation of anticancer and ACE-inhibiting activity, α-amylase and α-glucosidase inhibition, and antioxidant activity of camel milk fermented with camel milk probiotic: A comparative study with fermented bovine milk. Food Chemistry. 2018b; 239:588-597. https://doi.org/10.1016/j.foodchem.2017.06.149

Berhe T, Ipsen R, Seifu E, Kurtu MY, Eshetu M and Hansen EB. Comparison of the acidification activities of commercial starter cultures in camel and bovine milk. LWT. 2018; 89:123-127. https://doi.org/10.1016/j.lwt.2017.10.041

Chaudhary JK and Sreeja V. Effect of incorporation of Finger millet (Eleusine coracana) on the antimicrobial, ACE inhibitory, antioxidant and antidiabetic potential of a milk-millet composite probiotic fermented product. Indian Journal of Dairy Science. 2020; 73(3):222-230. https://doi.org/10.33785/IJDS.2020.v73i03.005

El Sayed I, Ruppanner R, Ismail A, Champagne CP and Assaf R. Antibacterial and antiviral activity of camel milk protective proteins. Journal of Dairy Research. 1992; 59(2):169-175. https://doi.org/10.1017/S0022029900030417

El Zubeir IEM, Basher MAE, Alameen MH, Mohammed MAS and Shuiep ES. The processing properties, chemical characteristics and acceptability of yoghurt made from non bovine milks. Development. 2012; 24:3.

El-Deeb A, Dyab A and Elkot W. Production of flavoured fermented camel milk. Ismailia Journal of Dairy Science and Technology. 2017; 5(1):9-20.

El-Salam MA and El-Shibiny S. Bioactive peptides of buffalo, camel, goat, sheep, mare, and yak milks and milk products. Food Reviews International. 2013; 29(1):1-23. https://doi.org/10.1080/87559129.2012.692137.

Farah Z, Streiff T and Bachmann MR. Preparation and consumer acceptability tests of fermented camel milk in Kenya. Journal of Dairy Research. 1990; 57(2):281-283. https://doi.org/10.1017/S002202990002690X

FSSAI. Lab manual of methods of analysis of foods milk and milk products. Food Safety and Standard Authority of India, Ministry of Health and Family Welfare, Government of India, New Delhi. 2015. https://www.fssai.gov.in/upload/uploadfiles/files/MILK_AND_MILK_PRODUCTS.pdf (Accessed on 12 April, 2023)

Gizachew A, Teha J, Birhanu T and Nekemte E. Review on medicinal and nutritional values of camel milk. Nature and Science. 2014; 12(12):35-41.

Habib HM, Ibrahim WH, Schneider-Stock R and Hassan HM. Camel milk lactoferrin reduces the proliferation of colorectal cancer cells and exerts antioxidant and DNA damage inhibitory activities. Food Chemistry. 2013; 141(1):148-152. https://doi.org/10.1016/j.foodchem.2013.03.039

Hassan RA, El Zubeir IE and Babiker SA. Effect of pasteurisation of raw camel milk and storage temperature on the chemical composition of fermented camel milk. International Journal of Dairy Science. 2007; 2(2):166-171.

Ibrahim AH and Khalifa SA. Improve sensory quality and textural properties of fermented camel's milk by fortified with dietary fibre. Journal of American Science. 2015a; 11(3):42-54.

Ibrahim AH. and Khalifa SA. The effects of various stabilisers on physiochemical properties of camel's milk yoghurt. Journal of American Science. 2015b; 11(1):15-24.

Ibrahim AH. Effects of exopolysaccharide-producing starter cultures on physicochemical, rheological and sensory properties of fermented camel's milk. Emirates Journal of Food and Agriculture. 2015; pp 373-384. https://doi.org/10.9755/ejfa.v27i4.19911

Ibtisam EM and Marowa II. Effect of pasteurisation of milk on the keeping quality of fermented camel milk (Gariss) in Sudan. Livestock Research for Rural Development. 2009; 21(2). Retrieved on March 8, 2023, from https://lrrd.cipav.org.co/lrrd21/2/zube21019.htm

IS 1479-3. Methods of test for dairy industry, Part III: Bacteriological analysis of milk, Indian Standards Institution, New Delhi. 1977. https://law.resource.org/pub/in/bis/S06/is.1479.3.1977.pdf (Accessed on 4 May, 2023)

IS 5838. Methods for estimation of Vitamin C in foodstuffs, Indian Standards Institution, New Delhi. 1970. https://law.resource.org/pub/in/bis/S06/is.5838.1970.pdf (Accessed on 3 June, 2023)

Jilo K and Tegegne D. Chemical composition and medicinal values of camel milk. International Journal of Research Studies in Biosciences. 2016; 4(4):13-25. http://dx.doi.org/10.20431/2349-0365.0404002

Jumah RY, Shaker RR and Abu-Jdayil B. Effect of milk source on the rheological properties of yogurt during the gelation process. International Journal of Dairy Technology. 2001; 54(3):89-93. https://doi.org/10.1046/j.1364-727x.2001.00012.x

Kaskous S. Importance of camel milk for human health. Emirates Journal of Food and Agriculture. 2016; pp 158-163. https://doi.org/10.9755/ejfa.2015-05-296

Lafta H, Jarallah EM and Darwash A. Antibacterial activity of fermented camel milk using two lactic acid bacteria. Journal of University of Babylon for Pure and Applied Sciences. 2014; 22:2377-2382.

Magdi OA, Ibrahim RAE and Hamid DA. Biochemical changes occurring during fermentation of camel milk by selected bacterial starter cultures. African Journal of Biotechnology. 2010; 9(43):7331-7336.

Mojtahedi SY, Izadi A, Seirafi G, Khedmat L and Tavakolizadeh R. Risk factors associated with neonatal jaundice: A cross-sectional study from Iran. Open access Macedonian Journal of Medical Sciences. 2018; 6(8):1387. https://doi.org/10.3889/oamjms.2018.319

Rahman IA, Dirar HA and Osman MA. Microbiological and biochemical changes and sensory evaluation of camel milk fermented by selected bacterial starter cultures. African Journal of Food Science. 2009; 3(12):398-405.

Shahein MR, Atwaa ESH, Elkot WF, Hijazy HHA, Kassab RB, Alblihed MA and Elmahallawy EK. The impact of date syrup on the physicochemical, microbiological, and sensory properties, and antioxidant activity of bio-fermented camel milk. Fermentation. 2022; 8(5):192.

SP: 18 (part XI). ISI Handbook of Food Analysis. Part XI. Dairy Products. 1981. Indian Standards institution, New Delhi, 43.

Thakkar PN, Patel AR, Modi HA and Prajapati JB. Evaluation of antioxidative, proteolytic and ACE inhibitory activities of potential probiotic lactic acid bacteria isolated from traditional fermented food products. Acta Alimentaria. 2018; 47(1):113-121. https://doi.org/10.1556/066.2018.47.1.14

Varga L, Süle J and Nagy P. Viability of culture organisms in honey-enriched acidophilus-bifidus-thermophilus (ABT)-type fermented camel milk. Journal of Dairy Science. 2014; 97(11):6814-6818. https://doi.org/10.3168/jds.2014-8300

Yoganandi J, Mehta BM, Wadhwani KN, Darji VB and Aparnathi KD. Evaluation and comparison of camel milk with cow milk and buffalo milk for gross composition. Journal of Camel Practice and Research. 2014; 21(2):259-265. http://dx.doi.org/10.5958/2277-8934.2014.00046.0