

Original Research Article

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## Assessment of Probiotic and Nutraceutical Properties of Camel Milk Yoghurt

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### ABSTRACT

In present generation, stress is considered as the root cause for development and progression of many diseases due to oxidative damage of the health. Supplementation of exogenous antioxidants from food substance has a promising approach to combat or act as shield against it. Probiotics can be define as the food substance which contain microorganisms that are claimed to provide health benefits when consumed directly, whereas nutraceutical food are the food or part of a food which allegedly provides medicinal or health benefits, including the prevention and treatment of disease. In the present study, probiotic and nutraceutical properties of fermented camel milk yoghurt were studied to determine the potential health benefits. The lactic acid bacteria (LAB) itself act as probiotic and considered as “good gut micro-flora”. The bioactive peptides which are produced by fermentation of camel milk have anti-oxidant nature. in the study, Evaluation of the product have been done for change in pH, TA, anti-oxidant activity (% inhibition of ABTS and DPPH activity), rancidity formation (TBA value) and microbiological parameters like Plate count, Coliform count, Yeast-Mold count and MRS count. The fermentative reactions was created using lactic acid bacteria (LAB); *Lactobacillus fermentum*. The organism shows vitality at day 7 of storage, which evinces the probiotic nature of the yoghurt. The study expresses the highest functional properties of the yoghurt in fresh conditions. It reveals that during storage, it diminished in terms of its functional potential.

#### Keywords

Camel milk,  
Nutraceutical properties,  
Functional food,  
Fermentative potential,  
*Lactobacillus fermentum*

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### Introduction

Camel plays a vital role in arid and semi-arid area by virtue of their adaptability and it concerned as an important component of Indian fragile desert ecosystem. However,

their contribution as dairy animals is less substantial but the camel milk act as piety due to its functional properties and nutraceutical values (Al-Shamsi *et al.*, 2018). The one-humped camels (*Camelus dromedarius*) are well-known producers of milk in the family

*camelidae*. The composition and technofunctional properties of camel milk have contrast from bovine milk due to the composition and structure of its protein content. It is widely admitted that the milk of dromedary camels have very good quality than other species in an environment the anti-oxidative properties in terms of fermentative potential (Singh *et al.*, 2018).

Normal camel milk is opaque and foamy nature. The taste of camel milk is usually sweet, when camels are fed on green fodder, but sometimes salty, due to feeding on certain shrubs and herbs in the arid regions (Yadav *et al.*, 2015).

The milk fat of camel milk mainly contains poly unsaturated fatty acids (PUFA). These polyunsaturated fatty acids are very essential to human nutrition. The content of long chain fatty acid (C-14 to C-18) is higher than short chain fatty acid (C-4 to C-14) (Narmuratova *et al.*, 2006). Caseins (CNs) are the major proteins in camel milk, and  $\alpha$ -,  $\beta$ - and  $\kappa$ -CN constitutes about 65, 21 and 3.47% respectively, of total caseins (Kappeler *et al.*, 2003). Camel milk shows similarity to human milk as it contains a high amount of  $\beta$ -CN; this could reflect its higher digestibility and lower incidence of allergy in infants, as  $\beta$ -CN is more sensitive to peptic hydrolysis than  $\alpha$ -CN (El-Agamy *et al.*, 2009). Camel milk  $\alpha$ -lactalbumin was reported to have a molecular weight of 14.6 kDa having 123 residues, which is similar to that of bovine, human and goat milk (Beg *et al.*, 1985).

Production of bioactive peptides can be done by controlled biochemical reactions like enzymatic hydrolysis and/or fermentative reactions by lactic acid bacteria (LAB). Bioactive peptides have been defined as specific protein fragments that have a positive impact on body functions or conditions and may ultimately influence health (Kitts and

Weiler, 2003). These are synthesised in the cell in the form of large prepropeptides, which are then cleaved and modified to give active products. Milk derived bioactive peptides play vital roles in human health and nutrition. They act as functional food and carry various neuraceutical properties (Haque *et al.*, 2008). Bioactive Peptides derived from the milk of camel exert multifunctional properties, including anti-microbial, immune modulatory, anti-oxidant, inhibitory effect and antagonistic activities against various toxic agents (Agarwal *et al.*, 2003).

Milk fermentation by proteolytic lactic acid bacteria (LAB) is one of the economical and practical methods for the production of fermented dairy products enriched in bioactive peptides (Hayes *et al.*, 2007). *Lactobacillus fermentum* is one of the keynote species of genus *Lactobacillus* which are used for a wide variety of applications; these applications include food and feed fermentation. It has been found that some strains for *Lactobacillus fermentum* have natural resistance to certain antibiotics and chemotherapeutics. They are considered potential vectors of antibiotic resistance genes from the environment to humans or animals to humans. *Lactobacillus fermentum* ME-3; an anti-oxidative lactobacilli showed very attractive probiotic nature on goat's milk (Kullisaar *et al.*, 2003). In the present research, the anti-oxidant potential and storage capacity of the camel milk fermented by *Lactobacillus fermentum* has been carried out.

## Materials and Methods

### Chemical and reagents

Fine chemicals such as ABTS (2,2'-azinobis (3-ethylbenzthiazoline-6-sulphonic acid), DPPH (2,2'-diphenyl-1-picrylhydrazyl), Potassium persulphate ( $K_2S_2O_8$ ), Tris-HCl were purchased from Sisco Research

Laboratories (SRL) Pvt. Ltd., India and TBA (2-Thiobarbituric acid) was obtained from Merck Life Science Private Limited, India. Other chemicals were of analytical grade from reputed companies and used without further purification. All solutions, prepared with double-distilled water, were kept at 4°C before further use.

### Collection of samples

About 2 liter of fresh camel milk was collected from camels maintained under the Camel Dairy at National Research Center on Camel (ICAR-NRC on Camel), Bikaner at weekly interval for period of 2 months to perform the different experiments as mentioned under the study. The milk was further inoculated with *Lactobacillus fermentum* and incubated for 8 hours for optimum functional properties to form bioactive peptide rich yoghurt.

### pH and Titratable Acidity (TA) measurement

The pH of samples was measured using combined glass electrode of Milkoscan at camel milk research laboratory, ICAR-NRC on Camel, Bikaner.

The pH of each sample was measured just before heating to inactivate the residual enzyme. Titratable acidity expressed as percentage of lactic acid, was determined by 10 ml of each sample titrating with 0.1 N NaOH using phenolphthalein as an indicator to an end-point of faint pink color.

### DPPH (2, 2'-diphenyl-1-picrylhydrazyl) radical-scavenging activity

The % inhibition activity of 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) was estimated following the method of Brand-Williams *et al.*, (1995), with slight modification. The

concentration of DPPH reagent was 100 which were mixed with 0.1 M molar buffer solution of Tris-HCl (pH 7.4). The hydrolysate of fermented camel milk was obtained after centrifugation at 10000 rpm for 10 minutes at 4 °C. The hydrolysate of fermented camel milk was analysed for DPPH activity. The absorbency was measured in zero minute ( $A_{t_0}$ ) and in 20 minute ( $A_{t_{20}}$ ) at 517 nanometer (nm) using a spectrophotometer. Ethanol was used as blank. The free radical-scavenging activity was calculated from the following equation:

$$\text{DPPH activity (\% inhibition)} = 100 - [(A_{t_{20}}/A_{t_0}) \times 100]$$

(Where,  $A_{t_0}$  = absorbency in zero minute and  $A_{t_{20}}$  = absorbency in 20 minute)

### ABTS (2, 2'-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) radical-scavenging activity

The % inhibition of ABTS activity was analysed according to method of Salami *et al.*, (2009) with slight modifications. The stock solution of ABTS was formed with 2.45mM potassium persulphate ( $K_2S_2O_8$ ) and kept in the dark place at room temperature for 16 hours before use. Prior to use, the stock solution was diluted with distilled water to get an absorbance of 0.70 at zero minute.

The hydrolysate of fermented camel milk was obtained after centrifugation at 10000 rpm for 10 minutes at 4 °C and used for % inhibition activity of ABTS. The absorbance was measured after 20 min ( $A_{t_{20}}$ ) at 734 nanometer (nm) using spectrophotometer. The ABTS activity was calculated by using the following formula:

$$\text{ABTS activity (\% inhibition)} = [(0.7 - A_{t_{20}}) / 0.7] \times 100$$

(Where,  $A_{t_{20}}$  = absorbency in twenty minute)

### **TBA (2-Thio Barbituric Acid) value**

The TBA value was determined according to Witte *et al.*, (1970) with slight modification. The filtrate was obtained using 20% trichloroacetic acids (TCA) in 2 M orthophosphoric acid and filtered through Whatman no.1 filter paper. The filtrate was then mixed with fresh chilled 2-thiobarbituric acid (0.005 M in 0.05 M NaOH). The test tube was shaken well and placed in the dark at room temperature (25°C) for 15- 17 hour to develop the color reaction. Formation of blank take place by mixing 3 ml of fresh chilled 2-thiobarbituric acid (0.005 M in 0.05 M NaOH) with 1.5 ml of cold solution containing 20% trichloroacetic acids in 2 M orthophosphoric acid and 1.5 ml of distilled water. After 15- 17 hour colour reactions was measured by spectrophotometer at 532 nm (A532). The TBA value was expressed as mg MDA/kg of sample, which was calculated by multiplying the absorbance by 5.2 factors as follows:

$$\text{TBA value (mg (MDA)/kg)} = A532 \times 5.2$$

### **Microbial count**

Microbial count was determined by using pour plate method. Standard plate count (SPC) was determined with plate count agar medium and the plates of different dilutions were incubated at 37°C for 24 hour. Violet Red Bile (VRB) Agar medium was used for determination of coliform bacteria. Man, Rogosa and Sharpe (MRS) agar medium used for enumeration and cultivation of *Lactobacillus* species and by pour plate method. Yeast molds (Y-M) count take place by Potato dextrose agar (PDA) medium by pour plate method.

### **Statistical analysis**

All the experiments of fermentation study were repeated three times and samples were drawn in duplicate. Data collected during the

present investigation were subjected to statistical analysis by using F-test and adopting appropriate methods of analysis of variance as described by Snedecor and Cochran (1994). Wherever, the variance ratio were found significant at 5 per cent and highly significant at 1 per cent levels of probability, the significance of mean differences were tested by Duncan's New Multiple Range Test (Duncan's Range Test) as modified by Kramer (1957).

### **Results and Discussion**

Table 1 illustrates changes in various biochemical properties of camel milk yoghurt during storage at 4°C for different periods. The results revealed that the stability of camel milk yoghurt decreased day by day with the storage period. Moreover, the good microbial load (MRS count) of the yoghurt decreased significantly during the whole storage period. However initially coliform count and yeast-mold (Y-M) count remains nil which show hygienic production of the products. Changes in pH and acidity of the products during storage, suggests effect of yoghurt microflora on its nutrient composition. Similar changes were observed by Eissa *et al.*, (2010), in goat milk yoghurt. Camel milk yoghurt shows an increased trend in acidity whereas it show continuous fall in pH during storage period.

According to Valli and Traill (2005), the activity and growth rate of the starter cultures are strain dependent. Hence, the acidification rate of lactic acid bacteria varied with the type of milk, being some yoghurt starters more active than others. Table 1 Shows changes in total viable count (plate count) of camel yoghurt during storage. The results obtained revealed that camel milk yoghurt contained highest total viable cells of bacteria initially. Increase in acidity of the growth media in camel milk yoghurt may retard bacterial growth.

**Table.1** Storage study (Mean ± SE) of fermented camel milk product

Parameter	Day 1	Day 3	Day 5	Day 7
TBA**	0.006 <sup>a</sup> ± 0.0001	0.12 <sup>b</sup> ± 0.002	0.16 <sup>c</sup> ± 0.002	0.29 <sup>d</sup> ± 0.025
pH <sup>NS</sup>	4.5 ± 0.02	4.30 ± 0.01	4.16 ± 0.01	4.02 ± 0.01
TA <sup>NS</sup>	0.68 ± 0.010	0.78 ± 0.005	0.89 ± 0.005	1.10 ± 0.010
ABTS**	20.15 <sup>d</sup> ± 0.45	19.55 <sup>c</sup> ± 0.09	15.05 <sup>b</sup> ± 0.19	13.12 <sup>a</sup> ± 0.03
DPPH**	8.15 <sup>d</sup> ± 0.02	7.80 <sup>c</sup> ± 0.04	5.05 <sup>b</sup> ± 0.22	3.15 <sup>a</sup> ± 0.04
Coliform count** log(CFU/ml)	nil	nil	1.42 <sup>a</sup> ± 0.3	1.64 <sup>b</sup> ± 0.4
Yeast Mold count** log(CFU/ml)	nil	nil	nil	0.78 ± 0.1
Plate count** log(CFU/ml)	9.47 <sup>d</sup> ± 0.8	9.35 <sup>c</sup> ± 0.8	9.18 <sup>b</sup> ± 0.7	8.85 <sup>a</sup> ± 0.6
MRS count* log(CFU/ml)	9.21 <sup>d</sup> ± 0.4	9.15 <sup>c</sup> ± 0.7	9.07 <sup>b</sup> ± 0.5	9.01 <sup>a</sup> ± 0.8

Note – Means bearing different superscripts differ significantly.

\*\* = Significant at 1% (P<0.01), \* = Significant at 5% (P<0.05), NS= Non Significant

The results obtained agree with the findings of Masud *et al.*, (1991). *Lactobacillus* count (MRS count) in camel milk yoghurt was decreased significantly during storage.

The load of lactic acid bacteria, however found in yoghurt at day 7 of storage, which stated as both yoghurts have probiotic nature. Tamime and Robinson (1999) reported that yoghurt should contain as minimum as 7 log(cfu/ml) or 10<sup>7</sup> viable cells of lactic acid bacteria per ml, to be stated as probiotic.

The change in chemical composition and lactic acid bacteria show parity with the results demonstrated by Ismaili *et al.*, (2017).

On the other hand, yeasts and molds were steadily increased significantly in camel milk yoghurt with storage period of day 7 but not found at day 1 to day 5 of storage period. An increase in acidity and/or reduction in potential oxygen during fermentation process may provide suitable state for growth of yeasts and molds. Contamination by yeasts

and molds during processing of yoghurt was reported by Karleskind *et al.*, (1993).

Coliforms were absent in fresh camel yoghurt, Coliforms viable cells were detected in camel yoghurt from 5 days of storage and increased significantly at 7 day of storage, which may be due to contamination or any other reason. However, many workers reported on the survival of coliforms, if present, in yoghurt to a maximum of 3 days, shown by Karleskind *et al.*, (1993).

Moreover, *E. coli* was observed by El-Agamy *et al.*, (1996) and Mainfreni *et al.*, (2002), to survive the low pH domestic yoghurt developed during cold storage and could tolerate lower acidity up to 6 days.

Usually, lipid per oxidation is not a major problem in yoghurt due to the low pH, the low storage temperature and low permeability to oxygen of packaging materials. Furthermore when lactobacilli are used as starter culture, it helps to reduce the rate of



lipid peroxidation by its natural anti-oxidant potential. Lipid peroxidation of camel milk products was determined by Thio Barbituric Acid (TBA) value, which gave the idea about rancidity of both milk products. In both products the initial TBA value at day 1 was very low and it slowly increased at day 3 and day 5 of storage which showed slow elevation till day 7 of storage, it increased significantly but the value was lower than 1 which can give an idea about no foul smell till 7 day of storage of camel milk product.

The results led to similar conclusion as provided by Saide and Gilliland (2005) that lactobacilli as a starter culture was helpful to prevent lipid peroxidation due to its anti-oxidant potential, however elevation of TBA value during storage was due to change in its physico chemical as well as microbial properties.

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