

Nutritive value and characterization properties of fermented camel milk fortified with some date palm products chemical, bacteriological and sensory properties

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Abstract: Camel milk and dates have been reported to be used for prevention and treatment of various diseases in Arab countries. A total of 40 milk samples after parturition were collected from different zones of Al-Ahsa area, KSA. The analyses of chemical and nutritional values of camel milk were performed. The samples were analysed for total solids, fat, total protein, sugars, ash, minerals as well as vitamins. Results showed 11.90± 0.11% total solids, 2.69± 0.28% protein, 3.26± 0.14 % fat, 1.13.22± 0.54 (g/100g), lactose 3.22±0.04%, glucose 3.22±0.07 % sucrose 1.93. ±0.03% and ±0.04 % 0.14 % acidity. The levels of ash, Ca, Mg, Na, vitamin C and B12 were higher. however concentration of Fe, vitamin A was relatively lower. Antimicrobial agents, lactoferrin (LF) were determined and the concentration was 1.61 ±0.19 mg/ml. Generally, camel milk presented high nutritive and therapeutic applications. Yoghurt made from camel milk was prepared by adding date palm syrup (Depis) to cultured milk. The main objective from this part was to investigate the influence of Depis on chemical characteristics (titrable acidity, total solids, fat, and protein content), microbiological and sensory properties and acceptability of the resultant yoghurt during storage for 21 days at 4° C. Addition of 1, 2.5 and 5 % of Depis did not affect yoghurt pH or protein and fat content, but decreased moisture and increased the total solids significantly. Moreover, addition of Depis improved the viability and stability of starter culture during storage and the total viable counts were 0.67, 7.4, 7.7 and 7.8 ×10⁻⁷ for control, 1, 2.5 and 5 % of Depis, respectively. Finally, addition of 5% Depis provided yoghurt with desired sensory properties.

Keywords: Camel Milk, Chemical and Nutritive Value, Fortification, Fermented Camel Milk

1. Introduction

According to [1] statistics, there are about 18 million camels in the world. Nowadays, camel milk production is in progress in many countries in both Asia and Africa due to increased demand. Raw and pasteurized milk and other dairy products made from camel milk are available in the markets of Gulf area and other countries [2]. Most of camel milk is consumed in the raw state without any heat treatments and kept at high temperature with lack of refrigeration facilities during milking and transporting [3]. Camel milk has properties that it can be kept for long periods than cow's milk when refrigerated and even with the desert heat it does

not spoil shortly [4]. Moreover, the milk composition of dromedary camel is excellent from a nutritional view point [5]. Camel milk also has valuable nutritional properties as it contains a high proportion of antibacterial substances and higher concentration of vitamin C in comparison with cow milk [6]. [7] have reported a unique camel milk health benefit in diabetic patients. Camel milk is much more nutritious than that of cow milk because it is low in fat and lactose contents, and higher in potassium, iron and vitamin C, [8]. Camel milk has medicinal properties and contains protective proteins, which may have a possible role for enhancing the immune defense mechanism [9]. The triglycerides, which contain a great variety of fatty acids, are

accompanied with small amounts of di-and mono-acylglycerols, cholesterol, free fatty acids and phospholipids [10]. In average, camel milk contains more proteins and whey protein than cowmilk [11]. The ability of camel milk to inhibit growth of pathogenic bacteria and its relation to whey lysozyme has been demonstrated by [12]. The reported average of Lysozyme content in human milk is 40 000 mg/100 ml and in cow milk 120 mg/100 ml [13]. The significantly very high level of Lysozyme in camel milk is of importance for the storage of milk and needs further investigation. [3] extracted Lysozyme (Lz), lactoferrin (Lf), lactoperoxidase (Lp), immunoglobulin G and immunoglobulin A from camel milk. [14] reviewed the ability of camel milk to inhibit growth of pathogenic bacteria and its relations to whey lysozyme. The lysozyme content of twenty samples showing growth inhibition was 648 -956 g/100ml which was significantly higher than the average in 38 samples (62.8 -956 g/100ml) with no inhibitory effect. The activity of these protective proteins was assayed against some pathogenic bacteria such as *Escherichia coli*, *Salmonella typhimurium* and rotavirus. [15] reported that the proteolytic activities of yogurt starters at (42°C for 4 h) were higher in camel milk than in cow milk. [16] observed that camel milk failed to form gel-like structure after 18 h incubation with lactic acid culture; this was attributed to the presence of antibacterial factors such as lysozymes, lactoferrin. Shubat is camel's sour milk from Kazakhstan [17]. Kefir is the Caucasian fermented camel's milk [9]. Lehban is fermented products from camel's milk in Syria and Egypt [18]. The date palm (*Phoenix dactylifera* L.) is the major fruit tree in KSA. In the Gulf region, in spite of the drastic socioeconomic changes, dates continue to play an essential role in the diet of the local people. Date fruits and consumed in large quantities in KSA [19]. Fortified fermented milk products are widely consumed for their benefits and refreshing effects. Their popularity is said to be attributed to the effective use of consumer-driven flavors and milder cultures [20]. These products already have a positive health image [21], which can be further enhanced by the addition of probiotic bacteria with therapeutic properties [22]. Supplementation or fortification with dates and other dates palm products like Depis may be beneficial during childhood and when diet is low in animal products and based on high-phytate cereals and legumes [23]. The objective of this work was to study the functional properties of camel milk, followed by isolation and assessment of the antimicrobial activity of isolated immunoproteins (Lf). The resultant fortified products (date-fermented camel milk) will be evaluated in terms of chemical, microbiological and sensory properties during the storage period.

2. Materials and Methods

2.1. Sampling

40 samples of camels milk were collected and stored in ice tank until reaching the laboratory, then stored at 4 oC if it

will be analyzed in 2-3 days or at 20oC if it will be analyzed immediately. The Camels were of indigenous breed fed extensively throughout the year. Each sample was analyzed in triplicate.

2.2. Materials

Camel milk samples were obtained from farms at Eastern Region around Al-Ahasa area. Lactoferrin standard was obtained from Sigma, Chemical Co, USA. Starter cultures *S. thermophilus* strain St4 and *L.bulgarcus* 92063 (DSM) were obtained from BafM, Kiel, Germany.

2.3. Methods

2.3.1. Chemical Analysis

Samples were analysed for total solids, total protein, ash, fat and sugar percentage according to [24]. Titrable acidity was determined according to [25]. The water soluble vitamins were determined by reverse phase HPLC technique by using High Performance Liquid Chromatography (HPLC): Hitachi (U.S.A). The minerals Calcium, Magnesium, Sodium, iron and concentrations were determined by Atomic Absorption Spectrophotometer (PG 990, England) according to the method of [26]. Lactoferrin was extracted from whey camel protein by procedure described by [27].

2.3.2. Microbiological Analysis

Fermented camel milk samples (10 ml) were homogenized for one minute in 90 ml (1/10) of a sterile solution of 0.1% (w/v) peptone water (Oxoid CM9) using a Stomacher Lab blender Model400, Seward Laboratory, London). From these samples serial decimal dilutions were prepared in sterile water. The microorganism's counts were carried out by the pour-plate method with duplicate plating on different selective agar media. Standard plate count agar was used to determine total viable bacterial count. All plates were incubated in an inverted position at 37 oC for 24–48 h. The antibacterial activity was determined using disc fusion method. 10 µl aliquots of (Lf) was added to the discs (Oxoid, UK) that had been arranged on sloppy agar plates previously seeded with the pathogenic indicator bacteria.

2.3.3. Preparation of Fermented Milk

Heat treated homogenized camel milk, dates Depis (Al Ahsa Food Industries Co.) at final concentration (1, 2.5 and 5%) were used to make fortified fermented camel milk. Fermented milks were made by a 1.5% inoculation of *S. thermophilus* strain St4 and *L.bulgarcus* 92063 (DSM) and incubated for 12 hour at 37°C. Samples then were taken and stored at 4°C for 21 days. Resultant fermented camel milk containing dates palm products (Depis) at different concentration (1, 2.5 and 5%) were compared to the control treatment (without fortification) during storage period.

2.3.4. Sensory Evaluation

Trained panelists from the staff of Food Science and Technology Department, Faculty of Agricultural and Food Sciences KFUPM, KSA performed the evaluation. All members have experience in evaluation of dairy products. Scores were on a 60-point scale with some modifications according to [28].

2.3.5. Statistical Analysis

Descriptive analyses between the different microbial parameters were performed using SAS, Statistical Analysis System software, release 9.3.1. (SAS Institute Inc. Cary, NC 27513, USA).

3. Results and Discussion

3.1. Chemical Properties of Camel Milk

The mean average of chemical properties of camel milk was presented in (Table 1). Data showed that, the total solids (TS) content was higher in camel milk and the following mean average was detected ($11.90 \pm 0.11\%$). These results agree with those obtained by and [30, 31]. Sugars content of camel milk were 3.22 ± 0.04 , 2.4 ± 0.07 and $1.9 \pm 0.03\%$ for Lactose, Glucose and Sucrose, respectively. Data showed also that, the camel milk contained low percentage of fat, protein and ash. The mean average of fat, protein and ash percentage was 3.26 ± 0.14 , 2.69 ± 0.28 and 1.1 ± 0.54 , respectively. These results are in agreement with those reported by [29].

(Table 1) shows the mean average of mineral contents of camel milk. The major salt constituents were Ca, Mg, Na, and Fe. Their concentrations were 131.18 ± 0.27 , 74.75 ± 0.19 , 67.4 ± 0.20 and 0.792 ± 0.12 , respectively. Data shows that camel milk represented a good source of minerals. Moreover (Table 1) shows the percentage of vitamins in camel milk. The results showed that camel milk contained high levels of vitamins C, B12; however concentration of vitamin A was relatively lower. These results agree with that obtained by [2]. The milk protein lactoferrin, which was found in large quantities in camel milk, does have anti-bacterial properties. The average of the isolated lactoferrin was 1.61 ± 0.11 . Data was higher than that reported by [30] which were found that the content of lactoferrin in camel milk is 0.24 ± 0.035 . Finally, data showed that camel milk is rich in lactoferrin. Moreover, the health-promoting properties of camel milk are strongly recommended.

3.2. Antibacterial Activity

Data in Fig (1) shows the effect of lactoferrin as antimicrobial agent. Results revealed that all camel milk samples were rich in lactoferrin. The final concentration of lactoferrin was 1.61 ± 0.19 mg/ml. Growth of bacterial strains were carried out in 40 ml medium. The isolated lactoferrin was collected and purified for determination of antibacterial substances. The study was achieved as

triplicates. The antibacterial spectrum of the isolated lactoferrin was determined using disc diffusion method. The samples were filter sterilized by passage through $0.22 \mu\text{m}$ pore size membrane. Ten μl aliquots of sterile samples were added to the discs (Oxoid, UK) that had been arranged on the Mueller-Hinton sloppy agar plates previously seeded with the indicator bacteria and incubated overnight at 37°C Fig (1). After 12-18 h of incubation, the diameters of the growth zones were measured. Our lactoferrin (LF) showed different diameters of inhibition zones against pathogenic bacteria. The inhibition zone's diameters ranged between 1.2 to 3.2 cm according to the isolate and the indicator strains. *Listeria monocytogenes* DSM 12464 and *Staphylococcus aureus* ATCC 6538 were highly affected with antimicrobial substance. The inhibition zone's diameter of *Staphylococcus aureus* were 3.0 to 3.2 cm. However, the inhibition zones of *L. monocytogenes* ranged between (2.7 to 3.0) cm by lactoferrin (Lf). Growth of *Bacillus cereus* ATCC 29212 was inhibited by LF, but the inhibition zones' diameters ranged between (1.2 to 2.4). The mechanism(s) of the antibacterial activity of lactoferrin LF appears to be multifactor, may be due to producing antibacterial peptides. [31] found that camel milk was containing high level of immunoproteins G in comparison to cow, buffalo, goat, sheep and human milk. The same study showed that camel milk contained also significantly higher level of lactoferrin than cow and goats milk. Exactly how lactoferrin functions is not entirely clear, but it is known to enhance the immune response, both directly and indirectly (passively) in reaction to a wide range of immune challenges and is an essential factor in the immune response in humans. Early studies attributed such effects to the acquisition of essential Fe from the environment, but more recent findings have implicated wider cell interactions. LF damages the outer membrane of bacteria, accompanied with the release of LPS from Gram-negative bacteria [32]. The ultra structure alterations caused by Lf to the bacteria enhance the activity of some antimicrobial agents, [33, 34], which could be approached to combine LF with antibiotics in treating infections.

3.3. Chemical Analysis of Fermented Camel Milk

Means for chemical composition (total solids, total protein, fat, ash and acidity) of fermented camel milk containing date products (Depis) at different concentrations (1, 2.5 and 5%); (T1, T2 and T3) are presented in (Table 2). Addition of Depis had no clear effect on total protein and fat content, while decreasing moisture content led to increasing in total solids of the treated camel milk (T1, T2 and T3). Data in (Table 2) showed that the means of the total solids were 12.41 ± 1.23 , 14.10 ± 2.10 , 14.21 ± 1.02 and $14.32 \pm 1.11\%$ for control and treatments (T1, T2 and T3), respectively. The average protein levels were $2.80 \pm 0.21\%$, 2.74 ± 0.19 , and 2.68 ± 0.33 and 2.54 ± 0.47 while fat % content revealed means of 4.11 ± 0.55 , 4.10 ± 0.76 , and $3.93 \pm 0.82\%$ and $3.87 \pm 0.77\%$, respectively. (Table 1) also shows that the

mean values of ash of fermented camel milk were 1.32 ± 0.77 , 1.39 ± 1.013 , 1.42 ± 0.89 and 1.50 ± 0.67 in control and treated samples (T1, T2 and T3), respectively. Fermented camel milk containing Depis had similar acidity values as control. The mean of acidity expressed as lactic acid percent of fermented camel milk revealed 1.18 ± 0.02 , 1.22 ± 0.16 , 1.29 ± 0.32 and 1.33 ± 0.41 in control and treated samples, respectively during storage. The results obtained from the analysis of chemical composition of the tested fermented camel samples (Table 2) were in the range of total solids content of fresh milk samples [35]. However, the fat percent in samples, which were found to be higher than the fat percent (2.8- 3.6%) in the fresh camel milk samples was reported by the same author. The variations might be due to the high fat percent of the camel milk used in the production of the fermented camel milk. Data also shows no significant changes in titratable acidities of fermented milk made from camel and Depis constituents during storage period. The acidity of treated camel milk was significantly higher than the control Fig (2). This may be attributed to the higher acidity of fortified fermented camel milk by Depis than control and to the lower buffering capacity of fortified fermented camel milk.

3.4. Microbiological Analysis of Fermented Camel Milk during Storage

Changes in the total viable counts during storage are presented in Fig (2). The initial viable cell counts of all samples ranged from 4.40×10^7 to 5.9×10^7 log₁₀ cfu/ml. These numbers indicated that the initial counts for the inoculated camel milk before fermentation were similar in the four treatments and remained stable with major increase during fermentation and incubation. The cell number increased slightly during fermentations and storage time (data not shown) only in treatments (T1, T2 and T3) however; the control did not grow significantly. Similar results was observed by [36] who found that, the total viable count for the all cultures increased during fermentation period of fermented camel and *L.bulgaricus* showed the fastest growth of all single cultures. The average of initial counts at the end of fermentation time and before storage were 5.41, 5.65, 5.79, 5.80×10^8 cfu/ml for control, T1, T2 and T3, respectively. Data showed that the addition of Depis to fermented camel milk during manufacture improves the viable and cell count compared to the control treatment (without addition of Depis). No clear differences between cell counts in the three Depis treatments were observed Fig. (2). At the end of the storage period only a slight decrease of the cell numbers was observed in control treatment Fig (2). However, samples treated by Depis (T1, T2 and T3) showed higher survival count. The reason for this positive effect on the survival of total bacterial count may be due to the sugar

content of Depis which improved the viability and stability of bacterial starter culture during storage for 21 days at 4°C.

3.5. Sensory Evaluation of Fermented Camel Milk

The mean values of sensory evaluation scores are summarized in Fig (3). The results show the values of color, firmness, taste, flavor, and over all acceptability of control and fermented milk containing Dipes. The overall acceptability scores of the sensory evaluation revealed that the camel milk fermented by yoghurt starter culture and fortified by different concentration of Depis was the most acceptable. While fermented camel without fortification was the least. The results showed also, that there were no significant differences ($p > 0.05$) in firmness of the four fermented products. Treated fermented camel milk had significantly higher rating for smell, taste and acceptability compared to the control one. However, the body and texture of all fermented camel milk products was weak and showed a brittle structure. Control fermented milk and treatments containing up to 5% Depis had similar ratings for flavour. However, addition of up 5% Depis to the yoghurt decreased the ratings for color significantly and increased flavor ratings significantly. Fermented milk containing 5% Depis had significantly higher ratings for overall score than the other treatments during storage up to 21 at 4°C. These finding agree with those of [37] who reported that acidophilus milk made from camel milk was watery and precipitated in the form of flocks. Similar results were obtained by [38] who observed that the fermentation of camel milk by starter culture did not reveal a good curd formation but indicated a fragile and heterogeneous structure. Also, the camel milk failed to reach a gel-like structure after sensory attributes 18h incubation [6]. Generally, participants found that the flavor of yoghurt with date products was very acceptable. Moreover, yogurt containing 5% Depis had better taste and flavor.

Finally, the present study increased the knowledge about the camel milk. Also, it could be concluded that, camel milk can be considered as a good source of minerals, vitamins and characterized by higher ratio of lactoferrin. Moreover, camel milk could meet a big part of the daily needs of humans from these nutrients because camel milk has most the essential nutrients. On the other hand, fermented camel milk fortified with different Depis concentration resulted in a good properties and higher acceptable sensory values in fresh and till the end of storage periods.

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Table 1. Chemical characteristics of camel milk, results are average of three replicates (n=40)

Parameters	minimum	Range maximum	Average \pm SEa
Total solids	10.84	13.25	11.90 \pm 0.11
Lactose %	3.90	4.29	3.22 \pm 0.04
Glucose	2.2	2.8	2.4 \pm 0.07
Sucrose	1.2	2.1	1.9 \pm 0.03
Fat %	3.44	3.89	3.26 \pm 0.14
Protein	2.39	3.09	2.69 \pm 0.28
Ash(g/100g)	0.90	1.73	1.1 \pm 0.54
Calcium(Ca) mg/100 ml	122	149.18	131 \pm 0.22
Magnesium (Mg)	70.9	80.75	74 \pm 0.19
Sodium (Na)	57.1	75.16	67.4 \pm 0.20
Iron (Fe)	0.47	0.99	0.76.2 \pm 0.12
Vit (C) mg/100 ml	114	128	117 \pm 0.26
Vit (A)	44.3	47.5	45.1 \pm 0.43
Vit (B12)	119	131	123.10 \pm 0.72
Lactoferrin mg/ml	0.49	1.84	1.61 \pm 0.19

a: Average and stander errors (SE)

Table 2. Average of Chemical composition of fermented camel milk fortified by different concentrations of Depis

Fermented Camel milk	TS %	TP %	Fat %	Ash %	Acidity %
Control	12.41 \pm 1.23	2.80 \pm 0.21	4.11 \pm 0.55	1.32 \pm 0.77	1.18 \pm 0.02
Depis 1% T1	14.10 \pm 2.1	2.74 \pm 0.19	4.10 \pm 0.76	1.39 \pm 1.01	1.22 \pm 0.16
Depis 2.5% T2	14.21 \pm 1.02	2.68 \pm 0.33	3.93 \pm 0.82	1.42 \pm 0.89	1.29 \pm 0.32
Depis 5% T3	14.32 \pm 1.10	2.54 \pm 0.47	3.87 \pm 0.77	1.50 \pm 0.67	1.33 \pm 0.41

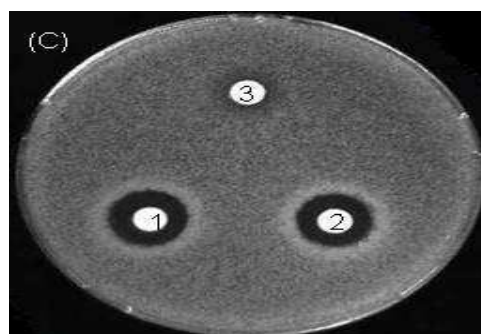


Fig 1. Antimicrobial activity of camel milk lactoferrin against pathogenic bacteria 1: *Staphylococcus aureus*, 2: *Listeria monocytogenes*, 3: *Bacillus cereus*.

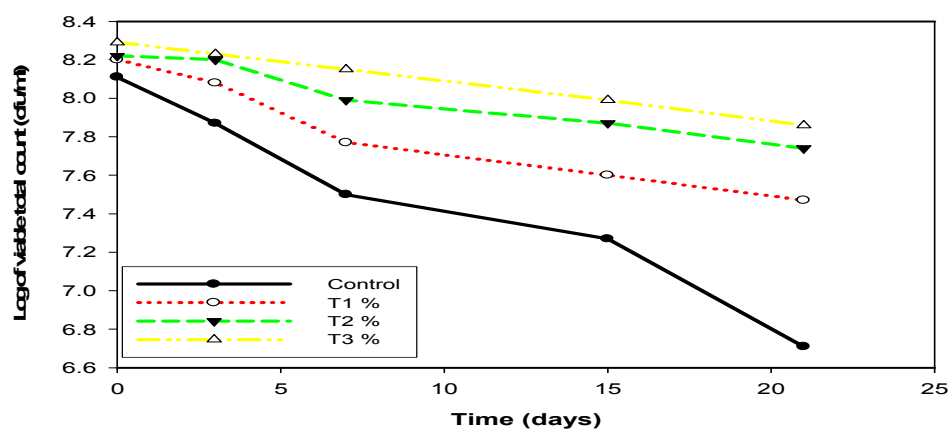
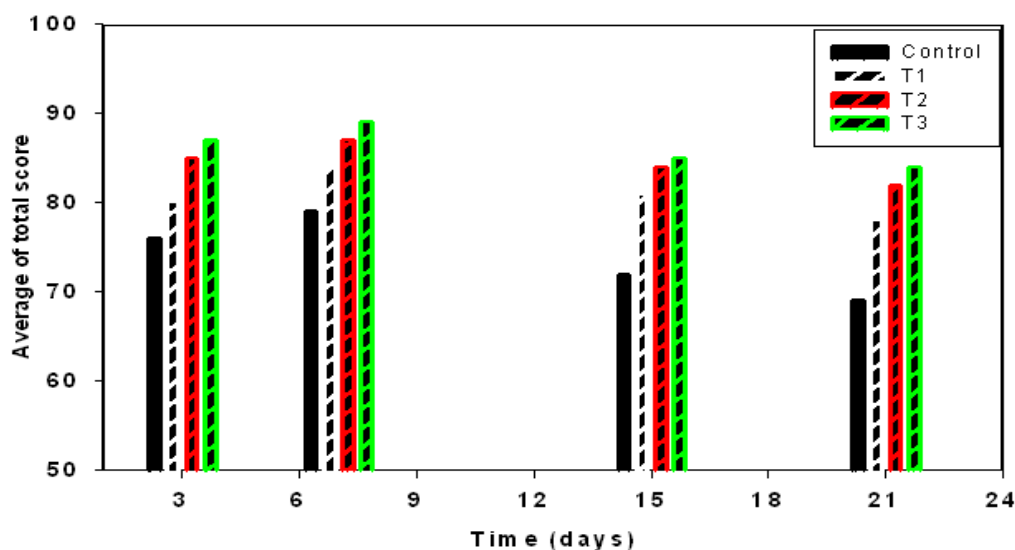


Fig 2. Changes in the total viable counts of fermented camel milk during (cfu/ml) storage at 4°C for 21 days. (Average of 3 replicates).



Control: untreated fermented camel milk T1: fortified fermented camel by 1% Depis
T2: fortified fermented camel by 2.5% Depis T3: fortified fermented camel by 5% Depis

Fig 3. Sensory evaluation and over all acceptability of fermented camel milk containing Depis during storage (4°C /21 days)

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