Journal of Pharmaceutical and Health Sciences 2016;4(3), 193-204.

JPHS

Original Article

Fermented Camel Milk (Chal): Chemical, Microbial and Functional Properties

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Abstract

The aim of this study was to determine physicochemical, microbial properties and antioxidant activity of fermented camel milk (Chal) and introduce it as a functional food. The protein content of the samples was determined using Kjeldahl method and total dry matter using oven drying method. The amount of fat content with Gerber method and pH was measured using a pH meter. Antioxidant activity was also determined using 2,2'-azino-bis-(3-ethylbensothiazoline-6- sulfonic acid) (ABTS) method. The mineral analysis was performed with atomic absorption spectroscopy and microbial count by pour plate method. Results revealed that fat, protein content and total solid determined 5.82±0.27%, 3.07±0.073%, and 12.24±0.16%, respectively. Acidity and pH determined 80±7 °D and 4.52±0.10, respectively. When a food has calcium by itself, this calcium is bonded with the protein of food, this calcium is more effective in our body than the calcium we add to food and they have not bonded any proteins. Adequate calcium consumption may support to decrease the risk of osteoporosis in life. Calcium ranged 103.29±3.87% and phosphorus 10.25±0.1% for Chal samples, respectively. The total counts were equal 6.54±0.19 log CFUmL^{-1}; Coliform count was determined in the ranges of 2.34±0.23 logCFUmL^{-1} for Chal samples. The results showed that Chal was rich in antioxidant. The antioxidant inhibitory activity of Chal was obtained 45.38%. Diets rich in antioxidants, can inhibit LDL oxidation, influence the activities of immune-competent cells and inhibit the formation of cell-to-cell adhesion factors. Therefore, Chal is introduced as a traditional functional food.

Keywords: Camel milk, Chal, Microbial quality, Physicochemical properties, Antioxidant activity, Functional food

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Introduction

Camel milk and fermented camel milk such as Chal, Shubat, and Grass has been consumed in different countries around the world. Chal is one of the fermented camel milk in Iran which has been traditionally prepared by fermentation of camel milk and addition of water. Chal and even camel milk have nutritional and medicinal features due to their functional properties as well as having some beneficial lactic acid bacteria. In many areas, Chal and camel milk are used for curing some maladies and to fight with health problems such as tuberculosis, asthma, anemia and piles . Previous studies revealed that the patients who have chronic hepatitis improved after using camel milk . Above all, foods that are healthier and prevent illnesses, are more acceptable for today's consumers. It should be highlighted that Chal is made of raw camel milk by spontaneous fermentation process; therefore this product may have some detrimental microorganisms. Fermented camel milk contains well-balanced nutrients and physicochemical components. Camel milk is high in vitamin C, folic acid, and niacinas and is richer in calcium and phosphorus than bovine milk. Previous research has recommended the potential use of camel milk in the manufacturing infant formula. The antioxidant and ACE-inhibitory property of casein-derived peptides of camel milk by digestive proteases have already been studied by Salami et al. (2011). The significantly greater antioxidant activity of camel milk -lactalbumin compared with that of bovine whey proteins was also observed in a previous study. The helpful outcome of camel milk, which cover the prevention and cure of diseases and condi¬tions such as gastroenteritis, diabetes, and hypertension, have been demonstrated experi¬mentally. Accordingly, improving the curative value of camel milk has received extensive attending. Casein amount to 75-80% protein of camel milk is very impressionable to proteolysis and intelligent to procreate peptides with various biological activities. Furthermore, the rates of osteoporosis and osteoporotic fractures are high and have been rapidly increasing worldwide. Camel and camel milk is a rich source of calcium. Calcium is the most abundant stored nutrient in the human body. In pregnancy when more calcium is required for the growing fetus the calcium absorption rate increases (31). At particular risk are female adolescents when bone formation and growth is most crucial. Later in the life cycle, women continue to be at highest risk and this risk is elevated if early baseline bone is not strong during adolescence. Postmenopausal women, due to hormonal changes that may affect bone mineralization processes, have also been widely studied for calcium deficiency risk. The priorities of bone formation differ by population group. In children and young adults, the emphasis is placed on strong baseline bone growth and structure as a foundation. Progressive bone mineralization loss over time increases the risk of bone fracture and falling. Amplification of food products with calcium is commonly practical as one of the strategies to raise calcium intake and recovery bone mineral density. However, it is hard to add calcium in noteworthy quantities, because it frequently gives a plaster taste and a bad mouth-feel or because of it sediment into a grayish mass within a liquid production. However, judicious mixing and the use of diverse sources of calcium, attractive tissue for the food itself, covering flavoring substances and diverse compositions of the component can recover the amiability of calcium tenacious foods. This clearly is a challenge for food technology vitamin and mineral rampant for special goals is formerly well known.

Therefore, camel milk and its products such as Chal were considered in the present study. Food is not only essential for body expansion, growth, and conservation but it is also accepted to play a key role in the quality of life. Functional food has the capability of having a useful effect on body functions to support improve the condition of well-being and health and decrease the risk of illness. Functional food is a conception aimed at sensational research in nutriment to support and authenticate the expansion of new foods and food components. It belongs to nutriment, not to pharmacology. All are functional foods with the message of a profit to health. A food can be regarded as functional if it is acceptable insignia to influence useful one or more target functions in the body, beyond enough nutritional influence. They are not pills or capsules, but then part of a normal food pattern.

Total antioxidant capacity is an arresting content to get a general picture of the antioxidant potential of a matter and it also requires much less work and methodological infrastructure than analyzing the often complex combination of individual antioxidants. An antioxidant is an applicable example of rampart that has the potential to consult health benefits as compounds of functional foods. Evidence indicates the probability of protection of vascular entirety through a useful compilation of risk factors such as high plasma homocysteine concentrations and high blood pressure. Camel casein is very susceptible to proteolysis and able to generate peptides with various biological activities such as antioxidant activity.

The aim of this study was to assay physicochemical and microbial properties, calcium, phosphorus content, and antioxidant activity of traditional fermented camel milk (Chal) in Golestan province of Iran and introduce it as a functional food.

Materials and Method

Materials

Trolox, 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma-Aldrich. Other chemicals such as Sodium hydroxide, Sulfuric acid, Hydrogen chloride, sodium carbonate, boric acid, nitric acid, ammonium heptamolybdate and lanthanum chloride were obtained from Merck, Darmstadt, Germany. All chemical agents were of analytical grade.

Sampling

Samples of Chal (traditional fermented camel milk) randomly collected from diverse household's rancher with various levels of sanitation in Golestan province. The areas under investigation were the city of Bandar Torkman in Iran. Samples were transported at 4 °C to the laboratory and subjected to determination of physicochemical, microbial properties, calcium, phosphorus and antioxidant activity.

Physicochemical Analysis

Compositional properties of Chal samples were analyzed in duplicate for contents of fat, protein, totally solid, acidity, pH, calcium and phosphorus. To determine the total protein content of fermented camel milk we have to use the Kjeldahl method, using a drying oven and acidity was compulsive total solid by titrating with 0.1 N NaOH using phenolphthalein and results were explicit in degrees (Dornic). Fat content was determined by the Gerber method, degree of pH were measured by the pH meter, calcium was determined by atomic absorption spectrometer in the presence of lanthanum oxide and concentration of phosphorus was measured by a colorimetric method with ammonium molybdate.

Mineral Analysis

Several techniques have been used to quantify mineral in milk and derivates, mainly flame atomic absorption spectrometry (FAAS). FAAS and titrimetry techniques have been mostly used in routine analysis of milk due to their simplicity, high accuracy and precision and relatively low cost. Atomic Absorption Spectrometry (AAS) is a material for measurement of the amount of chemical elements present in environmental samples by measurement the absorption irradiation by the chemical element of interest. This is done by reading the spectra constructed when the instance is excited by irradiance. Atomic absorption technique measures the quantity of energy in the form of photons of glory that are absorption instance. A signal mainframe then merges the changes in wavelength absorption, which become visible in the readout as peaks of energy absorbed at distinct wavelengths. The energy necessary for an electron to leave an atom is known as ionization energy and is special to each chemical element. When an electron moves from one energy degree to another within the atom, a photon is emitted with energy. Atoms of an element release a characteristic spectral line. Every atom has its own different template of wavelengths at which it will absorb energy due to the unique layout of electrons in its outward shell. The concentration is measured based on the Beer-Lambert law. Absorbance is straight proportionate to the concentration of the analyte absorbed for the existing set of qualification. The concentration is generally distinctive from calibration curves, obtained using standards of known concentration. However, utilization the Beer-Lambert law straight in AAS is arduous due to changes in atomization organization from the instance matrix, non-monotony of concentration and route length of analyte atoms. The chemical techniques used are based on material fundamental interactions, i.e. chemical reactance. For a long period of time, these methods were necessary experiential, inclusive, in most instance, great experiential proficiency. In analytical chemistry, AAS is a method used often for designation of the concentration of a particular matter metallic element within an instance. AAS can be an application to analyze the concentration of over 62 dissimilar metals in a dilution. To measure how much of a given element is ubiquitous in an instance, one must first create a foundation for collation using known quantities of that element to produce a calibration curve. To generation this curve, a special wavelength is chosen, and the detector is set to mensuration only the energy transmission at that wavelength. As the concentration of the target atom in the instance increscent, the absorption will also increscent pro rata. A series of instance comprising known concentrations of the composition of interest are analyzed, and the correspondent absorption, which is the inverted percent of glory transmission, is recording. The measurement absorbency at each concentration is then drawing so that an undeviating line can be drag middle of the outcome points. By this line, the density of the material under verification is extrapolated from the material's absorption. The utilization of specific glory sources and the choice of special wavelengths permit for the slight specification of the sole ingredient in a multielement admixture.

Microbiological Assay

Microbiological characteristics of Chal including a total count and coliform counts were determined based on Standard Methods for the Examination of Dairy Product. Plate Count Agar for total counts and plates were incubated at 30°C for 72 h. Violet Red Bile Agar applied for coliform count and plates were incubated at 37°C for 24 h.

Antioxidant activity

Oxidation is a vital process in all living organisms even though its side effects are the producing free radicals. The action mechanism of oxidation is related to the inactivation of reactive oxygen species (ROS) by scavenging of free radicals, chelation of pro-oxidative transition metals and re-duction of hydroperoxides. It is well documented that free radical formation is a major factor involved in the progression of many hu¬man diseases, such as heart diseases, stroke, athero-sclerosis, and cancer, which have been considered as the leading causes of human death.

Antioxidant activity of fermented milk samples was evaluated by a method based on scavenging the 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS; SigmaeAldrich) as described previously by Re et al. (1999) and Salami et al. (2011). The $ABTS^+$ radical cation was produced by dissolving 11 mg of cation $ABTS^+$ in 2 mg po¬tassium persulfate and 3 ml deionized water to stand in the darkness for 12-16 h at room temperature before use. The $ABTS^+$ was then diluted with 2750 μL sodium phosphate buffer, pH 7.4, to reach an absorbance of ca. 0.7 at 734 nm. A volume of 15 μL of sample was added to 50 μL of the $ABTS^+$ and 2750 μL sodium phosphate buffer. The mixture was incubated for 5 min at 25 °C. All the analyses were performed in triplicate. The absorbance was measured at 734 nm. The antioxidant activity of the samples, explicit as an inhibition per¬centage (%), was calculated with the calculation:

$$\text{Activity (\%)} = [1- (A_a - A_b) / (A_c - A_b)] \times 100$$

A^c is the absorbance of the beginning $ABTS^+$ radical cation, A_a is the absorbance of the remaining radical, and A_b is the absorbance of the blank (in the case of phosphate buffer). When the absorbance of the remaining radical (A_r - A_b) was equal to that of the scavenged radical (A_c - A_a).

Statistical Analysis

One-way analysis of variance (ANOVA; $P<0.05$) was used to analyze data using SPSS 20.0 software (SPSS INC., Chicago, IL, USA). Significant different were calculated by Duncan's New Multiple Range Test. The data reported as mean±SD (Standard Deviation).

Results and Discussion

Physicochemical Composition

Physicochemical properties of Chal were determined and the results are shown in Table 1.

Table1: The mean values for physicochemical properties of Chal (g/100mL)

physicochemical properties	Mean ±SD*
Fat (%)	5.8±0.27
Total Solid (%)	12.24±0.16
Acidity(Dornic)	80±7
Protein (%)	3.07±0.073
pH	4.52±0.10

pH and acidity levels for Chal obtained 4.52±0.10 and 80±7 °D, respectively. Titratable acidity of Chal was higher, while pH was similar to fermented bovine milk. According to the research by Teklemichael et al. (2015), fat in raw bovine milk was 3.862±0.412 and protein 3.420±0.139, therefore fat and protein Chal was high than fermented bovine milk.

The obtained data revealed that fat and protein content and dry matter in cold season was more than heat season; this may be explained by the low accessibility of water for a camel in the warm season and antidiuretic hormone secreted in this season; therefore the amount of water increased in the warm season. The increased water may cause a reduction in fat, protein, and dry matter. However, there was a significant difference between fat content in the cold and warm season ($p < 0.05$) but there was no significant difference in protein, dry matter, acidity and pH between the two seasons ($p \geq 0.05$) (Table 2).

Table2: Physicochemical properties (Mean ±SD*) of Chal in cold and warm season (g/100 mL)

Parameter	Cold season	Heat season
Fat (%)	6.21±0.29 b	4.87±0.14 a
Protein (%)	3.14±0.73 a	2.89±0.15 a
Total Solid (%)	12.50±0.17 a	11.60±0.12 a
Acidity(Dornic)	0.89±0.08 a	0.59±0.01 a
pH	4.37±0.11 a	4.92±0.10 a

**SD: Standard Deviation*
Values with different small letters in a row are significant difference ($p < 0.05$)

Results revealed that fat and total solid in this study were higher than in the study by Hassan, et al. (2003) Ibtisam and Marowa (2009), Olfa Samet Bali et al. (2012), while protein content in this study was in agreement with those reported by camel milk processing plant in Kenya (Vital Camel Milk Limited based in Nanyuki) (2006).

Mineral analyses

The results showed that Chal can provide a great amount of calcium and phosphorus and the mean value of these minerals of Chal was determined and results are shown in Fig. 1. In comparison calcium and phosphorus of Chal with kefir produced from bovine milk, the mineral content for Chal was more than kefir. Both elements are needed for tissue and bone development while deficiency results in slow growth, depraved appetite and rickets. The results indicated that Chal could provide a great amount of calcium and phosphorus as reported in this study.

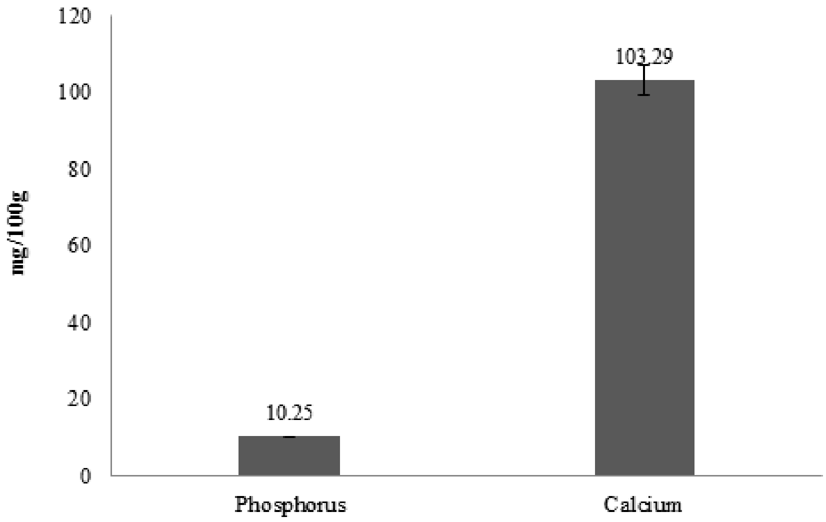

Figure1: The mean value for calcium and phosphorus of Chal (mg/100g)

Calcium and phosphorus in a warm season were higher than in winter ($p<0.05$), this may be due to feed with green grass in spring. In general, there was no significant difference in calcium and phosphorus content between hot and cold season (Fig. 2).

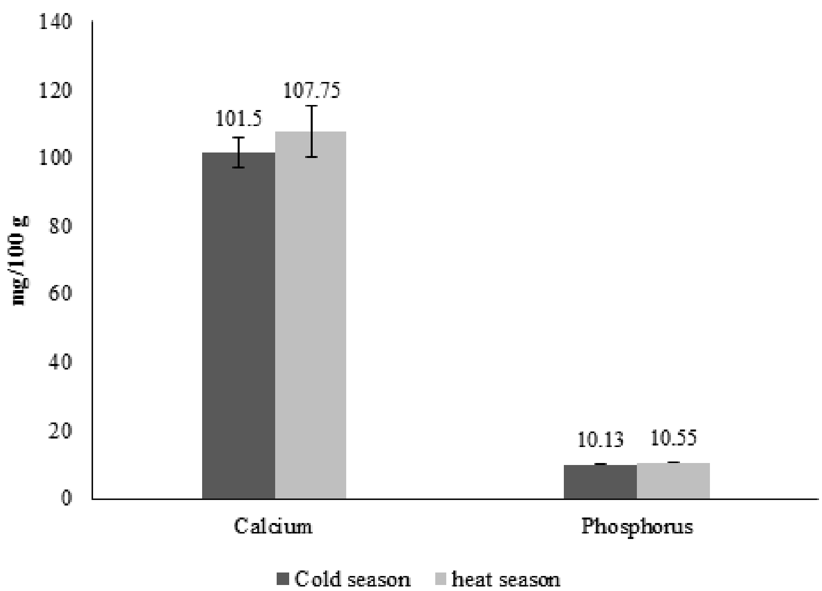

Figure2: Analysis for calcium and phosphorus of Chal in cold and hot seasons

Calcium and phosphorus content of Chal in this study was higher than previously reported for bovine milk. Calcium in milk is distributed between the milk serum and the casein micelles. Calcium is an essential macronutrient for humans, which represents approximately 2% of body weight in an adult person. This element has mainly a structural function in bones and teeth, besides to regulate many vital biological functions. More recently, interest in calcium has centered on its role in preventing osteoporosis. It is known that the highest demands for this element are produced during the periods of maximum growth such as in childhood and adolescence, and also during lactation and in the elderly. The need recommended dietary allowance for calcium by Food and Nutrition Board is approximately 1300 mg per day for adolescents and 1100 mg per day for adults. Samet-Bali et al. (2012) reported that calcium and phosphorus in traditional Leben were 0.75 ± 0.02 and 1.17 ± 0.06 g/kg, respectively. As reported by Ot1es and Cagindi (2003) calcium and phosphorus in kefir obtained 0.12 and 0.10 g/100g, respectively. In this study, calcium in Chal 103.29 mg/100g and phosphorus 10.25 mg/100g, therefore, Chal was rich in Calcium.

Phosphorus had a greater effect on growth, feed conversion and bone development than calcium and that the calcium-phosphorus ratio was more important than dietary levels of phosphorus for optimum over-all performance.

The kidney is the primary mechanism for rapid release or absorption of calcium through the filtration and urine excretion functions. Approximately 200 mg per day is typically excreted by adults through the kidneys via urine but varies by diet and serum parameters. The second organ system, the intestines, is slower in response. A daily dietary intake of 1,000 mg of calcium would potentially result in 800 mg available for tissue nutrient requirements and 200 mg to maintain serum calcium levels. Extraintestinal calcium can be processed through the kidneys and removed from the body through urinary excretion. In the third system, calcium can move both into and from bone matrix. The flexible bone pool, which varies by body size and bone density, typically has available calcium of approximately 150-200 mg. If more is required, actual bone calcium must be released ("borrowed") from the bone matrix and used to maintain serum calcium. Replacement of "borrowed" calcium does not always ensure similar bone composition.

Nutrient recommendations for healthy adults for calcium in France 1200 mg/day, Korea 700 mg/day, United Kingdom 700 mg/day and United States 1000 mg/day and for phosphorus in France 750 mg/day, Korea 700 mg/day, United Kingdom 550 mg/day and United States 700 mg/day.

A recent meta-analysis by Fenton et al. (2009) illustrated the relationship between phosphate and changes in calcium balance that occur primarily in the kidney. During growth period food that containing both calcium and phosphorus can lead to positive effects on bone health.

Most dietary Ca is absorbed in the small intestine, but results of some studies have indicated that Calcium might have been also be absorbed in the colon. The specific region in the small intestine where Ca is absorbed is not well defined because although the absorption of Ca is greater in the proximal fourth of the small intestine than in the remaining part, the type of diet that is fed may affect the location in the intestine where Ca is absorbed. The presence of Ca-binding proteins in the enterocytes and the pH of the duodenum and jejunum explain the efficacy of Ca absorption in the proximal small intestine. Therefore, camel milk and fermented camel milk (Chal) can be introduced as a remarkable source of Ca.

Microbiological Quality

Microbial quality of Chal samples is shown in Tables 3. Coliform counts obtained 2.34±0.23 log CFU mL^{-1} for Chal samples.

Comparison between the microbial count of

Table3: The mean value (Mean ±SD*) for microbial count of Chal (log CFUmL^{-1})

Microbial count	Count (log CFU mL-1)
Total count	6.54±0.19
Coliform count	2.34±0.23

**SD: Standard Deviation*

Chal samples in the hot and cold season is shown in Table 4. Coliform count in spring and winter was the same and there was no significant difference between hot and cold season (p≥0.05).

Table4: Analysis for microbial count (Mean ±SD*) of Chal between two seasons (log CFUmL^{-1})

Parameter	Cold season	Heat season
Coliform count	2.41±0.31 a	2.17±0.20 a
Total count	6.73±0.20 a	6.07±0.40 a

**SD: Standard Deviation*
Values with different small letters in a row are statistically significant difference at p< 0.05

According to Codex standard, microorganisms for fermented milk were 106 CFU mL-1. The result of total count for Chal was high; this may be because of the fermentation process to let the high yeast content of molds and LAB (lactic acid bacteria) growth in Chal. In this study, the total count of Chal samples ranged from 5×10^5 to 3.2×10^7 CFUmL^{-1} (Tassew et al 2010). Till now there is no standard for the microbial count for camel milk and its products; therefore, quantities for the limit of microbiological of bovine milk have been used to assess the quality of camel milk and its products.
Total counts were determined as 6.54±0.19 log CFUmL^{-1} for Chal samples, As shown in Table 4, there was no significant difference between hot and cold season (p≥0.05) regarding total count. Total count ranged in Shubat 6.8-7.6 log CFUmL^{-1}, in Gariss 7.3–8.7 log CFUmL^{-1} and in Sussa 9.03 log CFUmL^{-1}.

Antioxidant activity

Antioxidant mechanisms include radical-scavenging (free radical quenching) activity. In this research, radical scavenging activity was defined using the radical cation $ABTS^{+\bullet}$ which is decreased by participant transformation to a colorless product in the attendance of antioxidants by hydrogen-donating or breaking the chain attributes. The mean inhibitory activity for Chal was determined 44.95±1.29%. Antioxidant activity of bovine milk (42.29%(which was lower than antioxidant activity obtained for Chal, in this study.
Antioxidant one of the main driving force, which helps to sustain human life, are the biochemical reactions which take place within the organelles and cells of the body. The laws of nature are such that one moves from infancy to childhood, then into adulthood, and finally one becomes a frail human being eventually leading to death. This aging process is a common feature of the life cycle of virtually all multicellular organisms. The number of people aged 65 and over is predicted to increase by approximately 53% in the United Kingdom by the year 2031 and similar changes are likely to be seen in other developed countries due to low birth rates and increasing life expectancy, which will lead to an increasingly elderly population. This predicted gain in life expectancy would potentially lead to an increase in the number of older people acquiring age-related chronic diseases of the cardiovascular, brain, and immune systems. This can cause loss of autonomy, dependence and high social costs

for individuals and society, and will impose increased workload and financial pressures on healthcare systems worldwide. Due to this there is a major interest in understanding of the biochemistry of aging and providing a database of "anti-aging" medicines, diet and commercial products which can provide safe, effective and practical methods for increasing longevity with a good quality of life during aging, and thus decrease the dependence of elderly people on expensive high-tech medicine .

Antioxidants can inhibit LDL oxidation, efficacy the actuality of immune worthy cells and prevent the organization of cell-to-cell adhere factors. Evidence indicates the probability of protection vascular entirety through a useful compilation of risk factors such as high plasma homocysteine concentrations and high blood pressure.

Conclusions

When a food has calcium by itself, this calcium is bonded with the protein of food, this calcium is more effective in our body than the calcium addition to food which not bonded any proteins, thus Chal the best idea for securement calcium. The present research recommended that by considering good hygienic practice during milking, the microbial count will be in proper range. The results suggest that different climate conditions in the different countries can cause microbial contamination and various reports show this subject. The significant difference between two seasons was found for fat content. Fermented camel milk has developed a high reputation as a healthy nutrition with most of its cure value ascribed to its biologic properties such as antioxidant activity and pregnant in calcium and phosphorus. Antioxidants inhibit LDL oxidation and increase immune worthy cells and prevent the organization of cell-to-cell adhere factors. Evidence indicates the probability of protection vascular entirety through a useful compilation of risk factors such as high plasma homocysteine concentrations and high blood pressure. Adequate calcium consumption may support to decrease the risk of osteoporosis in life. Amplification of food products with calcium is commonly practical as one of the strategies to raise calcium intake and recovery bone mineral density. However, it is hard to add calcium in noteworthy quantities, because it frequently gives a plaster taste and a bad mouthfeel or because of it sediment into a grayish mass within a liquid production. However, judicious mixing and the use of diverse sources of calcium, attractive tissue for the food itself, covering flavoring substances and diverse compositions of the component can recover the amiability of calcium tenacious foods. This clearly is a challenge for food technology vitamin and mineral rampart for special goals is formerly well known. According to the points that mentioned in this study, we can introduce Chal as a functional food.

ACKNOWLEDGEMENTS

The support of pharmaceutical Sciences Branch, Islamic Azad University (IAUPS) are gratefully acknowledged.

Conflicts of Interest

None of the authors have any conflict of interest associated with this study.

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