Physico-Chemical Analysis and Composition of Camel Milk of Bangladesh

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Abstract: Camel farming is increasing in Bangladesh but the nutritious value of the produced milk has not been studied in this geological location. The milk was coagulated by citric acid and the coagulated solid *i.e.* the casein (7%) and pure serum (14%) were obtained. Fat content was determined by extracting casein and the aqueous serum, separately with n-hexane and found 2.59% and 5.79%, respectively. The fatty acids in the fat from casein and serum were made into their methyl ester by saponification followed by esterification and analyzed by GC-FID. Palmitoleic, oleic and linoleic acids were predominant fatty acids found in the analyzed samples while stearic, arachidic, behenic and myristic acids were present as minor acids. Water, ash, nitrogen and lactose contents in the milk were 84%, 0.88%, 1.62% and 9.32%, respectively. The presence of vitamin B_1, B_2 and B_6 were estimated by UV-VIS spectrophotometer and found 388, 64 and 116 ppm, respectively.

Keywords: Bangladesh, Camel milk, Casein, Fatty acids, Vitamins.

INTRODUCTION

Camel is a desert animal which has been used from ancient time as transport in the desert areas. Camel driven cart is usually common there. Camel farming can be done in dry and salty regions. Its farming is an alternative to cow dairy farming in dry regions of the world where cattle farming consumes large amount of water [1]. Camel milk is very similar to goat milk and compares very favorably with human milk [2]. Camel milk is renowned for its health-giving qualities, which includes good bone growth [3, 4]. The camel produces nutritious milk for human consumption. Most of their milk is drunk fresh. It is also consumed when slightly sour or strongly soured. Normally it has a sweet and sharp taste but sometimes it is salty [5]. Compared to cow, buffalo and ewe milk, camel milk fat is found to have higher concentrations of linoleic acid and the polyunsaturated acids, which are essential for human nutrition [3]. It is low in lactose compared with cow's milk. However, levels of potassium, magnesium, iron, copper, manganese, sodium, zinc and vitamins, especially vitamin C are higher than in cow's milk. Cholesterol in camel milk is lower than cow or goat milk. Its milk has more fat and protein than cow's milk [6]. The composition of camel milk mainly depends on its feed and species.

Camel farming is increasing in many different countries including USA, Netherland, China, Pakistan and India considering low cost of farming, nutritional & medicinal value of the milk [7]. There are some areas in Bangladesh where the land is getting salty and dry. It is difficult for cattle to graze there and as a result milk production is decreasing. Camel farming can be popular there to balance the milk supply. At present, there are some small farms in the country and owner is selling the camel milk for medicinal and nutritional purposes. However, there is no study of chemical composition of the milk of our country. The objective of the work is to determine chemical composition of camel milk produced in Bangladesh. The study will help the consumers to use it as a source of nutrition for health management.

MATERIAL AND METHODS

Instruments

UV-visible spectrum was recorded on Shimadzu UV 1800. Gas chromatography (GC) analyses were performed on a Shimadzu 2025 GC connected with a flame ionization detector (FID). Nitrogen was used as carrier gas. Hydrogen and air were used for flame. Separations were performed on HP-5 capillary WCOT quartz columns (30 m long & 0.25 inner diameter; film thickness was 0.25 µm). Column flow rate was 2 mL/min. Injected volume was 1.0 µl. The column oven temperature was programmed for analysis: 120 °C (1 min) in a rate 7 °C/min to 270 ^{o}C (6 min). Injector and detector temperature were 280 ºC and 290 ºC respectively.

Collection of Sample

Three samples of camel milk labeled as CM1, CM2 and CM3 were purchased from a local farm located in Dhaka and the milk was immediately stored in deep

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freezer at -20 °C to control souring of the milk and to stop attack by microorganism.

Separation of Casein and Serum from Milk

The whole liquid milk (250 mL) was boiled and 2M citric acid was added drop wise to the hot milk until coagulation completed followed by cooling at room temperature. The suspended protein *i.e.,* casein part and serum part were visible. The casein was collected by filtering through a pre-cleaned cotton filter while serum was collected (~150 mL) in another beaker. The casein part was washed for several times with distilled water until it was acid free. The acid free casein was collected in round bottomed flask and was dried in a freeze-dryer until constant weight was obtained. The weight of the casein of the camel milk was found to be 16 g (7%). The total serum (~150 mL) was taken into a separatory funnel and 5 mL of saturated NaCl solution was added to it and the mixture was mixed thoroughly. Then the serum was partitioned with n-hexane (50 mL x 3). The hexane soluble part was taken in a beaker, treated with anhydrous sodium sulphate, filtered and the transparent filtrate was taken into a pre-weighed round bottomed flask and was evaporated until constant weight was obtained. Amount of fat (*ie* butter oil) obtained was 14 g (6%). The remaining aqueous part (*ie* fat free serum) was neutralized with sodium hydrogen carbonate, filtered and was centrifuged. 10 mL of the centrifugate was freeze dried and 1 g of solid material was obtained. The dried serum was stored in the freezer. Rest of serum which was not freeze dried also saved in the freezer. Casein (16 g) was taken in a 250 mL a round bottomed flask and was refluxed with 100 mL of n-hexane in a heating mantle for 30 minutes. The n-hexane soluble part was collected by decantation. The process was repeated two more times. The combined n-hexane part was evaporated and 6 g (2.59%) of fat (ghee) was obtained.

Saponification and Esterification of Fatty Acids

Fat from serum (50 mg) or casein (50 mg) was taken in a pear shaped flask and 1.0 ml of 0.5 M methanolic NaOH solution was added to it. The mixture was ultrasonicated for 1 min and then refluxed on a boiling water bath at about 96 °C for 40 minutes. The mixture was acidified with 0.5 mL of 0.2M HCl. The acidified mixture was partitioned with n-hexane. The n-hexane soluble part was collected in another pear shaped flask and was evaporated to dryness by a rotavapour.

Borontrifluoride-methanol (BF_3-MeOH) complex was added into the pear shaped flask containing saponified hexane soluble fat from serum and the mixture was heated in a boiling water bath for 30 minutes. The methyl ester of fatty acid mixtures was then evaporated to dryness by a rotavapor. n-Hexane (1.0 mL) was added and filtered through cotton filter by a pasture pipette and transferred into a GC vial for analysis by GC-FID.

Estimation of Protein in Camel Milk

Nitrogen content of dried camel milk was determined by Kjeldahl method and then the value was multiplied by a factor 6.37 [8].

Determination of Total Solid Content

Camel milk (10 mL) was taken in a porcelain crucible and heated at 100 ^{0}C until constant weight was observed.

Determination of Ash Content

After removing the moisture content from 10 mL of camel milk it was heated at a temperature of 750 ^{0}C for about 2 hours in a high temperature muffle furnace.

Mineral Content

Serum (10 mL) was freeze dried to obtain 1.40 g solid and 1.0 g solid material was analyzed for the quantity of sodium and potassium ions by atomic absorption spectrophotometer (Thermo scientific ICE 3000). Sodium content was determined using sodium hollow cathode lamp as the source and potassium was determined by using potassium hollow cathode lamp as a source [9].

Determination of Lactose Content

Standard lactose (10 mg) was dissolved in distilled water (10 mL) and diluted to obtain concentration of 0.5 mg/mL. Then, the solution was serially diluted to 4 different concentrations, 0.15, 0.10, 0.075, and 0.05 mg/mL and distilled water was taken as blank. Lactose solution (300 µl) and 200 mL water were taken in a test tube and 80% aqueous phenol (50 µL) was added to it and vortexed for 30 sec. Conc. H_2SO_4 (3 mL) was added to the mixture with gentle shake and reddish brown color was observed. The absorbance of the reddish brown color of this solution was measured at 489 nm by a UV-visible spectrophotometer. Similar way, absorbance of other lactose solutions was

measured. A calibration curve was made by plotting absorbance against different lactose concentrations and a straight line was obtained (Figure **1**).

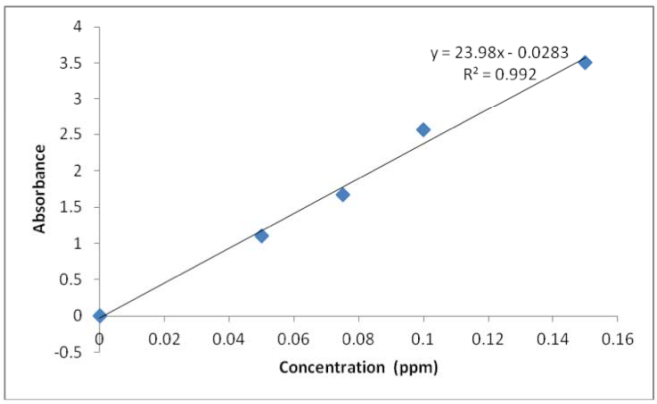

Figure 1: Calibration curve of standard lactose solution.

Serum of each camel milk (500 µL) was diluted to 100 mL with distilled water. Diluted serum (500 µL) was taken in a test tube for phenol-sulphuric acid test and absorbance was measured. Amount of lactose in camel milk solutions were determined using the standard calibration curve made for lactose [10]

Determination of Vitamin Contents

Procedure for the Spectrophotometric Determination of Vitamin B_1, B_2 and B_6

Standard vitamin B_1 (thiamine hydrochloride) solution (100 ppm) in deionized water was prepared and diluted to 50 ppm. The diluted solution (20 mL) was mixed with ammonia solution (20 mL), *p*-amino phenol solution (10 mL of 100 ppm) in a separating funnel and was vigorously shaken for 5 minutes. After addition of 40 mL of chloroform solution 2 layers were obtained and the bottom layer (chloroform layer) contained the yellowish colored vitamin B_1- p-amino phenol complex which was collected and diluted serially to obtain 15, 10, 8, 5, 2, 1 ppm vitamin B_1-p–amino phenol complex and finally absorbance was measured at 490 nm. A calibration curve was made by plotting absorbance against different concentrations and a straight line was obtained (Figure **2**). Absorbance of serum of each camel milk was measured and amount of vitamin B_1 in camel milk solutions were determined using the standard calibration curve [11].

Standard vitamin B_2 (riboflavin) solution (1000 ppm) was prepared in deionized water and diluted to 500, 100, 50, 40, 20, 10, 5, 2.5, 1, 0.5 ppm. Absorbance (deionized water was taken as reference) of these solutions were measured at 445 nm by a double beam UV spectrophotometer and a calibration curve was made.The absorbance of pure serum of camel milk was measured at 445 nm wavelength and the amount of vitamin B_2 in camel milk solutions were determined using the standard calibration curve made for vitamin B_2 [12].

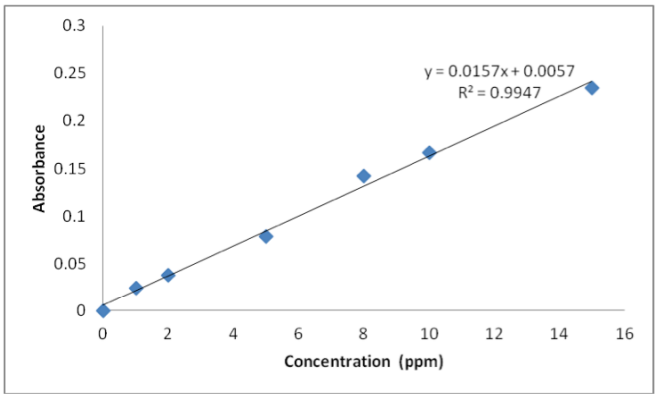

Figure 2: Standard Calibration curve for the determination of vitamin B_1.

Standard vitamin B_6 (pyridoxine hydrochloride) solution (50 ppm) was prepared in deionized water and diluted to 25, 15, 10, 5, 2.5, 1 ppm. 10 mL of each solution was made to 50 mL with 3.5 mL acetic acid, 25 mL methanol and deionized water. Absorbance of each solution was recorded at 292 nm. Absorbance of serum of each camel milk was measured at 292 nm and amount of vitamin B_6 in camel milk solutions were determined using the standard calibration curve [12].

RESULTS AND DISCUSSION

Camel milk was coagulated by citric acid and the coagulated solid *i.e.* the casein and the aqueous part were separated. Casein found in the milk was 7%. Serum was defatted to get pure serum for determination of minerals. Fat content was determined by extracting casein and the aqueous serum, separately with n-hexane. Percentage of fat from casein (ghee) and serum (butter oil) was 3 and 6, respectively. Total fat was the sum of the fat from casein and serum which is 9%. The percentage of fat from camel milk of Bangladesh was higher than those of other countries [5]. The fatty acids in the fat from casein and serum were made into their methyl ester by saponification followed by esterification and analyzed by GC-FID. The methyl esters of fatty acids in fat were identified by comparing their retention time with retention time of methyl ester of standard fatty acids. Palmitoleic, oleic and linoleic acids were predominant fatty acids found in the analyzed camel milk samples while stearic, arachidic, behenic and myristic acids

were also present as minor acids. The range of relative percentage of palmitoleic, oleic and linoleic acids in the fat of serum were 41-43, 19-24 and 13-18%, respectively while they were in the range of 32-34, 15-25 and 1-2% in the fat of casein (Table **1**). Relative percentage of unsaturated fatty acids was found higher in the serum (73-85%) than in the casein (48-61%). Water content in the camel milk was found 84% which is very close to the range of other finding (86-91%) [13-16] and the farming condition play important role for this value. Ash content was found 0.88% which is also within the range of other countries (0.35-0.95 %) [14-16]. Nitrogen content of dried camel milk was determined by Kjeldahl method and then multiplying that by a factor 6.37. Protein contents in the camel milk of CM1, CM2 and CM3 were 1.62, 1.60 and 1.64%, respectively which is much lower than that of the camel milk of other countries (Table **2**). Generally, the protein percentage in the milk is 2.5-4.5 [14-16]. Lower value of protein content might be due to high fat found in camel milk of Bangladesh. The level of sodium and potassium in the camel milk were found 0.15% and 0.08%, respectively. Our finding of mineral in the camel milk correlates with the findings from other countries [17].

Table 1: Range of Relative Fatty Acids (%)

Fatty acids	Serum	Casein
Palmitoleic	41-43	32-34
Oleic	19-24	15-25
Linoleic	13-18	1-2
Saturated	27-15	52-39
Unsaturated	73-85	48-61

Table 2: Nutritious Values in Camel Milk

Parameters	Values	Reported values
Water content	84%	86-91 [13-16]
Ash content	0.88%	0.35-0.95 [14-16]
Protein content	1.62%	2.5-4.5 [14-16]
Lactose content	9.32%	Not available
Vitamin B_1	388 mg/mL	Not available
Vitamin B_2	64 mg/mL	Not available
Vitamin B_6	116 mg/mL	Not available

The presence of lactose in the milk was estimated following phenol sulphuric tests by UV-VIS spectrophotometer. A calibration curve of standard lactose was made using absorbance against concentration of standard lactose (Figure **1**). The curve showed straight lines with r^2 = 0.992 (Figure **1**). From the calibration curve, the amount of lactose of CM1, CM2 and CM3 was found 8%, 9% and 11%, respectively indicating average amount of lactose 9% which is much higher than values reported by others. Lactose contents in the earlier reported experiments were estimated by titrimetric method but spectroscopic method was used for the present experiment which seems to be more reliable and accurate.

The presence of vitamin in the milk was estimated by UV-VIS spectrophotometer. Calibration curves of standard vitamin B_1, B_2 and B_6 were made using absorbance against their concentration and the curve showed straight lines with r^2 = 0.995, 0.999 and 0.995 for vitamin B_1, B_2 and B_6, respectively (Figures **2-4**). The concentration of vitamin B_1, B_2 and B_6 were 388, 64 and 116 ppm in camel milk. Vitamin contents in the earlier reported experiments were estimated by HPLC but spectroscopic method was used for the present experiment which seems to be more reliable and accurate.

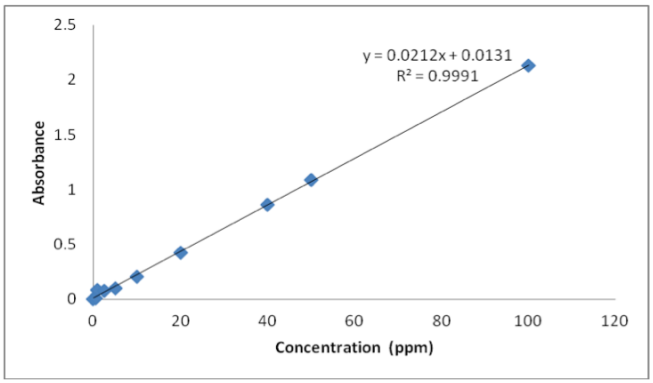

Figure 3: Standard calibration curve for the determination of vitamin B_2.

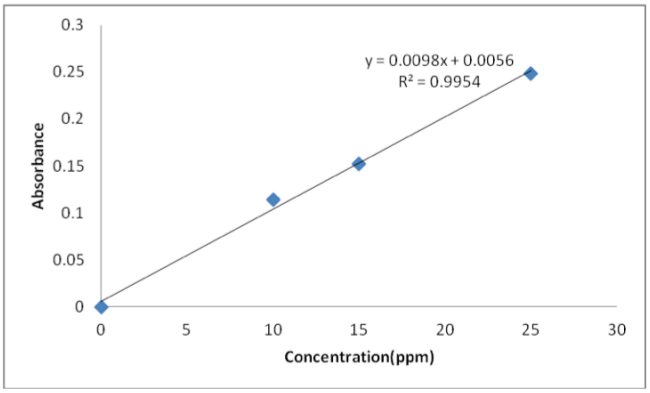

Figure 4: Standard calibration curve for the determination of vitamin B_6.

CONCLUSIONS

Casein (7%) and pure serum (14%) were separated from camel milk. Fat content was determined and palmitoleic, oleic and linoleic acids were predominant fatty acids found in the fat from casein (3% fat) and serum (6% fat) while stearic, arachidic, behenic and myristic acids were present as minor acids. Water, ash, nitrogen and lactose contents in the milk were 84%, 0.88%, 1.62% and 9.32%, respectively. Vitamins *i.e.,* B_1, B_2 and B_6 were 388, 64 and 116 ppm, respectively. Our finding suggests that camel milk from Bangladesh contains nutritious value for human health and camel farming can be beneficiary for the people who have less access to the cow milk farming.

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