

Research Article

Chromatographic appraisal of fatty acid profile in camel's milk at different temperatures

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Abstract

Milk is an important component of humans' diet. Milk fats provide the essential fatty acids important for health and normal growth of the body. The aim of the study was to determine the fatty acid profile of camel's milk through gas chromatography mass spectrometry (GC-MS) at different temperatures i.e. 4°C, 7°C, and 16°C. A total of 18 milk samples were obtained from lactating local camels available in Peshawar, Pakistan. The results demonstrated a higher proportion of saturated fatty acids (SFA) than unsaturated fatty acids (USFA) in milk samples. The predominant fatty acids were Palmitic acid (C16:0), Myristic acid (C14:0) and Stearic acid (C18:0), where they were 48.86%, 23.5% and 15.26% of the total fatty acids in milk respectively. The SFA were dominated by Myristic acid (C14:0) and Palmitic acid (C16:0) and the major monounsaturated fatty acids (MUFA) were Heptadecenoic (C17:1) and Eicosanoic acid (C20:1) and the major polyunsaturated fatty acid (PUFA) was Linoleic acid (C18:2c). The Medium-chain fatty acids (MCFA) which was represented by Lauric acid (C12:0) only was 1.51% in milk. The current study further elucidated differential expression of diverse fats with temperature changes. The statistical analysis showed that Myristic acid (C14:0), Pentadecanoic acid (C15:0), Palmitoleic acid C16:1c, Stearic acid (C18:0), and Linoleic acid (C18:2c) varied significantly ($p < 0.05$) in response to change in temperature.

Key words: Camel; Chromatography; Fatty acids; Milk

Introduction

Milk is one of the most significant foods for human beings containing lactose, protein, fat,

vitamins and minerals in simple, digestible and acceptable form. It is regarded as complete diet for pregnant mothers and

young children [1]. According to recent statistic of the Food and Agriculture Organization, the entire population of camel in the world is approximately 20 million and Somalia has the leading herds worldwide [2]. Camel is regarded as an excellent resource of meat and milk. All the vital nutrients present in bovine milk are found in camel milk [3]. Record shows that a good fed camel yield 10-15 L milk per day. In desert areas and those regions where there is scarcity of fodder, camels yield an average of 4 L milk per day [4]. Camel is regarded as an excellent resource of meat and milk. It is also used for many other activities including transport purpose, racing and dancing. The nutrition of peoples existing in the dry hot regions mainly depends on camel milk. In Saudi Arabia, camel's milk is mostly common and is taken as fresh and sour milk [5]. The countries including Russia, Sudan and India generally use fresh and fermented camel's milk for the treatment different diseases such as tuberculosis, jaundice, dropsy, leishmaniasis (kala-azar) and asthma [6].

Records show that keeping quality of camel's milk is better than cow milk, because of its absolute protein content which has antibacterial effects making it a profitable product [7]. Camel milk is more heat resistant due to its antimicrobial agents when compared with that of cow and buffalo milk [8]. The difference between camel's milk and milk produced by other animals is that it has lower level of sugar, protein and cholesterol but increased levels of insulin, vitamins and minerals [9]. The milk of camel contain 86.3 to 88.5% water, 8.9 to 14.3% solid-non-fat, 2.9 to 5.8% lactose, 2.9 to 5.5% fat, 2.5 to 4.5% protein and 0.35 to 0.90% ash [10]. The composition of lipids are cholesterol (C, 0.5%), phospholipids (PL, 0.8%) and triacylglycerol (TG, 98%). In the aqueous phase of milk the globules of lipids (3-5%) are present in emulsified form [11]. Based on the chemical structures, lipids can be

categorized into many classes [12]. The lipid class which is found mostly is triacylglycerol (TAG) leaving behind phospholipids. Despite the aforementioned facts regarding camel milk composition, comprehensive appraisal of the lipid profile of camel milk has not been fully reported. Accordingly, attempt has been made here to elucidate the fatty acid profile of camel milk and determine milk fat percentage at different temperatures.

Materials and methods

Collection of milk samples

A total of 18 milk samples (150 ml each) were collected in sterilized bottles from the local lactating camels in District, Peshawar. These camels are easily found throughout the province. Samples were shifted to laboratory in ice box to maintain the cold chain during transportation. The milk samples were divided into three portions under sterile condition. The 1st portion stored at 4°C, the 2nd stored at 7°C while the 3rd portion stored at 16°C. Samples stored at 4°C were analyzed at three-week interval, sample stored at 7°C at two-week interval while sample stored at 16°C were analyzed on day 0.

Lipid profile determination

The lipid profile was determined through GC-MS (QP-2010, Shimadzu, Japan, ID=PLC/FTC/Chem, MB/05) at Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories, Peshawar, according to the optimized protocol [13].

Extraction

Briefly, 30 ml milk sample was added into 112.5 ml Chloroform-Methanol solution (1:2 v/v) in a 300 ml conical flask and vortexed the mixture for 2 min., followed by centrifugation with 37.5ml chloroform and vortexed the mixture again for 2 min. Distilled water (37.5 ml) was added into mixture and vortexed, followed by centrifugation at 1000 RPM for five min at room temperature. The mixture was separated into two layers (aqueous at top and organic at the bottom). The sample was

evaporated (Rotavapour, Japan) after careful removal of bottom layer through pasture pipette without disturbing the upper phase.

Fatty acids methyl ester (FAME) preparation

Fatty acids methyl ester was prepared for GC-MS analysis according to the standard procedure [14]. Briefly 20 ml sample in a test was added with 1.5 ml of 0.5% solution of sodium hydroxide (NaOH) and boiled for 5 min. It was followed by addition of Borontrifluoride-methanol (BF₃-CH₃OH) 2.5 ml, saturated solution of Sodium Chloride (NaCl) 5 ml and 1ml Hexane (twice), each for specific time in the sample test tube. At the last upper layer was removed and FAME tubes were closed tightly and kept in refrigerator.

GC-MS conditions

The composition of Fatty acid methyl ester was determined by using Shimadzu, gas chromatograph mass spectrometer (model GC-MS QP 2010. plus Japan) having electron impact detector. Helium gas was used as carrier having a linear velocity of 65.8 cm/sec. The column temperature was held at 50°C for 1 min and was increased at a rate of 5°C to 150°C. Then it was kept constant at 175°C for 5 min and finally increased to 220°C at the rate of 10°C /min and was held for 5 min. The injector and ion source temperature was maintained at 240°C and 250°C respectively. The inter face temperature was kept at 240°C on scan mode with M/z from 85 to 380. Standard mixtures of identified fatty acids were used for the determination of peaks and compared the retention time of unknown sample with standard for identification of fatty acids.

Determination of fat% in milk

Determination of fat% was carried out by standard protocol of Gerber method of fat determination in milk. Briefly, 11ml milk sample, 10ml sulfuric acid (85%) and 1ml amyl alcohol were mixed in butyrometer, and closed with rubber cork. The mixture was

centrifuged in Gerber centrifuge machine at 1100 rpm for 5 min. The butyrometer scale determined the total fat%.

Statistical analysis

Data were statistically analyzed by applying Complete Randomized Design (CRD) and means were compared by using LSD test at 5% level of significance [15].

Results and discussion

Total of 11 fatty acids of different saturation levels were detected in milk samples (Table 1). Out of 11, six were saturated (SFA) and five were unsaturated fatty acids (USFA). The major SFA were Myristic acid (C14:0) and Palmitic acid (C16:0) and the major MUFA were Heptadecenoic (C17:1) and Eicosanoic acid (C20:1) and the major PUFA was Linoleic acid (C18:2c). The predominant fatty acids were Palmitic acid (C16:0), Myristic acid (C14:0) and Stearic acid (C18:0), where they formed 48.86%, 23.5% and 15.26% of total fatty acids in milk, respectively. These findings are in close association with [16], who found that Palmitic acid (C16:0), Myristic acid (C14:0) and Stearic acid (C18:0) were major fatty acids in concentration 31.5%, 12.5%, and 12.5% respectively. The findings of [6], showed that camel milk has 26.6% Palmitic acid (C16:0), 10.1% Myristic acid (C14:0) and 12.2% Stearic acid (C18:0) respectively. The Medium-chain fatty acids (MCFA) represented by a sole member Lauric acid (C12:0) was 1.51% in milk. These results are against the findings of [17], who reported that Lauric acid (C12:0) accounts in the range of 0.35 to 0.41%. This could be due to difference in feed intake by the camel and to difference in breeds, geographic locality, lactation stage and the environment [18]. The omega 6 (N-6) fatty acids Linoleic acid (C18:2c) and g-linolenic acid (C18:3n6) formed 1.39% and 0.29% of total fatty acids in milk respectively. These findings are also similar to the results of [17].

Table 1. Concentration (%) of different fatty acids in camel's milk

| Fatty acids | Concentration (%) |
|----------------------------|-------------------|
| Lauric acid (C12:0) | 1.51 |
| Myristic acid (C14:0) | 23.25 |
| Pentadecanoic acid (C15:0) | 3.28 |
| Palmitic acid (C16:0) | 48.86 |
| Palmitoleic acid C16:1c | 0.17 |
| Margaric acid C17:0 | 1.59 |
| Heptadecanoic acid (C17:1) | 3.93 |
| Stearic acid (C18:0) | 15.26 |
| Linoleic acid (C18:2c) | 1.39 |
| g-Linolenic acid (C18:3n6) | 0.29 |
| Eicosenoic acid (C20:1) | 0.41 |

The proportions for saturated (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUSFA) were 93.75%, 4.53%, and 1.68%, respectively (Table 2). Compared to the present results different contents of (66.1%) saturated fatty acids and (30.5%) unsaturated fatty acids were reported in camel milk [19]. This could be due to difference in feed intake by the camel and to difference in breeds, geographic locality, lactating stage and the environment [18]. Fatty acids and cholesterol contents in camel milk of Bactrian, dromedary and hybrids from different regions of Kazakhstan, within different seasons were determined. Fatty acids composition greatly varied in different regions [18]. The short chain fatty acids C6:0 and C8:0 were not detected in the samples of the current study. These findings are similar to the results of another study [20], who reported that even by decreasing the column temperature to 140°C,

the short chain fatty acids such as 6:0 and 8:0 were not detected in camel milk sample. It was concluded [16] that short chain fatty acids of camel milk are less than 1.2%. Other scientists [21] studied that factors such as animal genetics, feed, seasonal e and regional effects can effect fatty acid composition of an animal. Even-numbered long-chain saturated fatty acids (Myristic acid C14:0, Palmitic acid C16:0 and Stearic acid C18:0) in camel milk accounts for 87.37% of total fatty acids, with Palmitic acid C16: 0, Stearic acid C18: 0 and Myristic acid C14: 0 the most important components, respectively. These results are closely related with the findings of [6, 22]. Palmitic acid (C16:0) was the major fatty acid and it accounted for 48.86% of the saturated fatty acids found in camel milk. These findings are in close association with [23], who reported that Palmitic acid (C16:0) accounts for 49.7% of the saturated fatty acids in adult camel milk.

Table 2. Concentration of fatty acids groups (%) in camel milk

| Fatty acids groups | Concentration % |
|-------------------------------------|-----------------|
| Total fatty acids (TFA) | 99.96% |
| Saturated fatty acids (SFA) | 93.75% |
| Mono unsaturated fatty acids (MUFA) | 4.53% |
| Poly unsaturated fatty acids (PUFA) | 1.68% |

In the present study, the fatty acid concentration showed variation with change in temperature. The mean values for Lauric

acid, Palmitic acid, Margaric acid, Heptadecanoic acid, g-Linolenic acid, and Eicosanoic acid at temperatures 16°C, 7°C,

and 4°C showed non-significant ($p < 0.05$) variation (Table 3, Figure 1, 2, 3). These results are in close association with the findings of earlier researchers [23, 24]. Results showed significant ($p < 0.05$) variations in concentration of Myristic acid,

Pentadecanoic acid, Palmitoleic acid, and Linoleic acid with changing temperature level (Table 3). These results are similar to the findings reported by earlier scientists [23, 24].

Table 3. Fatty acid composition of camel milk (%) at various temperatures (16°C, 7°C and 4°C)

| Fatty acids | Mean value at 16°C | Mean value at 7°C | Mean value at 4°C | P value |
|----------------------------|--------------------|-------------------|-------------------|---------|
| Lauric acid (C12:0) | 1.51 | 1.59 | 1.51 | 0.0525 |
| Myristic acid (C14:0) | 23.25 | 24.07 | 24.66 | 0.0149 |
| Pentadecanoic acid (C15:0) | 3.28 | 3.37 | 3.58 | 0.0293 |
| Palmitic acid (C16:0) | 48.86 | 47.76 | 46.70 | NS |
| Palmitoleic acid C16:1c | 0.17 | 0.12 | 0.11 | 0.0424 |
| Margaric acid C17:0 | 1.59 | 1.64 | 1.74 | NS |
| Heptadecanoic acid (C17:1) | 3.93 | 4.32 | 4.09 | NS |
| Stearic acid (C18:0) | 15.26 | 14.83 | 15.26 | 0.0131 |
| Linoleic acid (C18:2c) | 1.39 | 1.60 | 1.59 | 0.0467 |
| g-Linolenic acid (C18:3n6) | 0.29 | 0.28 | 0.29 | NS |
| Eicosenoic acid (C20:1) | 0.41 | 0.43 | 0.48 | NS |

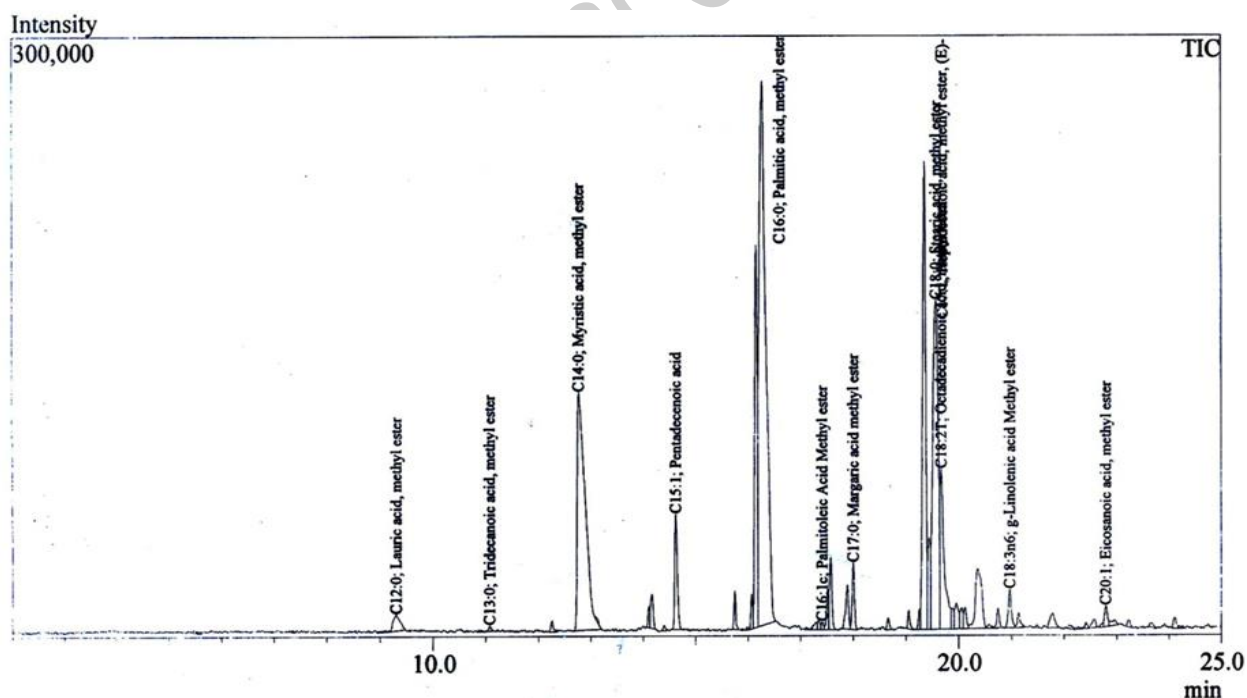


Figure 1. Gas chromatogram of camel milk fat at 16°C.

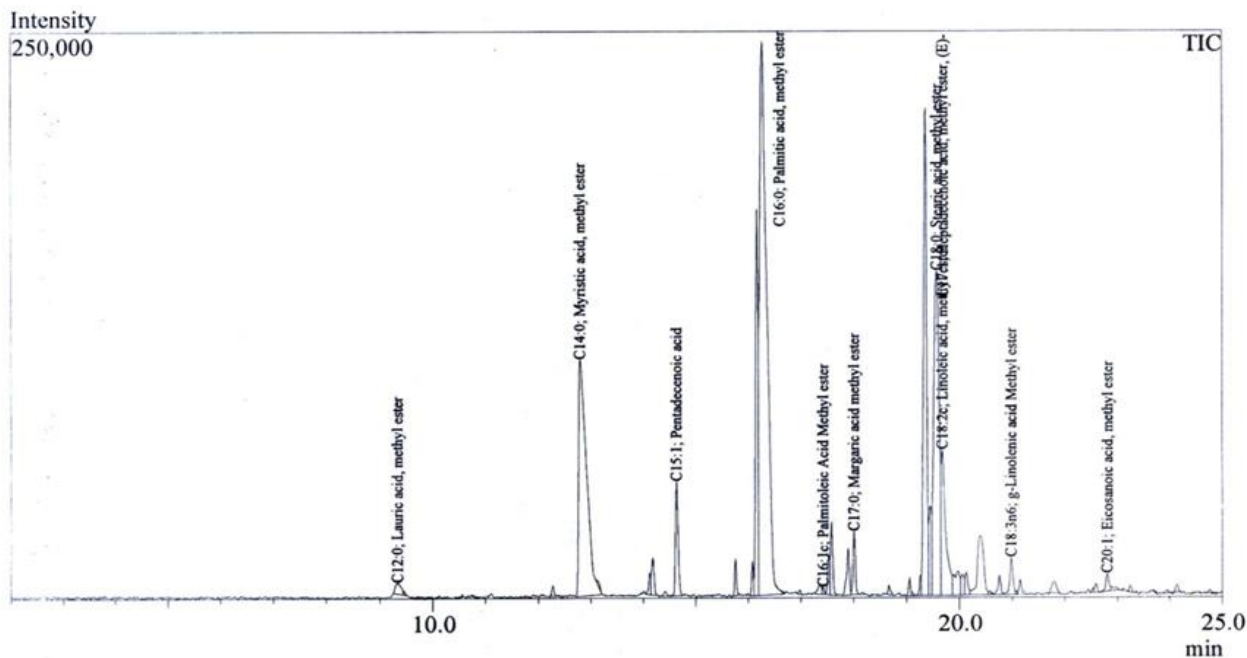


Figure 2. Gas chromatogram of camel milk fat at 7 °C

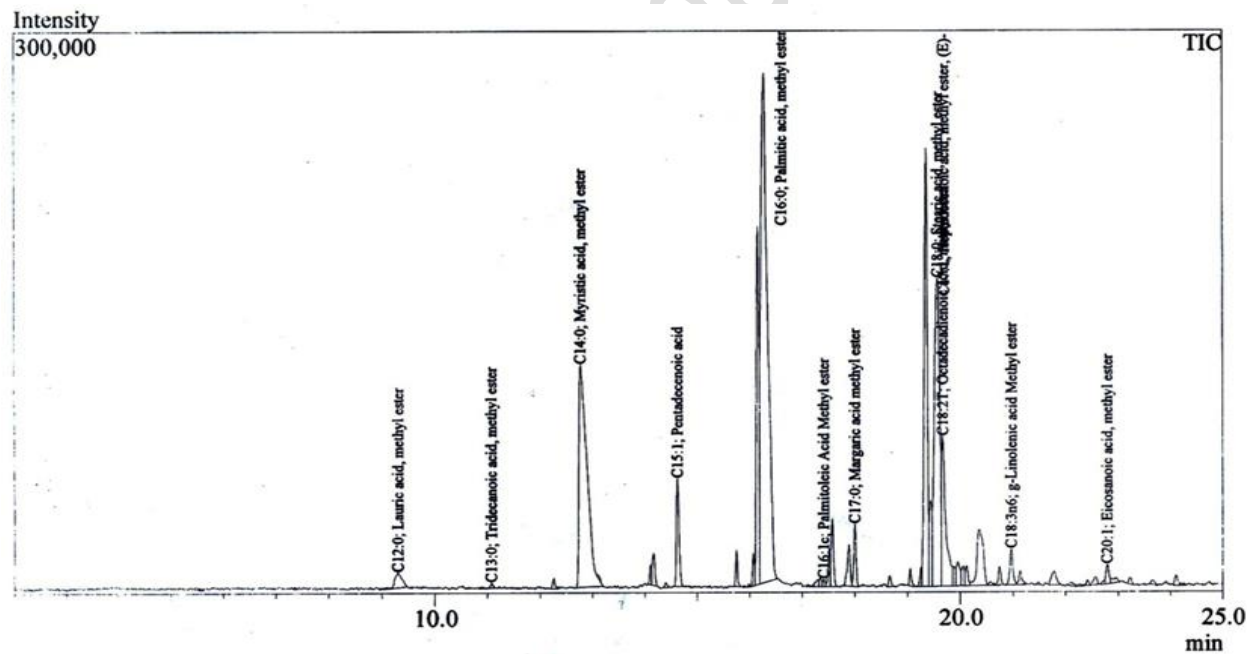


Figure 3. Gas chromatogram of camel milk fat at 4 °C

Conclusion

In conclusion, camel milk is having a higher proportion of saturated fatty acids than unsaturated fatty acids. The current study further elucidated differential expression of

diverse fats with changing temperature. The statistical analysis showed that Myristic acid (C14:0), Pentadecanoic acid (C15:0), Palmitoleic acid C16:1c, Stearic acid (C18:0), Linoleic acid (C18:2c)

concentration change significantly ($p < 0.05$) in response to change in temperature.

Authors' contributions

Conceived and designed the experiments: ZU Aamir, H Khan & S Wahab, Performed the experiments: ZU Aamir, Analyzed the data: S Wahab, MT Tunio, O Aimen & SM Khan, Contributed materials/ analysis/ tools: S Wahab, SM Khan & K Shah, Wrote the paper: H Amanullah & H Khan.

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