Effect of animal origin and farm altitude on some physicochemical properties and minerals of milk samples

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Abstract

The physicochemical properties of milk depend on many factors, including the animal origin, animal health status, size, age, and nutrition, genetics, and environmental factors. This study investigated the effect of animal origin and altitude on some physicochemical properties of milk. The studied parameters were the pH, conductivity, moisture and ash percentages, total dissolved solids (TDS), specific gravity, sodium, potassium, and calcium. The animals from where the milk samples were collected were camels (8), goats (5) and sheep (6) and from two altitudes; 14 and 2110 meters above sea level. Standard methods were used to determine the physicochemical properties of milk samples while the flame photometer technique was used for the determination of the studied mineral concentrations. At the low altitude the conductivity was significantly different between the milks of the three ruminants and the moisture, TDS, specific gravity and calcium were significantly different between the camel and sheep milks. With regard to the animals living at the high altitude, the moisture, TDS, specific gravity and the ash were significantly different between the camel and sheep milks and between the sheep and goat milks while the minerals were insignificantly different between the three milk samples. Concerning the effect of altitude on each milk, it significantly affected the pH, conductivity, ash percentage, and potassium in the camel milk samples, while it significantly affected the conductivity, specific gravity, ash percentage and the calcium in goat milks whereas it significantly affected the ash percentage in the sheep milk samples. The animal origin and the altitude have significant effects on the majority of the studied parameters.

Introduction

The physicochemical properties of milk include the pH, conductivity, specific gravity, ash and water and total solids (TDS) percentages, viscosity and the optical characteristics such as the refractive index and the optical activity. With regard to the chemical composition of milk, it is majorly composed of proteins, minerals, and vitamins (FAO, 2020; Zhao et al., 2020).

Different factors are well known to affect the physicochemical properties of milk such as the origin of the milk, breeding and genetic, health, age and size of the lactating animal, environment, nutrition of the lactating animal and stage of lactation (Rahli et al., 2013; Ahmad et al., 2016).

The nutritional value of human and animal milk includes its concentration of proteins, lipids, carbohydrates, vitamins, minerals, and energy. The milk nutritional value is affected by altitude (Quinn et al., 2016), breed and genetics, environment, animal health and physiology, and nutrition (National Research Council (US) Committee on Technological Options to Improve the Nutritional Attributes of Animal Products, 1988).

Milk nutritional composition has different impacts on human health such as the unmodified cow milk which is deficient for the nutrition of infant and its proteins causes allergy with prevalence ranging from 2% to 7%. On the other hand goat milk can a good source for infant nutrition. Milk allergy is well known to be because of its proteins and lactose. However, fermented dairy products possess anti-inflammatory activities in humans not suffering from milk allergy (Truck, 2013; Bordoni et al., 2017). Full fat milk has no
threatening effects on the cardiovascular health and it is an important source of fat soluble vitamins such as vitamin D and vitamin K (Lordan et al., 2018).

This study investigated the effect of lactating animal and altitude on some physicochemical properties of milk and the concentration of three major minerals. The studied parameters were the pH, conductivity, moisture and ash percentages, total dissolved solids (TDS), specific gravity, sodium, potassium, and calcium.

**Material And Method**

**Ethical clearance**

This study was academically, legally and ethically approved from the department of chemistry at King Khalid University and the samples were collected from the farmers after obtaining an oral informed consent.

**The study samples**

Nineteen fresh milk samples were collected from three lactating animals; camels (*Camelus dromedaries*) (8), goats (*Capra aegagrus hircus* - Nagdi breed) (5) and sheep (*Ovis aries* - Rudi breed) (6). The samples were collected from farms located at 14 meters above sea level (4 camel milk samples, 2 goat milk samples, and 3 sheep milk samples) and at 2110 meters above sea level (4 camel milk samples, 3 goat milk samples, and 3 sheep milk samples). The farms were in Abha (Asir region) and Aldarb (Jazan region) at the south western part of Saudi Arabia [Fig.1]. The altitudes were determined using the Google earth software (Jarvis et al., 2008).

The samples were collected from during October – December 2019, the animals were fed on similar diet and their milk cycle was the same.

The analysis of all the studied parameters was done twice for the purpose of excluding the hand and machine errors.

**Measurement of the pH**

The determination of the pH of the milk samples was carried out using a calibrated pH meter (HI 8314 HANNA, Italy). 30 mls of each milk sample were used for the determination of its pH value and the pH meter was calibrated by two buffers with pH 9 and pH 7.

**Measurement of conductivity**

Twenty milliliters of each milk sample were used to measure its conductivity value using a calibrated conductometer (Metrohm, 712 conductometer, Switzerland).

**Determination of the moisture percentage**
Five grams of each milk sample was weighed (A), heated at 70°C for one hour and at 105°C for six hours (Bradley, 2017). After that, the milk was weighed (B) and the moisture percentage was determined following the bellow equation:

\[
Moisture\% = \left( \frac{A - B}{A} \right) \times 100
\]

**Determination of total dissolved solids (TDS) Percentage**

To measure the TDS of the milk samples, 5 grams of each milk sample (A) was heated for one hour at 70°C and for six hours at 120°C (Bradley, 2017). The water evaporated milk was weighed (B) and the TDS was calculated according to the equation:

\[
TDS\% = \left( \frac{B}{A} \right) \times 100
\]

**Determination of the ash percentage**

To determine the ash percentage, the starting milk sample was the sample of the moisture determination. The moisture determined sample was heated to 600° C in a furnace oven and the ash percentage was calculated by dividing the weight of the ash (C) by the weight of the milk sample (A) as follows (Marshall, 2017):

\[
Ash\% = \left( \frac{C}{A} \right) \times 100
\]

**Calculation of the specific gravity**

The ratio of the milk density to the water density is known as the specific gravity of the milk. The specific gravity of the milk samples was determined by weighing 50 ml of each milk sample (A). The density of the milk was calculated by dividing the weight of the milk sample by the volume (50). The density of the milk was divided by the density of the water (1) to obtain the specific gravity of the milk samples (Williams et al., 2012).

**Measurement of calcium, potassium and sodium**

Two steps were followed to measure the concentration of the calcium, potassium and sodium according to the method of Singh et al. (2015). The minerals analysis was divided to two steps; digestion and measurement.

The digestion step was carried out using the microwave (Anton Paar Multiwave ECO). 0.5 ml of each milk sample was mixed with 4 ml of concentrated nitric acid and 2 ml of hydrogen peroxide. The mixture was introduced into the microwave and the temperature was set at 125°C and the power was 800 Watt for one
hour as follows; increase of the power to 800 Watt for five minutes, application of constant pressure for 40 minutes at 800 Watt and cooling for 15 minutes. Finally, the digested samples were diluted with 1% nitric acid to 25 milliliters using volumetric flasks.

The three minerals were measured in the digested and diluted milk samples using the JENWAY flame photometer (PFP7 Flame Photometer).

For the creation of the standard curves the following standards were prepared; the standards of the Calcium were 12.5, 25, 50, 100 and 200 part per million (ppm), Potassium standards were 2, 4, 8, 16, 32 ppm, while the standards of Sodium were 1.25, 2.5, 5, 10 ppm. The emission wavelengths of the calcium, potassium and sodium were 622nm (orange), 766nm (violet), and 589nm (yellow), respectively.

Quality control samples were prepared using any sample (C ppm). A spike concentration (S ppm) was added to the sample as follows: 50 ppm of Calcium, 16 ppm of Potassium and 5 ppm of Sodium. The concentration of the prepared quality control samples was determined (Q ppm). The recovery percentage was calculated following the bellow equation:

\[
    \text{Recovery}\% = \left( \frac{Q - C}{S} \right) \times 100
\]

The standards, quality control samples and the milk samples were introduced to the flame photometer and the emitted wavelength was measured. The concentration of the quality control samples and the samples was determined from the created standard curves. The results of the samples was approved if the \( R^2 \) of the standard curves was more than 0.98 and if the recovery percentage of the quality control samples was more than 75%.

Statistical analysis

The ANOVA test of the SPSS statistical program was used for the analysis of the results. The difference between the means of the parameters was considered significant if the \( p \)-value was \( \leq 0.05 \).

Results

pH

The pH values of the camel, goat and sheep milk samples increased in the high altitude, but the increase was insignificant and the effect of animal origin was also, insignificant (Table.1 and Table.2).

Conductivity

The conductivity mean values were decreased in the high altitude milk samples. However, the significant decrease was reported in the camel milk samples only. Concerning the effect of animal origin at the two altitudes, it insignificantly affected the conductivity at low altitude while it significantly affected the conductivity at high altitude (Table.1 and Table.2).
Moisture %

The moisture percentage was insignificantly decreased in the high altitude camel and sheep milk samples while it insignificantly increased in the high altitude goat milk samples. When comparing the camel and sheep milk samples, their moisture percentages were significantly different at high and low altitudes while the moisture percentage of the goat and sheep milk samples were significantly different at high altitude only (Table.1 and Table.2).

TDS %

The TDS% insignificantly increased in the high altitude camel and sheep milk samples while it insignificantly decreased in the goat milk samples from high altitude. There significant variation between the camel and sheep milk samples in the low and high altitude while the significant variation between the goat and sheep milk in the low altitude only (Table.1 and Table.2).

Ash percentage

The high altitude significantly increased the ash percentage in the camel, goat and sheep milk samples. There were significant variations between the mean ash percentage of the milk samples from the camels and sheep and between the goats and sheep living at the high altitude (Table.1 and Table.2).

Specific gravity

The high altitude significantly increased the specific gravity in the camel, goat and sheep milk samples. There were significant variations between the specific gravity of the milk samples from camels and sheep and between goats and sheep living at the low and high altitudes (Table.2 and Table.3).

Minerals

*Standard curves.* The R2 values of the standard curves of the calcium, potassium and sodium were 0.9923, 0.9995, and 0.9867, respectively [Fig. 2, Fig. 3 and Fig. 4].

*Recovery percentages of the quality control samples.* The recovery percentages of the quality control samples for the calcium, potassium and sodium were 76.92%, 89.9% and 86.4%, respectively.

*Calcium.* The calcium concentration in the camel and sheep milk samples was insignificantly increased in the high altitude compared to its concentration in the milk samples from the low altitude. The altitude significantly increased the concentration of calcium in the goat milk samples. The concentration of the calcium in the camel milk and goat milk samples from the low altitude were significantly different (Table.1 and Table.2).

*Potassium.* The concentration of the potassium increased in the high altitude milk samples from goats and camels with significant increase in the camel milk samples. There was insignificant variations between the potassium concentrations in the milk samples from camels and goats in the high and low
altitudes while there was significant variation between the milk samples of camels and sheep living at the high altitude. The potassium concentration in the milk samples was very high compared to the calcium and sodium. (Table.1 and Table.2).

**Sodium.** The altitude insignificantly affected the concentration of the sodium in the different milk samples. However, the concentration of the Sodium decreased in the high altitude in the camel and sheep milk samples while it increased in the goat milk samples. Significant variation between the sodium concentration was reported between the milk samples from camels and goats living at high altitude (Table.1 and Table.2).

**Discussion**

The high altitude was characterized by increased pH, ash percentage, specific gravity, calcium, and potassium while it was characterized by decreased conductivity in all the milk types. However, the pH and the potassium concentration difference was insignificant while the ash percentage and the specific gravity difference was significant. The variation of the high and low altitude calcium and potassium was significant in the goat and camel milk samples, respectively. The animal origin had significant (conductivity, moisture%, TDS%, ash%, specific gravity, and calcium) and insignificant effects (pH, potassium and sodium).

Concerning the Effect of animal origin on the physicochemical properties of milk samples; Legesse et al. (2017) studied camel, goat and cow milk samples from Ethiopia and found that the pH of the goat and camel milk samples were 6.38 ± 0.08 and 6.13 ± 0.11, respectively with insignificant difference while the specific gravity values were 1.04 ± 0.00 and 103 ± 0.00, respectively and also with insignificant variation. The TDS% of the goat and camel milk samples were 14.25 ± 1.16 and 13.65 ± 1.40, respectively. The ash percentage of the Ethiopian goat and camel milk samples were 0.73 ± 0.07 and 0.73 ± 0.03, respectively. The conclusion of Legesse et al. (2017) study is that there is no variation between camel goat milk except the TDS% which is high in the goat milk compared to the camel milk. However, this study reported insignificant variation between the TDS% of the camel and goat milk samples whereas there was significant variation between the TDS% of the camel and sheep milk samples. As general the study of; Legesse et al. (2017) is comparable to the samples from the high altitude because the altitude of the study area of their study is 1803 meters above sea level. A Pakistani research (Zhao et al., 2020), studied buffalo, cow and goat milk samples with regard to their pH, conductivity, moisture%, TDS%, specific gravity, calcium, potassium, and sodium. The findings of the Pakistani study were to some extent similar to the findings of this study with slight differences except for the conductivity (10.8 ± 2.07 Pakistani compared 6.93 ± 0.60 in low altitude of our study and 5.56 ± 0.15 at the high altitude), TDS% (12.9 ± 1.01 Pakistani study compared to 18.43 ± 0.25 at low altitude and 14.07 ± 6.43 at high altitude) and ash% (1.04 ± 0.13 Pakistani compared to 0.58 ± 0.15 at low altitude and 3.51 ± 0.65 at high altitude of our study). The differences between the Pakistani study and this study may be due to the different geographical and environmental conditions. As mentioned by the authors of the Pakistani study, their mineral results are less than the WHO standards and comparable to some previous studies. Compared to the results of our study,
the results of the minerals of the Pakistani study were comparable with regard to the calcium concentration only (644 ± 76.6 in the Pakistani study compared to 521.75 ± 0.35 and 568.5 ± 0.50 in the low and high altitude milk samples, respectively). Sabahelkhier et al. (2012), compared the pH, moisture%, TDS%, ash% and specific gravity of milk samples from camel, goat, sheep and cow. The results of our study is different that the results of Sabahelkhier et al. (2012) with respect to the values of the sheep milk TDS (19.3% in Sabahelkhier study compared to 25.80 ± 0.00 or 26.50 ± 7.85 in our study), and moisture% (80.7% in Sabahelkhier study compared to 74.20 ± 0.00 or 73.57 ± 7.90 in our study), while the ash% of the high altitude milk samples (2.64%, 3.51%, and 4.67%) are not comparable to the ash% of Sabahelkhier study (≤ 0.9%). The differences between the study of Sabahelkhier et al. (2012) and our study may be due to the different breeds, environmental conditions and altitude. Depending on the review article of Abbas et al. (2014), about the physicochemical properties of goats milk, the calcium concentration of this study is very low compared to the previous studies which may be referred to the recovery percentage in our assay (76.92%) and to the differences in the breeds and altitudes. However, the ranges of the calcium, potassium and sodium in goats milk were (850–1980 mg/l), (1400–2420 mg/l) and (380–580 mg/l), respectively (Abbas et al., 2014). The results of the potassium and sodium of this study are compatible to the results of the previous studies. In a review article about the nutritional value of milk from different origin, Barłowska et al. (2011) reported that the source of the milk determines its nutritional value and its industrial uses. According to Barłowska et al. (2011) the concentration of calcium in the camel, goat and sheep are (1140–1160 mg/l), (1320–1340 mg/l) and (1950–2000 mg/l), respectively. The calcium results of this study are less than the ranges of the previous studies as mentioned by Barłowska et al. (2011). The potassium and sodium results of this study are within the ranges mentioned by Barłowska et al. (2011). The potassium ranges in the milk of camels, goats and sheep in the review article of Barłowska et al. (2011) were (1440–1650 mg/l), (1510–1820 mg/l), and (1360–1400 mg/dl), respectively. The sodium ranges in camel, goat and sheep milk samples of the mentioned review article were (≥ 590 mg/l), (410–594 mg/l), and (440–580 mg/l), respectively. The results of the potassium and sodium of this study are comparable to the results mentioned in the review article of Barłowska et al. (2011). Another review article reported that the calcium, potassium and sodium concentration ranges in goats milk are (1060–1920 mg/l), (1350–2350 mg/l), and (340–500 mg/l), respectively while their concentration ranges in the sheep milk are (1360–2000 mg/l), (1740–1900 mg/l), and (290–310 mg/l), respectively (Zamberlin et al., 2012). The results of the minerals concentration in the milk samples proves the effect of the milk animal source. Similar to the finding of this study about the concentration of the milk potassium, all the reviewed previous studies reported high range of potassium concentration compared to the calcium and potassium.

Any region with elevation more than 1500 meter above sea level is considered as high altitude area. High altitude is characterized by low atmospheric pressure, hypoxia, low temperature, high amount of rain falls and ultraviolet radiation compared to low or sea level altitudes (West, 2012; Crocker et al., 2020). The climate conditions of high altitude areas affects the health, physical and physiological activities of the living animals which affects the amount and quality of their products (Qiu et al., 2012; Bharti et al., 2017; Holt, 2020). This study reported significant effects of high altitude because of its climate conditions on the physicochemical properties and mineral content of different sources milk samples. Previously, different studies reported significant effects of the geographical origin and the seasonal variations on the pH,
Conclusions

The following are the conclusions of this study: 1) The altitude had significant effects on the conductivity, ash%, specific gravity, calcium and potassium; 2) The animal source significantly affected the conductivity, ash%, specific gravity, calcium, potassium, moisture percentage, TDS%, and sodium.

Declarations

Ethics approval and consent to participate

Not Applicable

Consent for publication

The authors agree that the BMC chemistry Journal has the right to publish this article

Availability of data and material

The data of this research is available for the journal

Competing interests

The author declare no conflict of interest

Funding

Not Applicable

Authors’ contributions

AA, QN and AM did the practical work and approved the final copy of the manuscript. MM and BE designed the research, statistically analyzed the results and revised and approved the final copy of the manuscript.

Authors’ information (optional)

Acknowledgement

Not Applicable

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### Tables

Table 1: The results of the studied parameters (Mean ± SD) in the milk samples from different origins and altitudes

| Parameter          | Sheep milk | Goat milk | Camel milk |
|--------------------|------------|-----------|------------|
|                    | High altitude | Low altitude | High altitude | Low altitude | High altitude | Low altitude |
| **pH**             | 6.54± 0.11 | 6.24± 0.37 | 6.54± 0.07 | 6.23± 0.26 | 6.64± 0.07 | 6.39± 0.16 |
| **Conductivity (mS/cm)** | 4.54± 0.51 | 5.29± 1.11 | 5.56± 0.15 | 6.93± 0.60 | 5.98± 0.85 | 9.55± 1.46 |
| **Moisture %**     | 73.57± 7.90 | 74.20± 0.00 | 84.3± 4.42 | 81.5± 0.14 | 88.17± 2.75 | 88.75± 1.06 |
| **TDS %**          | 26.50± 7.85 | 25.80± 0.00 | 14.07± 6.43 | 18.43± 0.25 | 12.24± 3.70 | 11.21± 1.09 |
| **Ash %**          | 4.67± 0.96 | 0.51± 0.00 | 3.51± 0.65 | 0.58± 0.15 | 2.64± 0.12 | 0.32± 0.07 |
| **Specific gravity** | 1.04± 0.000 | 1.03± 0.000 | 1.027± 0.001 | 1.013± 0.004 | 1.025± 0.002 | 1.015± 0.007 |
| **Calcium (Ca) ppm** | 574.5± 10.39 | 543.17± 37.53 | 568.5± 0.50 | 521.75± 0.35 | 568.5± 0.41 | 564.87± 30.64 |
| **Potassium (K) ppm** | 1129.8± 237.9 | 1404.7± 309.3 | 1610.8± 472.3 | 1147± 509.8 | 2149.5± 172.9 | 1532.3± 413.5 |
| **Sodium (Na) ppm** | 348.17± 98.3 | 408± 125.5 | 253.8± 133.1 | 246.5± 140.0 | 500.0± 66.4 | 519.8± 102.8 |
The pH, ash percentage, specific gravity, calcium, and potassium was increased in the high altitude while the conductivity decreased in all the milk types. The moisture percentage and sodium decreased in the high altitude in the camel and sheep milk samples whereas it increased in the goat milk. The TDS % increased by the altitude increase in the camel and sheep milk while it decreased in the goat milk samples. The potassium concentration increased in the high altitude milk samples from the camels and goats while it decreased in the sheep milk. The results showed that the milk samples are rich in potassium rather than the calcium.

Table 2: The variations between the mean values of the studied parameters in the different study groups using the LSD post hoc test of ANOVA test

| Parameter   | p-value |
|-------------|---------|
|             | Goat−  | Camel− | Sheep+ | Goat+ | Camel+ |
| pH          | 0.95   | 0.33   | 0.33   | 0.09  | 0.97   | 0.10   | 0.61   | 0.59   | 0.15   |
| Conductivity mS/cm | 0.024  | 0.001  | 0.001  | 0.31  | 0.224  | 0.087  | 0.09   | 0.61   | 0.001  |
| Moisture %  | 0.14   | 0.01   | 0.14   | 0.88  | 0.02   | 0.51   | 0.003  | 0.32   | 0.89   |
| TDS %       | 0.18   | 0.02   | 0.19   | 0.88  | 0.02   | 0.37   | 0.008  | 0.67   | 0.83   |
| Ash %       | 0.91   | 0.74   | 0.66   | 0.001 | 0.001  | 0.001  | 0.002  | 0.087  | 0.001  |
| Specific gravity | 0.001 | 0.001  | 0.41   | 0.004 | 0.001  | 0.001  | 0.001  | 0.42   | 0.005  |
| Calcium (Ca) ppm | 0.29  | 0.20   | 0.035  | 0.09  | 0.74   | 0.03   | 0.72   | 1      | 0.81   |
| Potassium (K) ppm | 0.44  | 0.64   | 0.23   | 0.36  | 0.12   | 0.17   | 0.002  | 0.07   | 0.03   |
| Sodium (Na) ppm | 0.12  | 0.20   | 0.012  | 0.51  | 0.30   | 0.94   | 0.09   | 0.01   | 0.8    |

+ high altitude milk.

− Low altitude milk
The significance level of the compared mean values of the studied parameters was set at the level of 95% (p-value ≤ 0.05).