Research Article

# Minerals Composition, Phenolic Compounds and Antioxidant Activity of Fresh and Processed Milk Consumed in Khyber Pakhtunkhwa, Pakistan

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**Abstract** | The study was determined mineral content, total phenolic compounds and antioxidant activity of fresh (cow, buffalo, goat, sheep and camel) and processed milk types (S1, S2, S3, S4 and S5) commonly consumed in Khyber Pakhtunkhwa (KP), Pakistan. Samples were collected from three different districts of KP and after composite sampling dried in oven and stored at room temperature for further biochemical analysis. Samples were then analyzed for minerals content, total phenolic compounds, total flavonoids and antioxidant activity. Physicochemical characteristics, proximate composition and energy density were also estimated. Statistical analysis showed significant differences ($p < 0.05$). Fat content (6.73±0.06%) and total solid (15.21±0.01%) were found highest in Buffalo milk. Mineral analysis revealed that Sheep milk contain marked level of calcium, magnesium and zinc that is (196.00±3.61, 19.00±1.73 and 0.55±0.03) respectively while, high iron content was found in Camel milk (0.22±0.00). Among processed milk types calcium and magnesium were high in S5 (138.00±2.00) and (18.83±0.21), respectively. Iron was recorded high in S3 (0.05±0.00) and zinc in S1 that is (0.51±0.01). Among fresh milk Buffalo milk showed significant levels of total phenolic compounds (78.31±0.97 mg GAE/L) while, marked antioxidant activity (DPPH) was detected in Sheep milk (27.36±2.02 mg VCE/L). While, S5 exhibited higher total phenolic compounds and antioxidant activity *i.e.* (42.99±0.75 mg GAE/L and 26.89±0.72 mg VCE/L) respectively, among processed milk types. Flavonoids were not detected in both milk types. Correlation coefficients demonstrated higher significance at r=0.856 ($P \leq 0.01$) for total phenols and antioxidant activity. These findings demonstrates that total phenolic compounds were found in all fresh milk types in significant amount and as well as in some processed milk types. It was concluded that overall fresh milk results are better than those of processed milk for all tested parameters. These findings related to fresh and processed milk can be used by nutritionists, dieticians and health care providers in planning normal and therapeutic diet.

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## 1. Introduction

Milk is a clear liquid produced by mammals mammary glands. Milk is a unique food for both children and adults. The physical properties and composition of milk vary depending on the species. Whey proteins and casein proteins, antioxidants, fat, minerals, enzymes, lactose, cells, hormones, vitamins, and immunoglobulins are all found in milk (Habib *et al*., 2013). Milk and milk products are consumed by six billion people worldwide, and the dairy industry employs up to 750 million people. Cow milk accounts for 85 percent of global milk output, followed by buffalo (11 percent), goat (2.3 percent), sheep (1.4 percent), and camel (0.2 percent) (Hallam, 2012). Pakistan, a well-known agricultural country, ranks second and fourth in goat and buffalo milk output, respectively. Many tetra pack firms, such as Nestle (4th largest milk processing group in the world), are also active in milk production (Douphrate *et al*., 2013; Hemme and Otte, 2010).

Milk offers various health benefits, including being a good source of calcium for bones and potassium, which can help lower blood pressure by dilating blood vessels Several recent investigations have confirmed the presence of tannins, trepens, monotrepenes, and sesquierpenes in the milk of grazing goats (De Feo *et al*., 2006). Milk contains exogenous antioxidants (vitamins A, E, and C), endogenous antioxidants (enzymes, glutathione peroxidase, catalase, and superoxide dismutase), and proteins such as lactoferin and coenzyme Q10 (Lindmark-Mansson and Akesson, 2000; Compagnoni *et al*., 2004).

Because of the multiple health benefits of milk, it is speculated that it contains several phenolic compounds and antioxidant characteristics. The public in Khyber Pakhtunkhwa (KP) consumes both fresh and processed milk, but information on its antioxidative and biochemical qualities is limited. We wanted to look into their possibilities in this way. Include information on processed milk as well. The antioxidant activity and connection with total phenolic components of the three milk types (fresh and processed) used in this investigation were not tested analytically (TPCs). As a result, we have determined for the first time, to the best of our knowledge, the antioxidant activity and its relationship with TPCs in milk varieties regularly consumed in KP. This study will add to our knowledge of TPCs, antioxidant activity, and mineral content in various milk kinds.

## 2. Materials and Methods

### *2.1 Sample collection*

Fresh milk samples of various species (cow, buffalo, goat, sheep, and camel) were purchased from various parts of Peshawar, Charsadda, and Mardan districts (Pakistan). District Peshawar provided all of the tetra packs of well-known brands. Identical samples were combined into a single sample. The composite sampling approach was adopted, which involved mixing and drying the same milk samples collected from several locations. The goal of the composite sample was to reduce variation and provide more exact results.

### *2.2 Sample drying and storage*

Fresh and tetra pack milk samples were dried in an oven at 70 degrees Celsius. For subsequent investigation, the dried milk samples were kept in plastic jars at room temperature.

### *2.3 Determination of proximate composition and physicochemical characteristics*

According to the approach used by Pavel and Gavan (2011), the composition and physicochemical parameters of fresh and tetra pack milk were determined using a milk analyzer (Ekomilk-Ultrasonic Milk Analyzers Model EON Trading INC 300, made in Bulgaria). All of the tests were done in duplicate.

### *2.4 Determination of carbohydrates*

Carbohydrates content of each sample of fresh and commercial milk was obtained by subtracting the sum of ash, moisture, fat and protein from 100. The values were expressed in percent.

$$\textit{Total carbohydrates (\%)} = 100 - (\textit{Moisture} + \textit{Ash} + \textit{Fat} + \textit{Protein})$$

### *2.5 Minerals determination*

The AOAC (2000) techniques for the evaluation of selected minerals in milk types, namely calcium, magnesium, iron, and zinc, were used to evaluate mineral content. A calibration curve's standard solution was made by diluting a standard solution containing 1000 mg L-1 (Perkin Elmer) of the particular element. The experiment was conducted using a Perkin Elmer Analyst PinAAcleTM 900T flame atomic absorption spectrometer (FAAS) with an AS 900 graphite furnace auto sampler and a deuterium background corrector.

### *2.6 Determination of phytochemicals*

### *2.6.1 Preparation of sample*

Dried milk samples weighing 1 gm were combined with 10 ml of methanol in Eppendorf centrifuge tubes (covered in aluminum foil). For 2 hours, samples were placed in a water bath shaker (model No. 1217-2E, produced in USA). After shaking, samples were centrifuged for 20 minutes at 3500 rpm using a Hermle Z 200 A centrifuge built in Germany. The samples were then pipette out and transferred to W/PL SC CAP tubes using a 1000 l pipette. After that, the tubes were stored at -200C for later investigation.

### *2.7 Determination of total phenolic content*

The total phenolic (TP) assay was performed using a slightly modified Folin-Ciocalteu (FC) method (Blainski *et al*., 2013). The phenomenon is based on the chemical reduction of tungsten and molybdenum oxides by the transfer of electrons from phenolic compounds to a mixture of phosphomolybdic and phosphotungstic acids in an alkaline media, resulting in blue complexes readable by a spectrophotometer. First, 10 fold FC solutions were made, then 10 ml of FC was extracted and 2 ml of sample was put to a conical flask. After 3 minutes, Na2CO3 was introduced. 5 mL distilled water was used to make a volume of 25 ml. The combination was then placed in the shade for an hour, and the absorbance was measured in a UV spectrophotometer against a wavelength of 765 nm. Gallic acid was prepared as a standard by dissolving 50mg of gallic acid in 100ml of Methanol. The reading was recorded in three different ways. The results were calculated as gallic acid equivalents (GAE) per 50g dry weight. Prior to the samples, the same technique was followed for the blank and standard.

### *2.8 Determination of total flavonoid content*

The total flavonoids content was determined using a slightly modified aluminum chloride colorimetric technique (Ghosh *et al*., 2014). First, make a 100 percent stock solution for Aluminum chloride, then make a 1M/L potassium acetate solution as directed in the reagent preparation section. Then, in a round bottom flask, 1 ml of already prepared samples was combined with 3 ml of methanol, 0.2 ml AgC13 from the stock solution, 0.2 ml potassium acetate from the 1M/L solution, and 5.6 ml distilled water. For 30 minutes, the round bottom flask was kept in the dark. The UV Spectrophotometer was used to examine the absorbance at 510 nm. The benchmark was quercetin, and the results were given in milligrams of quercetin equivalents (QE) per 100 grams of dry weight. The results were triple-checked.

### *2.9 Determination of antioxidant activity*

DPPH is a dark violet-colored organic nitrogen molecule that is extremely stable. An antioxidant ingredient transforms the dark violet hydrazine into a pale-yellow hydrazine in this technique. Electron spin resonance or monitoring the absorbance at 515-528 nm can be used to measure how much DPPH is reduced by antioxidants. The antioxidant activity was determined using the approach of (Ahmed *et al*., 2010). For the standard curve, DPPH stock solution and various concentration gradients were generated. After 30 minutes in the dark, the absorbance of the ready sample was measured at 517nm using a Thermo spectrophotometer (Geneys 10UV) made in the United States; if the absorbance was less than 1, it was declared normal. The experiment was carried out three times. The benchmark was ascorbic acid (vitamin C), and the results were presented as vitamin C equivalents (VCE).

The proportion of scavenging activity was calculated using the formula below.

$$\% \, DPPH = [A_{control} - A_{sample} / A_{control}] \times 100$$

### *2.10 Statistical analysis*

All the analysis was carried out in triplicate for different parameters. Statistic 8.1 version was used for analysis of all milk samples. Each parameter for composition of milk was compared among various milk samples by using one way analysis of variance (ANOVA). The results were represented by mean and standard deviation. The significant p-value of <0.05 was used to indicate statistical significance.

## 3. Results and Discussion

### *3.1 Proximate composition of fresh and processed milk types*

Table 1 shows the approximate compositions of several milk varieties typically eaten in Khyber Pakhtunkhwa. The maximum moisture level was observed in S4 (89.570.36%), while the lowest was recorded in Buffalo milk (84.90.12%). The results for ash were not statistically different. Goat milk had the highest ash concentration (0.80.01%), while Camel milk, S4 and S5 had the lowest (0.70.00%). Buffalo milk had the highest fat level (6.70.06%), whereas S3 milk had the lowest (2.60.10%). Sheep milk had the highest protein content (3.80.00%), whereas S3 had the lowest (2.10.06%). Carbohydrate content varies from 7.80.11% in S3 to (3.90.05%) in S4.

### *3.2 Physicochemical characteristics of fresh and processed milk types*

Table 2 lists the physicochemical parameters of both fresh and processed milk types (pH, total solids, viscosity, Titratable acidity, specific gravity, and conductivity). Camel milk had the greatest pH (6.80.10) while Cow milk had the lowest pH (4.80.06). S4 milk had a total solids content of 10.640.00, while Buffalo milk had a total solids content of 15.210.01. S3 (1.550.01) had the highest viscosity, whereas S4 (1.020.00) had the lowest. Cow milk has a Titratable acidity of 1.810.01, while goat milk has a Titratable acidity of 0.560.01. Buffalo Milk and S3 (1.040.00) had the highest specific gravity, whereas Goat Milk (1.010.00) had the lowest. Goat milk had the highest conductivity (10.770.06) and Buffalo milk had the lowest (6.550.00).

**Table 1: Proximate composition (%) of fresh and processed milk types commonly consumed in Khyber Pakhtunkhwa.**

| Samples | Moisture | Ash | Fat | Protein | CHO |
|---|---|---|---|---|---|
| **Fresh milk** | | | | | |
| Goat milk | 88.30±0.01 | 0.81±0.01** | 3.33±0.06 | 2.70±0.00 | 4.85±0.06 |
| Sheep milk | 86.05±0.31* | 0.72±0.01 | 5.67±0.06 | 3.80±0.01** | 4.14±0.23 |
| Cow milk | 86.75±0.65 | 0.77±0.01 | 3.00±0.00 | 3.17±0.11 | 5.92±0.11 |
| Camel milk | 87.67±0.28 | 0.71±0.01* | 2.70±0.06* | 2.73±0.06 | 6.35±0.08 |
| Buffalo milk | 84.92±0.12 | 0.72±0.01 | 6.73±0.06** | 3.47±0.58 | 4.29±0.10 |
| **Processed milk** | | | | | |
| S1 | 87.03±0.89 | 0.75±0.05 | 3.00±0.00 | 2.83±0.06 | 5.86±0.09 |
| S2 | 86.22±0.02* | 0.72±0.01 | 3.30±0.12 | 3.00±0.00 | 6.76±0.01 |
| S3 | 87.22±0.68 | 0.80±0.01** | 2.60±0.10* | 2.13±0.06* | 7.79±0.11** |
| S4 | 89.57±0.36** | 0.71±0.01* | 3.20±0.06 | 2.87±0.06 | 3.87±0.05* |
| S5 | 86.49±0.43 | 0.71±0.01* | 3.27±0.15 | 3.13±0.06 | 6.66±0.17 |

S1: Sample 1; ….. and S5: Sample 5. ** Highly significant * Least significant. Values are expressed as Mean ±SD of triplicate analysis. All the means have different letters are significantly different from each other (*P<0.05*).

**Table 2: Physicochemical characteristics of fresh and processed milk types.**

| Samples | pH | Total solids (%) | Viscosity (cp) | Titratable acidity (%lactic acid) | Specific gravity | Conductivity (mS) |
|---|---|---|---|---|---|---|
| **Fresh milk** | | | | | | |
| Goat milk | 5.27±0.06 | 11.65±0.00 | 1.01±0.01* | 0.56±0.01* | 1.01±0.00* | 10.77±0.06** |
| Sheep milk | 5.60±0.00 | 13.26±1.67 | 1.31±0.01 | 0.77±0.01 | 1.03±0.01 | 8.02±0.00 |
| Cow milk | 4.87±0.06* | 12.88±0.01 | 1.37±0.01 | 1.81±0.01** | 1.03±0.01 | 9.17±0.06 |
| Camel milk | 6.80±0.10** | 12.43±0.01 | 1.03±0.01 | 1.21±0.00 | 1.02±0.00 | 8.98±0.00 |
| Buffalo milk | 6.67±0.06 | 15.21±0.01** | 1.51±0.01 | 0.87±0.06 | 1.04±0.00** | 6.55±0.00* |
| **Processed milk** | | | | | | |
| S1 | 6.17±0.06 | 12.45±0.01 | 1.22±0.01 | 1.55±0.01 | 1.03±0.00 | 9.12±0.00 |
| S2 | 6.07±0.06 | 13.54±0.00 | 1.23±0.01 | 1.33±0.01 | 1.03±0.00 | 8.70±0.00 |
| S3 | 6.23±0.06 | 13.32±0.01 | 1.55±0.01** | 1.49±0.01 | 1.04±0.00** | 8.03±0.01 |
| S4 | 5.50±0.10 | 10.64±0.00* | 1.02±0.00 | 1.34±0.01 | 1.02±0.00 | 9.02±0.01 |
| S5 | 6.17±0.06 | 13.75±0.01 | 1.25±0.01 | 1.27±0.01 | 1.03±0.00 | 8.56±0.01 |

S1: Sample 1; …………….. and S5: Sample 5. ** Highly significant * Least significant. Values are expressed as Mean ±SD of triplicate analysis. All the means have different letters are significantly different from each other (*P<0.05*).

*3.3 Mineral composition of fresh and processed milk types*

Macro minerals (Calcium and Magnesium) and micro minerals (Iron and Zinc) composition of fresh and processed milk types commonly consumed in KP are shown in Table 3. Ca and Mg were found high in Sheep milk (196±3.61) and (19.00±1.73), respectively while, least Ca was found for S4 (88.34±5.40) and Mg for Camel milk (7.50±0.70). Fe content was observed highest in Camel milk (0.22±0.00) while, lowest was found in S2 and S4 that is (0.03±0.01). Content of Zn was found high for Sheep and Goat milk with similar content that is (0.55±0.01) while lowest amount of Zn was recorded for S4 that is (0.19±0.01).

**Table 3: Mineral analysis (mg/100ml) of fresh and processed milk types.**

| Samples | Calcium | Magnesium | Iron | Zinc |
|---|---|---|---|---|
| **Fresh milk** | | | | |
| Goat milk | 130.09±4.00 | 14.29±1.54 | 0.06±0.02 | 0.55±0.01** |
| Sheep milk | 196.00±3.61** | 19.00±1.73** | 0.06±0.03 | 0.55±0.03** |
| Cow milk | 120.63±1.18 | 13.14±1.03 | 0.06±0.02 | 0.52±0.04 |
| Camel milk | 110.79±1.58 | 7.50±0.70* | 0.22±0.00* | 0.52±0.08 |
| Buffalo milk | 166.06±3.53 | 17.6±1.13 | 0.14±0.001 | 0.23±0.02 |
| **Processed milk** | | | | |
| S1 | 129.33±3.51 | 16.50±0.50 | 0.04±0.01 | 0.51±0.01 |
| S2 | 106.43±1.27 | 13.84±0.58 | 0.03±0.00* | 0.22±0.02 |
| S3 | 123.22±1.68 | 11.16±0.74 | 0.05±0.00 | 0.32±0.01 |
| S4 | 88.34±5.40* | 10.48±0.73 | 0.03±0.00* | 0.19±0.01* |
| S5 | 138.00±2.00 | 18.83±0.21 | 0.04±0.00 | 0.44±0.01 |

S1: Sample 1; …….. and S5: Sample 5. ** Highly significant * Least significant. Values are expressed as Mean ±SD of triplicate analysis. All the means have different letters are significantly different from each other ($P<0.05$).

*3.4 Total Phenolic compounds and antioxidant activity of fresh and processed milk types*

Table 4 shows the total phenolic content and antioxidant activity of 10 distinct milk kinds, including both fresh and tetra packs. Buffalo milk (78.310.97mgGAE/L) and Sheep milk (27.362.02 mg VCE/L) had the highest total phenolic compounds (TPCs) and antioxidant activity, respectively. S2, on the other hand, had the lowest TPCs and antioxidant activity, with 12.410.60 mg GAE/L and 5.870.43 mg VCE/L, respectively.

*3.5 Correlation between total phenolic (TP) and antioxidant activity (AA)*

Table 5 reveals correlation between total phenols and antioxidant activity of fresh and processed milk types. Positive significant correlation was found ($R^2$= 0.856: $P<0.01$) between total phenols and antioxidant activity.

**Table 4: Total Phenolic compounds and antioxidant activity of fresh and processed milk types.**

| Milk type | Total phenolics (mg GAE/L) | Antioxidant activity (mg VCE/L) |
|---|---|---|
| **Fresh milk** | | |
| Goat milk | 72.57±0.66 | 24.58±0.53 |
| Sheep milk | 63.97±0.29 | 27.35±2.02** |
| Cow milk | 73.12±0.25 | 22.24±0.87 |
| Camel milk | 59.86±0.72 | 20.53±0.26 |
| Buffalo milk | 78.31±0.97** | 20.24±1.01 |
| **Processed milk** | | |
| S1 | 22.99±0.53 | 9.56±1.08 |
| S2 | 12.41±0.60* | 5.87±0.43* |
| S3 | 29.37±0.91 | 9.74±0.14 |
| S4 | 20.95±0.17 | 8.04±0.64 |
| S5 | 42.99±0.75 | 26.89±0.72 |

S1: Sample 1; …….. and S5: Sample 5. ** Highly significant * Least significant. Values are expressed as Mean ±SD of triplicate analysis. All the means have different letters are significantly different from each other ($P<0.05$).

**Table 5: Correlation between total phenolic (TP) and antioxidant activity (AA).**

| | AA |
|---|---|
| TP | 0.856 ($P < 0.01$) ** |

**Correlation coefficients are significant at ($P \leq 0.01$).

*3.6 Proximate composition of fresh and processed milk types*

Milk is considered as a good source of nutrients essential for human health. The energy in milk is provided by means of protein, fat and carbohydrates.

Results of current study for Goat, Cow, Buffalo and Camel milk agreed with findings of Soliman (2005) for moisture, ash and carbohydrate. Protein content of Buffalo and Camel milk were in line with results evaluated by Singh *et al.* (2013). Our proposed results for fat in Sheep milk were lower than those presented by Mehmood and Usman (2010) whereas, results for Cow, Buffalo and Goat milk were comparable to our research conclusion with minor difference. The decline and changes in values might be due to stage of lactation, fodder, different breeds and seasonal variations. Processed milk as they are UHT and are considered safe, hygienic and of good quality with extended shelf life Velero *et al.* (2001). Moisture content reported by Imran *et al.* (2008) for S3, S5 and

S4 was in strong harmony with our findings except for S5. Protein content repels our results except for S3. Ash content of S3, S5 and S4 were in strong resemblance to our results. Our results for fat content were analogous with those of Awan *et al.* (2014) for S1, S2, S3, S4 and S5.

*3.7 Physicochemical characteristics of fresh and processed milk types*

The pH values found by Singh *et al.* (2013) for Camel milk were close to ours, but not for Buffalo milk. Imran *et al.* (2008) showed conductivity, viscosity, and specific gravity for Buffalo, Cow, and Goat milk, and their results were very similar to ours. Our findings on the Titratable acidity of cow milk differed from Tascis (2011). The total solids content of sheep milk was similar to Mehmood and Usman (2010). The findings of Awan *et al.* (2014) for pH in tetra packs were somewhat close to ours. The total solids values for S4, S3, and S5 were extremely similar to those of a prior study conducted by Imran *et al.* (2008). Our findings were strikingly similar in terms of viscosity, Titratable acidity, specific gravity, and conductivity.

*3.8 Mineral composition of fresh and processed milk types*

Soliman (2005) reported calcium and magnesium levels for Camel, Buffalo, Cow and Goat milk which, were in good match with our findings. Magnesium content when compared with results presented by Raynal-Ljutovac *et al.* (2008) in Sheep milk was equivalent to our results whereas, for Goat and Cow milk results claimed were not exactly the same. Elbagermi *et al.* (2014) concluded iron for Cow milk was close to our results. Shamsia (2009) presented iron result for Camel milk was analogous with our results. Park *et al.* (2007) results for zinc levels were close to our results in Goat, Sheep and Cow milk. Imran *et al.* (2008) data for calcium and magnesium in tetra packs S3, S4 and S5 were nearly equivalent to our findings.

*3.9 Total Phenolic compounds and antioxidant activity of fresh and processed milk types*

Vazquez *et al.* (2015) reported total phenolic compounds in Goat and Cow milk which were somehow in line with our results; they investigated different samples for each milk type and then reported results in terms of means.

More interest is developed by researchers in study of antioxidants because of their therapeutic value and safety. Milk has many healthy effects on human health and its disease preventive nature lies with those of antioxidant properties (Halliwell *et al.*, 1992; Singh and Sharma, 2009).

Antioxidant activity of Buffalo milk measured by Sreeramulu and Raghunath (2011) was not in good agreement with our results. Balakrishnan and Agarwal (2014) investigated antioxidant activity of Cow, Goat and Camel milk before and after fermentation and presented values in % radical scavenging activity their observed values strongly repelled our results. Simos *et al.* (2011) results for Cow and Goat milk were not analogous with that of our findings. The main reason for change in values might be different processing techniques and methodology, climate, fodder, breed etc.

## Conclusions and Recommendations

In Khyber Pakhtunkhwa (Pakistan), there is a scarcity of information on the chemical composition and other characteristics of fresh milk and common tetra pack brands. The energy content of processed milk, which is extensively consumed in KP, has yet to be determined. Our findings may be useful in providing enough information about their nutritional values and composition. Nutritional data on milk products is currently available and might be used to construct both normal and therapeutic diets. It will assist individuals and dietitians in making better decisions by evaluating milk varieties' macro content and addressing main nutrition issues linked to community diet. Furthermore, health care practitioners and nutritionists can use the information on nutritional content of milk kinds to determine energy and nutrient intakes.

## Novelty Statement

Many work is done in contest of mineral contents of milk but there is not much evidence for phenolic compounds in milk specially in Pakistan.

## Author's Contribution

**Juweria Abid:** She have done research, Conceptualization, Methodology, Formal Analysis, Writing-original draft.
**Shakeel Ahmad:** help in results analysis.
**Humaira Wasila:** Supervisor.
**Attaullah Jan:** Review article.

**Muhammad Farooq:** Conceptualization, Review the manuscript and amp; Editing.

*Conflict of interest*

The authors have declared no conflict of interest.

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