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Research Article

Proximate Analysis, Phenolic Compounds and Antioxidant Activity of Milk Products Commonly Consumed in Khyber Pakhtunkhwa, Pakistan

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Abstract | The current study was designed to determine the proximate composition, phenolic compounds and antioxidant activity of milk products commonly consumed in Khyber Pakhtunkhwa, Pakistan. Fifteen different milk product samples were collected from 3 different markets in the study area to get a composite sample. AOAC 15th edition methods were used to analyze moisture, ash, carbohydrates, fats and protein contents of milk products. Atomic absorption spectroscopy and flame photometry were used for mineral analysis. Total phenolic compounds were evaluated by the Folin-Ciocalteu method. The aluminum chloride colorimetric method was used for evaluating total flavonoids contents. Antioxidant activity was assessed by the DPPH method. The results showed that moisture was high in Buttermilk (92.15±0.13 g/100g), ash and proteins in Manpasand (5.71±0.03, 25.36±0.04 g/100g respectively), carbohydrates in Mardani Paida (79.69±0.14 g/100g), and fats in Butter (81.71±0.04 g/100g). Khoa showed the highest amount of calcium and iron (612.00±0.01, 2.756±0.04 mg/100g respectively), while maximum zinc was observed in Cheese (4.80±0.05 mg/100g) and phosphorus in Mardani paida (401.60±0.04 mg/100g). The highest total phenols (37.04±0.61 mg GAE^{b}/100g), total flavonoids (12.027±0.06 mg QE^{c}/100g), and antioxidant activity (18.44±0.05 mgVCE^{d}/100g) were investigated in Green tea with milk. Macro and micronutrient composition of milk products commonly consumed in Khyber Pakhtunkhwa is available now and can be used by nutritionists, dieticians and health care providers in planning a normal and therapeutic diet. Milk products contained phenolic compounds which show antioxidant activity.

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1. Introduction

Milk is defined as the whole, clean and fresh lacteal secretion obtained by the complete milking of one or more animals (Hui and Yiu, 1993). In fresh form, milk has maximum nutritious value because it contains a high amount of proteins, fats, carbohydrates, vitamins and minerals in comparison with other food items (Neumann *et al*., 2002).

Pakistan is one of the major milk-producing countries. The main sources of milk include buffalo, cow, camel, sheep, and goat. Currently, Pakistan has 27.33, 29.56, 0.92, 26.49, and 53.79 million buffalo, cows, camels, sheep, and goats, respectively (GOP, 2006). Among the topmost milk-producing countries, Pakistan lies in 4th while America is first, Russia in 2nd and India lies in 3rd position. On an annual basis, Pakistan produces about 45 billion liters of milk (Iqbal *et al*., 2011).

Milk products are a rich source of minerals like zinc, phosphorus, calcium, potassium, magnesium and selenium, as well as vitamins, like vitamin A and vitamin B complex (Iqbal *et al*., 2011). Due to the presence of a significant amount of phenolic compounds, the importance of milk products is increased, which maximized their production (O'Connell and Fox, 2001). Many studies confirmed that milk products contain a variety of antioxidant molecules like carotenoids, retinol, thiols, tocopherol and ascorbate (Nozière *et al*., 2006).

Milk products are extensively eaten worldwide and have financial value. Milk products contribute about 10% of total energy and 15-25% of fat and protein requirements (Yildiz, 2009). Milk products are commonly consumed in the Khyber Pakhtunkhwa district of Pakistan and have high demand. Due to increased demand, this study was designed to find out the composition of macronutrients, minerals, phenolic compounds and antioxidant activity of milk products commonly consumed in Khyber Pakhtunkhwa Pakistan.

2. Materials and Methods

2.1 Study site

The study was conducted in the laboratory of Human Nutrition, The University of Agriculture Khyber Pakhtunkhwa, Pakistan.

2.2 Sample collection

Fifteen different milk products locally produced and consumed were selected for this study, including yogurt, cream, butter, buttermilk, cheese, khoa, black tea with milk, green tea with milk, milky ladu, burfi, sohan halwa, mardani paida, rasgulla, gulabjamun and manpasand.

Milk products were purchased from three different local markets in Khyber Pakhtunkhwa by simple random sampling technique. Composite samples were made for further laboratory work.

2.3 Sample drying and storage

Samples were dried in an oven at 65^{o}C temperature for three days. A commercial grinder was used to grind milk products. Then samples were stored at room temperature in plastic jars.

2.4 Proximate composition of milk products

Association of official analytical chemists 15th edition procedures were followed for proximate analysis of milk products. It assumed the amount of carbohydrates, protein, fats, ash and moisture contents. All chemical analysis was conducted in triplicate (Association of Official Analytical Chemists and Helrich, 2000).

2.5 Minerals determination

To determine elements like calcium, phosphorus, iron and zinc, the method of (Polyakova and Shuvaeva, 2005) was used. Atomic absorption spectroscopy and flame photometry were used for mineral analysis.

2.6 Phenolic compounds and antioxidant activity

Total phenolic compounds were evaluated by the Folin-Ciocalteu method with slight modification (Singleton *et al*., 1998). The aluminum chloride colorimetric method of (Chang *et al*., 2002) was used for the evaluation of total flavonoid content. Antioxidant activity was assessed according to the procedure of (Molyneux, 2004).

2.7 Statistical analysis

Statistical software statistix 8.1 was used in which one-way repeated ANOVA was used for multi comparison, and all the results were represented by mean±SD.

3. Results and Discussion

Milk products are commonly consumed in the Khyber Pakhtunkhwa district of Pakistan and have high demand. Due to increased demand, this study was designed to determine the composition of

macronutrients, minerals, phenolic compounds and antioxidant activity of milk products commonly consumed in Khyber Pakhtunkhwa.

The proximate composition of milk products commonly consumed in Khyber Pakhtunkhwa is given in Table 1. Moisture contents ranged from 92.15±0.13 g/100g seen in Buttermilk to 0.84±0.06 g/100g seen in Manpasand. The highest ash contents were observed in Manpasand (5.71±0.03g/100g), while the lowest was observed in Butter (0.11±0.42g/100g). Similarly, maximum carbohydrates were present in Mardani paida (79.69±0.14g/100g), and the lowest carbohydrates were present in Buttermilk (2.13±0.02 g/100g). Protein in the highest amount was examined in Manpasand (25.36±0.04g/100g), and protein in the lowest amount was examined in Sohan halwa (0.43±0.25 g/100g). In the same way, the topmost fats were noted in Butter (81.71±0.04g/100g), and the lowermost fats were noted in Yogurt (0.77±0.04g/100g).

Minerals contents of milk products commonly consumed in Khyber Pakhtunkhwa are presented in Table 2. Maximum calcium (612.00±0.01mg/100g) was observed in Khoa, and the least calcium (15.22±0.03 mg/100g) was observed in Butter. The highest amount of zinc was seen in Cheese (4.80±0.05^{a} mg/100g), while the lowest zinc was seen in Butter (0.109±0.02 mg/100g). Similarly, the utmost quantity of iron was examined in Rasgulla (5.60±0.05 mg/100g), and the least quantity of iron was examined in Buttermilk (0.110±0.05mg/100g). Maximum phosphorus content was determined in Mardani paida (401.60±0.04 mg/100g) while, the lowest phosphorus content was determined in Butter (25±0.01 mg/100g).

Phenolic compounds of milk products commonly consumed in Khyber Pakhtunkhwa are shown in Table 3. The highest total phenolic compounds were seen in Green tea with milk (37.04±0.61 mg GAE^{b}/100g), while the lowest total phenolic compounds were seen in Manpasand (12.07±0.86 mg GAE^{b}/100g). In the same way, the topmost flavonoids were observed in Green tea with milk (12.02±0.06 mg QE^{c}/100g) and the lowermost flavonoids were observed in Cream (3.45±0.04 mg QE^{c}/100g). Due to the presence of phenolic compounds, the highest antioxidant activity was detected in Green tea with milk (18.44±0.05 mgVCE^{d}/100g) and the lowest antioxidant activity was detected in Rasgulla (7.59±0.23 mgVCE^{d}/100g).

The correlation between total phenolic compounds, total flavonoids and antioxidant activity is given in Table 4. A significant correlation was seen (0.676 (P < 0.01)) between total phenolic compounds and total flavonoids. In the same way, a strong significant correlation (0.805(P < 0.01)) was observed between total flavonoids and antioxidant activity. Similarly, a significant positive correlation was noted (0.667 (P < 0.01)) between total phenolic compounds and antioxidant activity.

Table 1: Proximate analysis of milk products commonly consumed in Khyber Pakhtunkhwa (g per 100g wet weight).

Milk products	Moisture	Ash	Carbohydrates	Protein	Fat
Butter milk	92.15±0.13^{a}	0.38±0.021^{jk}	2.13±0.02^{m}	3.71±0.14^{h}	1.53±0.01^{n}
Black tea with milk	79.09±0.09^{d}	4.80±0.04^{b}	5.04±0.10^{k}	5.06±0.05^{g}	5.92±0.21^{k}
Green tea with milk	80.21±0.18^{c}	4.60±0.05^{c}	4.70±0.12^{l}	5.26±0.42^{f}	5.10±0.30^{l}
Yogurt	86.46±0.32^{b}	0.42±0.01^{j}	11.32±0.40^{i}	1.02±0.02^{l}	0.77±0.04^{o}
Cream	54.47±0.09^{e}	0.70±0.54^{i}	4.78±0.24^{kl}	2.41±0.01^{j}	25.33±0.22^{c}
Butter	15.80±0.01^{h}	0.11±0.42^{l}	2.19±0.12^{m}	0.98±0.04^{l}	81.71±0.04^{a}
Cheese	54.47±0.06^{e}	3.91±0.14^{d}	5.73±0.21^{j}	15.01±0.05^{d}	20.86±0.05^{f}
Khoa	17.41±0.01^{g}	3.31±0.22^{e}	31.77±0.10^{h}	17.76±0.12^{c}	35.73±0.13^{b}
Milky ladu	14.29±0.26^{i}	0.90±0.24^{h}	66.33±0.20^{d}	1.66±0.13^{k}	16.63±0.15^{i}
Burfi	7.23±0.03^{k}	0.37±0.02^{jk}	72.34±0.10^{b}	1.90±0.43^{k}	18.26±0.43^{h}
Sohan halwa	11.43±0.01^{j}	0.21±0.03^{kl}	68.15±0.10^{c}	0.43±0.25^{m}	19.77±0.14^{g}
Mardani paida	2.35±0.02^{m}	1.30±0.01^{g}	79.69±0.10^{a}	3.06±0.17^{i}	13.60±0.02^{j}
Rasgulla	51.27±0.03^{f}	2.01±0.11^{f}	34.35±0.70^{g}	8.24±0.07^{e}	4.15±0.10^{m}
Gulabjamun	6.22±0.02^{l}	4.57±0.10^{c}	48.27±0.10^{e}	19.43±0.19^{b}	21.51±0.03^{e}
Manpasand	0.84±0.01^{n}	5.71±0.03^{a}	45.34±0.21^{f}	25.36±0.04^{a}	22.73±0.32^{d}

Values expressed as Mean±SD. Values with same letter(s) in each column are not significantly different at P < 0.05.

Table 2: Mineral analysis of milk products commonly consumed in Khyber Pakhtunkhwa (mg/100g).

Milk products	Calcium	Zinc	Iron	Phosphorus
Butter milk	90.99±0.01^{l}	0.388±0.02^{e}	0.110±0.05^{n}	68.72±0.03^{k}
Black tea with milk	140.04±0.02^{k}	0.117±0.03^{l}	0.730±0.03^{d}	102.28±0.05^{j}
Green tea with milk	148.06±0.05^{j}	0.123±0.02^{k}	0.680±0.02^{e}	107.5±0.01^{i}
Yogurt	201±0.05^{i}	0.750±0.05^{d}	0.127±0.04^{m}	173±0.04^{h}
Cream	51±0.02^{n}	0.228±0.021^{f}	0.202±0.01^{l}	51±0.03^{l}
Butter	15.22±0.03^{o}	0.109±0.02^{m}	0.201±0.02^{l}	25±0.01^{n}
Cheese	520.02±0.02^{b}	4.80±0.05^{a}	1.55±0.02^{c}	392±0.02^{b}
Khoa	612.0±0.01^{a}	3.605±0.04^{b}	2.75±0.04^{b}	364.0±0.03^{c}
Milky ladu	392.8±0.04^{f}	0.180±0.02^{g}	0.391±0.01^{k}	312.04±0.01^{f}
Burfi	464±0.12^{d}	0.175±0.01^{h}	0.589±0.02^{f}	392.20±0.02^{b}
Sohan halwa	316.05±0.01^{h}	0.116±0.01^{1}	0.512±0.03^{h}	288.50±0.01^{g}
Mardani paida	515.02±0.04^{c}	0.133±0.05^{j}	0.484±0.02^{i}	401.60±0.03^{a}
Rasgulla	63.04±0.01^{m}	1.245±0.03^{c}	5.60±0.05^{a}	45.56±0.05^{m}
Gulabjamun	410.04±0.01^{e}	0.171±0.02^{h}	0.411±0.03^{j}	360.40±0.02^{d}
Manpasand	390.5±0.05^{g}	0.151±0.04^{i}	0.569±0.05^{g}	344.60±0.05^{e}

Values are expressed as Mean±SD. Values with same letter(s) in each column are not significantly different at P < 0.05

Table 3: Phenolic compounds and antioxidant activity of milk products commonly consumed in Khyber Pakhtunkhwa.

Milk products	Total phenolic compounds (mgGAE^{b}/100g)	Total flavonoids (mg QE^{c}/100g)	Antioxidant activity (mg-VCE^{d}/100g)
Butter milk	29.93±0.83^{c}	7.50±0.02^{d}	13.78±0.93^{c}
Black tea with milk	34.84±0.24^{b}	11.20±0.07^{b}	17.50±0.32^{b}
Green tea with milk	37.04±0.61^{a}	12.02±0.06^{a}	18.44±0.05^{a}
Yogurt	18.58±0.64^{i}	9.05±0.32^{c}	13.77±0.20^{c}
Cream	20.82±0.61^{h}	3.45±0.04^{o}	7.78±0.87^{m}
Butter	24.63±0.52^{e}	4.61±0.05^{k}	8.99±0.38^{l}
Cheese	13.61±0.53^{m}	5.99±0.02^{f}	12.20±0.26^{f}
Khoa	20.84±0.84^{h}	7.04±0.05^{e}	12.41±0.35^{e}
Milky ladu	17.91±0.68^{j}	4.12±0.04^{m}	9.48±0.25^{k}
Burfi	26.16±0.82^{d}	4.92±0.02^{i}	11.80±0.23^{g}
Sohan halwa	16.50±0.34^{k}	3.82±0.03^{n}	9.95±0.46^{j}
Mardani paida	21.80±0.38^{g}	4.75±0.12^{j}	11.40±0.11^{h}
Rasgulla	15.34±0.45^{l}	5.57±0.02^{g}	7.59±0.23^{n}
Gulabjamun	22.29±0.25^{f}	4.52±0.07^{l}	10.61±0.30^{i}
Manpasand	12.07±0.86^{n}	5.08±0.04^{h}	12.56±0.45^{d}

Values are expressed as Mean±SD. Values with same letter(s) in each column are not significantly different at P < 0.05.

Knowledge of the macromineral and trace element composition of milk products is important because of its wide use and nutritional importance. The macro density of milk products depends on factors like the genetics of lactating animals, environmental conditions, lactation stage, pasture type, manufacturing procedures and added ingredients in the processing of milk products (Sola-Larrañaga and Navarro-Blasco, 2009).

Table 4: Correlation between total phenolic compounds (TP), total flavonoids (TF) and antioxidant activity (AA).

	TF	AA	TP
TP	0.676 (P < 0.01)**		
TF		0.805(P < 0.01)**	
AA			0.667 (P < 0.01)**

**Correlation coefficients were significant at (P ≤ 0.01).

Moisture contents of the current study on yogurt were supported by (Olugbuyiro and Oseh, 2011). Ash contents in Buttermilk were closely related to the results of (Munde, 2015). Similarly, carbohydrates in Gulabjamun were confirmed by the findings of (Kumar, 2005). Correspondingly, protein in Cheese was matched with observations of (Sameen *et al.*, 2008). Likewise, protein in Khoa was the same as determined by (Sameen *et al.*, 2008). The findings of fats in Rasgulla were parallel to figures of (Puniya, 2015).

Milk products are rich in micronutrients like calcium, phosphorous, iron, and zinc (Ataro *et al.*, 2008). National dairy council provided mineral contents

such as phosphorus, calcium, magnesium, zinc, iron, manganese, selenium, copper, iodine, chloride, potassium and sodium levels in milk products (Dairy Products and Foods U.S. Dairy, n.d.). Fermented milk products contain more than 20 different elements. Elements like copper, zinc, manganese, and iron are essential, and they are very important for normal metabolism, growth, and development (Khan *et al.*, 2014). On the other hand, elements such as lead, chromium, mercury, and cadmium are very important because of their toxicity and metabolic roles (Dervisoglu *et al.*, 2014). The most vital mineral in milk products is calcium. National Academies of Science indicates the dietary reference intake for calcium that 800 mg per day for 3–8 years old, 1300 mg per day for 9–17 years old, and 1200 mg per day for people over 50 years. Vitamins and minerals are important for human life. They play vital roles in metabolic functions like maintenance of pH, nerve conductance, osmotic pressure, muscle contraction, bone health, energy production and in almost all aspects of body growth (Institute of Medicine US, 1997). Calcium in yogurt, butter and cream was endorsed by (Sameen *et al.*, 2008). Similarly, zinc in rasgulla was proven by (Prodhan *et al.*, 2017). Iron in cheese was supported by the results of (Singh *et al.*, 2016). Phosphorus in cheese, yogurt, cream and butter was seen as the same as calculated by (Zamberlin *et al.*, 2012). Zinc in Cheese was the same as observed by (Singh *et al.*, 2016).

Dairy products are one of the most interesting and promising foods with regard to their potential antioxidant activity due to their wide diversity of antioxidant molecules such as milk caseins and whey proteins (Pihlanto, 2006). Furthermore, milk contains a variety of antioxidant molecule traces i.e. low molecular weight thiols (Niero *et al.*, 2015), ascorbate, tocopherol, retinol and carotenoids (Niero *et al.*, 2017). Total phenolic compounds in Yogurt was somehow related to results of (Ramos *et al.*, 2017); minor changes might be due to the selection of raw material for Yogurt preparation. Total flavonoids in Yogurt were supported by the findings of (Qureshi *et al.*, 2017). Total phenols in Black tea with milk were the same as proven by (Liebert *et al.*, 1999). Antioxidant activity in green tea with milk was matched with the results of (Mahmood *et al.*, 2014). Medicinal plants rich in natural antioxidants and phenolics are progressively applied in dairy food manufacturing to improve nutritional and therapeutic properties, which may help in the prevention and control of different diseases (Bertolino *et al.*, 2015).

Conclusions and Recommendations

Nutritional information regarding milk products is available now and would serve as a basis for planning normal and therapeutic diets. It will help individuals to make a better choice by comparing milk products for their macronutrient contents and addressing major nutrition disorders that are related to the diet of the community. Milk products contain phenolic compounds which show antioxidant activity. Data on the nutritional composition of milk products can be used by health care workers and nutritionists to calculate energy and nutrients intakes.

Novelty Statement

This is the first ever study which observed the nutritional composition of milk products commonly consumed in Khyber Pakhtunkhwa, as well as phenolic compounds and antioxidant activity of these products, which makes it a novel study.

Author's Contribution

All authors equally contributed to this research.

Conflict of interest

The authors have declared no conflict of interest.

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