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Comparative study of nutritional status, minerals and heavy metal contents in tetra pack branded milk samples with fresh milk from selected milk producing animals

Tahira Batool[1], Zahed Mahmood[2], Muhammad Riaz[3*], Muhammad Zahoor ul Hassan Dogar[4], Saba Irshad[1], Mian Anjum Murtaza[5], Khalid Mehmood[6], Ghulam Rasool[3], Asma Irshad[7], Haleema Sadia[8]

[1]*Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan*

[2]*Department of Biochemistry, Government College University Faisalabad, Pakistan*

[3]*Department of Allied Health Sciences, Faculty of Medical and Health Sciences, University of Sargodha, Sargodha, Pakistan*

[4]*Department of Biochemistry, Sargodha Medical College, Faculty of Medical and Health Sciences, University of Sargodha, Sargodha, Pakistan*

[5]*Institute of Food Science and Nutrition, University of Sargodha, Sargodha, Pakistan*

[6]*Technology Education Department, University of Management and Technology, Lahore, Pakistan*

[7]*Center of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan*

[8]*Department of Biotechnology, Balochistan University of Information Technology, Engineering and Management Sciences (BUITEMS), Quetta, Pakistan*

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Abstract

Milk contains the key ingredients of nutritional importance required for growth; however, increased milk demand and consumption makes essential to use tetra pack market milk to fulfill the required milk demand. Therefore, the current study was planned to analyze and compare the proximate composition of collected fresh and tetra pack milk samples including Cow (S1), Buffalo (S2), Camel (S3), Goat (S4), Haleeb (S5), Dairy pure (S6), Good milk (S7), Milk pack (S8), Umung (S9) and Olper (S10), and to determine the minerals and heavy metal contents in studied milk samples. Proximate analysis was performed following the standard methods while the metals content were determined through Atomic Absorption Spectrophotometer analysis. Our results revealed significant ($p<0.05$) differences in proximate composition of most of the studied milk samples. Mineral analysis revealed significant ($p<0.05$) differences in mineral contents (Calcium, Iron and Zinc) of studied milk samples. Among the studied heavy metals, lead (Pb) content was significantly ($p<0.05$) different in all the milk samples while Nickel (Ni) and Cadmium (Cd) were not detected in any studied milk sample. All the fresh milk samples from selected animals have significantly ($p<0.05$) higher minerals (Ca, Fe and Zn) contents as compared to branded tetra pack milk samples. The study concluded that tetra pack milk have poor nutritional values compared to fresh milk, especially with respect to lower protein, fat, calcium, iron and zinc that are the major constituent of milk and made it a unique source of nutrients in all foodstuffs.

* **Corresponding Author:** Muhammad Riaz ✉ riazmlt786@gmail.com

Introduction

Milk is a vital component of diet because of essential nutrients and minerals which provide energy and promote growth of all the newborn mammals. The milk varieties differ in composition but holding all the fundamental elements required for growth and development. Pakistan ranked 7th among world's top milk producing countries regardless of the socioeconomic constrains faced by the dairy industry (Shahnawaz *et al.*, 2011). Buffalo and cattle are contributing the milk requirements in Pakistan by producing 26.4 million tons of milk. Per capita annual availability of milk is 82.4 kg. Overpopulation and rapid urbanization increased the milk production demand. In Pakistan, raw milk is being used by almost 97% of the people in their daily life with poor hygienic conditions. Raw milk carries the risk of microbial pathogens leading to human illnesses in addition to poor hygienic quality (Awan *et al.*, 2014). Milk and dairy products contain all the essential elements required for healthy life particularly for the development, growth and body metabolism. Minerals such as calcium, selenium, zinc and copper are vital for normal functioning of the body because of their involvement in various physiological processes. However, milk may also contain variety of heavy metals such as cadmium (Cd), lead (Pb) and arsenic (As). If heavy metal contaminated milk is consumed in excessive amount, then this milk can lead to severe systematic health problems due to the excessive concentration of such heavy metals from the production environment (Zhou *et al.*, 2019). A previous study suggested that fodder contamination might be a factor responsible for increased Pb concentration in blood and milk sample of buffalos (Shailaja *et al.*, 2014). Other heavy metal contamination might show similar observations such as As. For instance, the most important routes of as exposure are contaminated foods and drinking water (Zhou *et al.*, 2019).

Presence of toxic heavy metals like Cd and Pb in milk as part of human diet has irrecoverable effects. Excessive Pb concentration in diet results in anemia and disorders of various organs like liver, kidneys, heart, reproductive organs, immunity and central nervous system (Correia *et al.*, 2000). Cd being carcinogenic agent causes tumors of the lungs and prostate leading to the failure of various organs such as kidneys, lungs, bones, vessels and hearts. Cd contamination affects the pregnant women resulting in malformations, reduction in fetal weight, abnormalities in baby's DNA and proteins as well as high metal contamination may lead to abortion (Delavar *et al.*, 2012).

From the standpoint of health and sanitation, the maximum allowable levels of toxins in food have already been set by global health organizations such as FAO and WHO. The amounts of lead and cadmium in milk have been determined to be 1000 ppb and 100 ppb, respectively. Hence, choosing a suitable method for measuring these toxic metals in food materials is highly important (Delavar *et al.*, 2012).

Milk quality and quantity is dependent on animal health condition. Animal health requires adequate amount of minerals for normal functioning. Some of the inorganic minerals such Ca, Fe, P, Na, K, Mg, Cl, Zn, Cu, I, Mn and Co are essential nutrients for animals nutrition (Farid and Baloch, 2012). The minerals concentration in animal's diet affects the quality of milk due to the removal of nutrients from blood by the mammary glands converting them into milk and thus secreted into the udder. Various environmental pollutants might reach to animal's milk through forage, as a result, worries about the quality of milk has been increased due to growing environmental pollution (Farid and Baloch, 2012). Several factors particularly the manufacturing processes, environmental settings and the possible contamination might lead to the presence of toxic metals in milk and dairy products (AsadiDizaji *et al.*, 2012).

Therefore, the current study was planned to analyze and compare the physicochemical properties, nutritional contents and selected heavy metals of various tetra packed milk samples with fresh milk from selected animals.

Materials and methods

For the current study, a total number of ten different milk (250 mL) samples were collected. Among the collected milk samples, four were of fresh milk from selected animals including cow, buffalo, camel and goat while six milk samples from different tetra packed milk. All the milk samples were collected from local market of Faisalabad and brought to the research laboratories of Department of Biochemistry and Central Hi-tech Lab of University of Agriculture, Faisalabad for further analysis. These collected tetra pack milk samples were available under the trade name of Haleeb (S5), Dairy pure (S6), Good milk (S7), Milk pack (S8), Umung (S9) and Olper (S10). Each sample was divided into two halves: one to study the proximate composition (Dry matter, Moisture, Fat, Ash and Crude protein) and other to determine the minerals and heavy metal contents (Calcium, Iron, Zinc, Copper, Lead, Nickel and Cadmium).

Proximate analysis

Proximate composition like percent moisture, dry matter, fat, ash and crude protein were determined following the methods of the Association of Official Analytical Chemists (AOAC, 2000). Duplicate determinations of all the parameters were performed and the proximate values were reported in percentage.

Sample digestion for mineral and heavy metal determination

Acid digestion method was used to obtain the analyte of interest following the standard method (AOAC, 2000). Milk sample (1 mL) was taken into a 100 mL conical flask and digested with conc. HNO_3 (10 ml) by heating on hot plate for 20-30 min. After cooling, perchloric acid (5 ml) was mixed into the mixture. The mixture was heated vigorously reducing the mixture to 2-3 ml and then diluted to 20 mL with redistilled water for minerals and heavy metal analysis.

Standards preparation and sample analysis

Different concentrations of standards were made using standard stock solution (1000 ppm) commercially available to calibrate the Atomic Absorption Spectrophotometer (AAS). Well prepared standard concentrations gave linear standard curves of studied parameters which were used to estimate metal contents in studied milk samples.

Determinations by Atomic Absorption Spectrophotometry (AAS)

Selected elements (Ca, Fe, Zn, Cu, Pb, Ni and Cd) concentrations in prepared samples were measured using Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) under the conditions as described in AOAC (1990) (Chemists, 1990).

Statistical analysis

The data obtained were analyzed by ANOVA (Analysis of Variance) to determine the significance of results using Minitab, version 16. $P<0.05$ value was considered statically significant. The data were presented as Mean ± SEM (standard error of mean) of duplicate determinations.

Results and discussion

The current research results depicted an overall wide variations in studied parameters of analyzed milk samples both the fresh milk from selected animals and those of tetra pack branded milk available in local market.

Proximate composition

Proximate compositions of studied ten milk samples S1-S10 are illustrated in Table 2. Dry matter content is responsible to provide essential nutrients necessary for providing energy, growth and health. Significant ($p<0.05$) variations in percent dry matter content was found in studied milk samples. Highest dry matter content was found in Buffalo milk (S2) while the lowest dry matter content was found in Dairy pure (S6). The mean percentage dry matter content in studied milk samples ranges from 9.77±0.075 – 18.48±0.031 %. Dry matter content of Cow milk in our study was found 13.52% that supported by the reported value 13.5% (Faverdin *et al.*, 2017). Among the tetra pack milk samples, highest dry matter content was found in Olper (16.62±0.045%). Lower content of dry matter in milk indicates reduced nutritional ingredients in that milk. Moisture content of cow milk (86.47±0.035%) in our study is nearly close to recommended value of moisture in dietary milk (86.5%). Moisture content significantly ($p<0.05$) variable in brands milk, dairy pure showed significantly ($p<0.05$) high moisture content while olper showed significantly ($p<0.05$) reduced moisture content from all selected brands.

Table 1. Operational conditions employed for the measurement of selected metals by Atomic Absorption Spectrophotometer.

Elements	Wavelength (nm)	Slit Width (nm)	Lamp Current (mA)	Burner Head	Flame	Burner Height (mm)	Oxidant gas pressure (Flow rate) (kpa)	Fuel gas Pressure (Flow rate) (kpa)
Calcium	422.7	0.4	7.5	Standard type	Air-C_2H_2	7.5	160	6
Cadmium	228.8	1.3	7.5	Standard type	Air-C_2H_2	5	160	6
Copper	324.8	1.3	7.5	Standard type	Air-C_2H_2	7.5	160	7
Iron	248.3	0.2	10	Standard type	Air-C_2H_2	7.5	160	6
Lead	283.3	1.3	7.5	Standard type	Air-C_2H_2	7.5	160	7
Nickel	232	0.2	10	Standard type	Air-C_2H_2	7.5	160	7
Zinc	213.9	1.3	10	Standard type	Air-C_2H_2	7.5	160	6

Increased percent moisture implies high water activity supporting the growth of microbes, as a result decreasing the shelf life of milk. Cow milk has 5% fat that is slightly high than recommended value 4.55%. Haleeb milk has significantly ($p<0.05$) lower fat content while Good milk has content of fat content among all tetra pack milk samples. The ash content which is an indication of minerals in milk samples and is higher in Goat milk while lowest in Dairy pure. The ash content of cow milk (0.73±0.04%) found in our study was nearly equal to the recommended value (0.7%) of ash content in Milk. The ash content showed non-significant differences in all he studied milk samples. Significant ($p<0.05$) variations in crude protein content were observed in studied milk samples. Crude protein content in Cow milk (3.29±0.06%) in our study was found slightly lower than recommended value (3.5%). Highest crude protein content was found in Good milk (3.59±0.07%). Lower protein content in milk indicates poor nutritional worth. Proximate analysis revealed that good milk has better nutritional values especially in respect to crude protein and fat contents.

Various studies reported the proximate composition of fresh and tetra pack milk samples. Braun and Stefanie (2008) studied the proximate composition of milk and found similar results of dry matter concentration in buffalo milk as we found in our study. But different studies reported variable proximate composition in variety of milk samples from different sources.

Minerals and heavy metals

The results of various metal elements detected in studied milk samples are illustrated in Table 3. Calcium plays pivotal role in bone formation, muscle contraction and metabolism. Calcium is one of the quality parameter of good quality milk. WHO recommended amount of calcium in milk is 1290.0 mg/L. In our study, overall, highest concentration of calcium (1625±25 ppm) was found in buffalo milk while the lowest (1625±25 ppm) was found in Umung. Among fresh milk samples collected from selected animals, buffalo milk has increased calcium (1625±25 ppm) while goat milk has lowest (541.7±14.4 ppm). On the other hand among studied tetra pack milk samples, dairy pure has high calcium while Umung has low calcium. Calcium concentration differ significantly ($p<0.05$) among all the studied milk samples. Cow milk has nearly the recommended concentration of Calcium (1166.7±14.4 ppm). The results of present study regarding Ca concentration are consistent with the findings of Sikiric *et al.* (2003).

Table 2. Proximate composition of various fresh milk and tetra pack milk samples included in this study. The values are mean ± SEM. Last column shows ANOV A results following the comparison of each parameter between various milk samples.

Milk sample	Dry matter (%)	Moisture (%)	Fat (%)	Ash (%)	Crude protein (%)
S1	13.523±0.035	86.47±0.035	5.0±0.1	0.73±0.04	3.29±0.06
S2	18.48±0.031	81.51±0.031	5.47±0.15	0.64±0.02	3.42±0.11
S3	11.95±0.131	88.05±0.131	3.03±0.15	0.76±0.14	3.43±0.18
S4	12.43±0.22	87.57±0.22	4.5±0.1	0.80±0.20	2.84±0.072
S5	12.43±0.152	87.75±0.152	1.77±0.53	0.68±0.12	2.87±0.12
S6	9.77±0.075	90.23±0.075	2.0±0.1	0.621±0.023	2.27±0.13
S7	13.33±0.041	86.47±0.04	3.03±0.12	0.73±0.03	3.59±0.07
S8	13.28±0.045	86.72±0.045	2.0±0.1	0.77±0.023	2.48±0.17
S9	10.68±0.023	89.32±0.023	2.0±0.2	0.63±0.017	1.71±0.21
S10	16.62±0.045	83.38±0.045	2.2±0.2	0.75±0.023	2.62±0.11
P-Value	<0.05	<0.05	<0.05	>0.05	<0.05
Reference value	13.0	87.8	3.3	0.7	3.3

$p<0.05$ significant, $p>0.05$ Non-significant Where S1= Cow, S2= Buffalow, S3= Camel, S4= Goat, S5= Haleeb, S6=Dairy pure, S7= Good milk, S8= Milk pack, S9= Omung, S10=Olper.

Iron (Fe) is an essential trace element for the normal functioning of the body, forming a part of the protein hemoglobin which supplies oxygen throughout the body from lungs.

In studied milk samples, Fe concentration ranged from 9.83±0.32 – 23.0±0.5 ppms with highest Fe concentration in Cow milk while the lowest in Olper. Ahmad *et al.* (2017) studied the metal concentration in different cattle's milk and found highest Fe concentration in Camel milk but lowest in Sheep milk whereas contrary o that we found high Fe in Cow milk and lowest in Goat milk. Zinc is a vital cofactor for number of enzymes involved in different biochemical reactions.

It acts as antioxidant, playing significant role in development, growth and reproduction. Significant variation in Zn concentration was found in studied milk samples with highest concentration recorded in Camel milk (11.16±0.4 ppm) while lowest in Umung (4.17±0.32 ppm). Elatrash and Atoweir (2014) in their study reported heavy metal conc. in milk samples that supports our present study findings. Copper (Cu) was found undetectable in fresh milk collected from Cow and Buffalo while highest concentration was found in Dairy pure. No significant ($p>0.05$) variation in Cu concentration was found in studied milk samples.

Several researchers have focused on studying the contents of metal elements in fresh and tetra pack milk samples for milk quality and toxic effect of studied heavy metals like Pb, Ni and Cd. Lead (Pb) is one of the elements naturally found in earth's crust and its extensive use has resulted widespread environmental pollution. Increased Pb concentration may reduce cognitive improvement in children, hypertension and cardiovascular disorders (Ogabiela *et al.*, 2011). Lead (Pb) concentration was also significantly variable in studied milk samples with minimum level in Camel milk (0.667±0.28 ppm) and highest in Cow milk (3.17±0.38 ppm). Pb level was undetectable in Milk pack. Nickel (Ni) and Cadmium (Cd) was undetectable in all the studied milk samples. Ni and Cd are highly toxic to all life forms. High Pb content in milk samples might be owing to contamination of fodder, climatic factors like wind, contaminated drinking water and use of Agro-chemicals.

Table 3. Mean ± SEM values of selected minerals and heavy metals contents of various fresh milk and tetra pack milk samples. P-values indicates statistically significant differences following the comparison of each parameter between various milk samples.

Milk sample	Ca (ppm)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Pb (ppm)	Ni (ppm)	Cd (ppm)
S1	1166.7±14.4	23.0±0.5	7.67±0.3	--	3.17±0.38	--	--
S2	1625±25.0	17.33±0.30	8.667±0.29	--	2.184±0.29	--	--
S3	1383.3±14	15.33±0.29	11.16±0.4	0.33±0.28	0.667±0.28	--	--
S4	541.7±14.4	11.5±0.5	6.18±0.35	0.33±0.27	3.167±0.41	--	--
S5	450±25.0	12.83±0.76	4.67±0.31	0.46±0.31	1.83±0.31	--	--
S6	641±14.4	11.0±0.5	5.66±0.29	0.66±0.36	1.67±0.29	--	--
S7	366.7±14.4	13.33±0.32	5.66±0.28	0.33±0.29	2.33±0.34	--	--
S8	83.3±14.1	10.67±0.76	4.33±0.27	0.32±0.28	--	--	--
S9	58.3±12.4	16.5±0.5	4.17±0.32	0.32±0.30	2.0±0.5	--	--
S10	508.3±15.4	9.83±0.32	5.33±0.29	0.37±0.28	2.167±0.29	--	--
P-Value	<0.05	<0.05	<0.05	>0.05	<0.05	--	--
Reference value	112	2.0	0.4	0.1	0.02	--	--

$p<0.05$ significant, $p>0.05$ Non-significant, -- Not detected, Where S1= Cow, S2= Buffalow, S3= Camel, S4= Goat, S5= Haleeb, S6=Dairy pure, S7= Good milk, S8= Milk pack, S9= Omung, S10=Olper, Ca=Calcium, Fe= Iron, Zn= Zinc, Cu= Copper, Pb= Lead, Ni= Nikle, Cd= Cadmium.

The study concluded that tetra pack milk have poor nutritional values compared to fresh milk specially with respect to lower protein, fat, calcium, iron and zinc that are the major constituent of milk and made it a unique source of nutrients in all foodstuffs.

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