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Original Article

Minerals and heavy metals in raw and ultra heat treated commercial milks in Pakistan

Saeed Akhtar[a], Tariq Ismail*[a], Muhammad Riaz[a], Muhammad Shahbaz[a], Amir Ismail[a], Khalid Amin[a]

[a] *Institute of Food Science and Nutrition, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan – Pakistan*

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Abstract

The study was aimed at assessment of heavy metals in street vended raw and branded milk to identify possible gateways of milk toxicity. 15 samples of processed and 24 of raw milk were collected from Multan city. Atomic absorption spectrometry revealed milk offered for sale in the region to be potentially contaminated with higher levels of some most critical elements i.e. Pb (0.048 – 0.418mgL^{-1}), Cd (0.0015 - 0.125 mgL^{-1}), Ni (0.044 - 0.294 mgL^{-1}), and Cu (0.0037 - 0.273 mgL^{-1}). Exceptionally higher levels of Cd (0.102 mgL^{-1}) were recovered in street vended raw milk as compared to processed branded milk (0.042 mgL^{-1}). The study signified deleterious outcomes of industrial and agricultural revolution parallel to poor phyto-sanitory and sanitary measures during animal feeding and milking. The study suggest comprehensive and nationwide survey to identify the metal polluted red zones, their toxicity levels and milk intoxicating channels either from environment, animal feed or water.

1. Introduction

Nutritional significance of milk is widely recognized and its consistent consumption at childhood, adolescence and at advanced stages of life as a growth promoting dietary factor is well established (IEA, 2007) The health benefits of milk may be achieved by assuring that it is free of heavy metals in addition to several other toxic compounds and environmental contaminants (Patra et al., 2008; Simsek et al., 2000; Soares et al., 2010; Tajkarimi et al., 2008). However, escalating population, urbanization and increased industrial and agricultural expansion lead to exceedingly higher degree of environmental pollution such as heavy metals in the air, water and soil. Metals find their way to the animals' tissues and milk in case animals are lactating through ingestion of heavy metal contaminated water and plants. Several researchers have demonstrated presence of heavy metals in milk (Caggiano et al., 2005; Fayed et al., 1995; Licata et al., 2004; Raghunath et al., 1997) suggesting dietary intake of heavy metals, a significant health problem (Muchuweti et al., 2006; Ogabiela et al., 2011; Yahaya et al., 2010).

Several studies confirmed heavy metals to be ubiquitous in environment especially those regions where higher industrial and agricultural wastes are existent (Boffetta, 1993; Hayes, 1997). Heavy metals being not biodegradable, with long biological half-life posses the ability to accumulate in various body parts. There is a compelling evidence to suggest deleterious effect of heavy metals on human health as many epidemiological studies confirmed prevalence of many types of cancers in regions of higher prevalence of heavy metals, radioactive elements and their derived products (Duruibe, Ogwuegbu, & Egwurugwu, 2007; Jarup, 2003; Sathawara, Parikh, & Agarwal, 2004; Zaidi et al., 2005).

Extensive body of literature demonstrates that majority of Pakistani foods are badly implicated with heavy metals (Akhtar, 2012). Besides, prevalence of numerous chemical toxicants as adulterants in raw street vended milk, heavy metal contamination has also been confirmed by several researchers in such milk samples. Previously, investigation of fresh, processed milk and milk powder samples from the country has shown

***Correspondence Author:** Tariq Ismail
e.mail: *ammarbintariq@yahoo.com*

drastically higher levels of heavy metals (Jaffar et al., 2004).

Data are scant to portray a candid picture about extent of prevalence of heavy metals in raw and branded processed milk in various parts of Pakistan. Therefore the present research aims at exploring the levels of minerals and heavy metals in raw and (Ultra High Temperature) UHT processed commercial milks in Multan, the fourth biggest city of Pakistan. The findings of current research explicit the level of food safety in milk samples available in the open market suggesting the local dairy industry, government and allied stakeholders to implement better hygiene and food safety practices throughout the milk supply chain. This research investigation further amasses the level of awareness among the consumers to demand raw and processed milk free of all types of toxicants.

2. Materials and methods

2.1. Sampling

Fifteen samples of UHT processed branded milks and 24 samples of raw milk were collected. Raw milk samples were drawn from the street shops of four towns of Multan city. Sampling sites were randomly selected focussing the slums of the old city. Samples were collected in clean, acid-washed polyethylene for subsequent determination of minerals and heavy metal analysis. All of the samples were kept in the refrigerator for subsequent analyses within 48 h after their collection

2.2. Minerals and heavy metals detection

Minerals and heavy metals including, Pb, Cd, Ni, Cu, Zn, Fe, Co, and Mn were determined in all milk samples after wet digestion (AOAC, 2003), using atomic absorption spectrophotometer (AAS) (Thermo Scientific iCE 3000 series), equipped with multiple hollow cathode lamps, by using method described by Chukwujindu et al. (2008). Electrolytes i.e. Ca, K and Na, were measured by flame photometry (Model 410) after calibrating the equipment using standard solutions of different strengths.

All experiments were conducted in duplicate. Means of the replicates were obtained and standard deviations were calculated.

3. Results and discussions

Data presented in Table 2 revealed higher concentration of heavy metals and minerals in different milk brands collected from the local market. Brands comparison revealed Brand 2 milk to be highly contaminated with lead and cadmium while Brand 1, Brand 3, Brand 4 and Brand 5 depict a wide difference for the concentration of lead and cadmium especially in Brand 4 bearing least level of Cd i.e. 0.0015 mgL^{-1}. The levels of nickel in Brand 1 milk was higher among the tested samples while remaining milk brands exhibited least difference for the Ni concentration. Cu seems to exist in concentration with slightly higher levels in Brand 1. Similarly, higher levels of zinc and iron were noticed in Brand 3 followed by Brand 1 and Brand 2.

The order of the levels of trace elements i.e. Co and Mn, from highest to the lowest in tested milk samples was noted to be Brand 1, Brand 4, Brand 3, Brand 2, Brand 5 and, Brand 1, Brand 3, Brand 2 , Brand 5 and Brand 4 respectively. Data presented in Table 4 indicate higher concentration of electrolytes in all UHT processed milk samples. A great variability for the levels of Ca, K and

Table 1. Instrumental parameters for determination of micro elements with Atomic Absorption spectroscopy

Element	Wave Length (nm)	Lamp Current (mA)	Fuel (Acetylene flow) L/min	Burner Height (mm)	Band Pass (nm)	Nebulizer Uptake Time (Sec)	Excess curvature Limit (%)	Detection Limit (ppm)
Pb	217	10	1.1	7	0.5	4	10-40%	0.07
Cd	228.8	10	1.2	7	0.5	4	10-40%	0.01
Ni	232	15	0.9	7	0.2	4	10-40%	0.05
Cu	324.8	6	1.1	7	0.5	4	10-40%	0.03
Zn	213.9	12	1.2	7	0.2	4	10-40%	0.01
Fe	248.3	15	0.9	7	0.2	4	10-40%	0.05
Co	240.7	8	1	7	0.2	4	10-40%	0.06
Mn	279.5	15	1	7	0.2	4	10-40%	0.02

Table 2. Minerals and heavy metal content (mgL^{-1}) of commercial processed UHT- Treated milks in Multan-Pakistan

Elements	Commercial UHT treated milk samples					
	Brand 1	Brand 2	Brand 3	Brand 4	Brand 5	Mean ± SD
Pb	0.0475	0.4175	0.1536	0.1704	0.1603	0.190±0.137
Cd	0.0593	0.0621	0.0379	0.0015	0.0489	0.042±0.025
Ni	0.2113	0.1498	0.1555	0.1132	0.1173	0.149±0.04
Cu	0.0558	0.0224	0.0043	0.0333	0.0037	0.024±0.022
Zn	7.930	6.910	9.530	5.320	4.330	6.804±2.064
Fe	1.6291	1.6734	1.7417	1.6345	1.6229	1.66±0.05
Co	0.1339	0.0280	0.0287	0.0507	0.0198	0.052±0.047
Mn	0.0419	0.0256	0.0271	0.0085	0.0213	0.025±0.012

Values are the means of two experiment (n=2) with ± SD. The milk samples represent the most popular and extensively consumed UHT-processed milk brands in Pakistan

Table 3. Mean Concentration of Microelements and Heavy Metal in raw milk (mgL^{-1})

Element	Spectrometry Method	Mean (mgL^{-1})	Standard Deviation	Range (Min-Max) (mgL^{-1})	Number of Samples
Pb	AAS	0.2	0.05	0.145-0.290	24
Cd	AAS	0.102	0.017	0.072-0.125	24
Ni	AAS	0.176	0.088	0.044-0.294	24
Cu	AAS	0.159	0.057	0.102-0.273	24
Zn	AAS	2.155	0.867	1.889-3.189	24
Fe	AAS	1.039	0.288	0.54-1.442	24
Co	AAS	0.318	0.153	0.064-0.516	24
Mn	AAS	0.356	0.232	0.007-0.601	24

Na was observed in all UHT processed milk samples e.g. a difference of 3.00 mg/ml for Na concentration was noted in fifteen UHT processed milk samples. Higher levels of heavy metals (lead, cadmium and cobalt) were detected in raw milk samples (Table 3). Concentrations of Pb were shown to be two-fold when comparison was made between the lowest and the highest levels. Likewise, the level of variation between the concentrations of Cd, Ni and Cu in raw milk samples was significantly higher. Relatively, higher levels of Cu, Fe and Zn were observed in all samples ranging 0.1016 - 0.2728, 0.5391 - 1.4419 and 1.889 - 3.189 mgL^{-1}, respectively. Appreciable amounts of Co and Mn were observed in 75% sample population while only 25% samples contained relatively lower levels of Co and Mn. Majority of the samples indicated higher levels of minerals and heavy metals in raw milk as compared to the concentrations indicated in UHT processed milk (Table 2). In the same way, raw milk samples were found to contain higher concentration of calcium and lower levels of sodium as compared to the processed samples of different brands (Table 4). Mean concentration of electrolytes i.e. calcium (2.063mg/mL), potassium (6.562 mg/mL) and sodium (2.938 mg/mL) in raw milk were significantly different from processed milk brands i.e. 1.7 mg/mL, 13.2 mg/mL, 8.7 mg/mL for Ca, K and Na respectively.

Comparison of average heavy metal's concentration in UHT processed and raw milk samples revealed a slight higher level of lead in the latter however; residual cadmium contents in street vended raw milk were almost two hundred times higher than in branded milk i.e. 0.042 and 0.102 mgL^{-1} respectively (Table 2 and 3). In the same way, identical trends were observed for Zn, Co, Cu, Mn and Na in raw milk as compared to UHT treated milk (Table 3).

Permissible limits of lead in milk have been reported as 0.02mg/kg while that off cadmium as 0.075 mgL^{-1} (CE Regulation, 2001). The present results depict significantly higher levels of Pb in all milk samples as compared to the given permissible limits. Cadmium contents of branded milk were found to be well in range of CE regulations as maximum contents were found as 0.0621 mgL^{-1} in Brand 2 followed by 0.0593 in brand

Brand 1 however, the given values were still higher than those reported as Egyptian standards i.e. 0.05 mgL^{-1}. Higher lead contents have been reported in raw milk collected from industrial and intense traffic areas of Basra, Turkey i.e. 0.032 and 0.049mg/kg, respectively (Patra et al., 2008) while our findings from the City of Multan are far higher than reported from Turkey. Lower levels of cadmium in branded milk are by some means associated with the process of milk selection carried out by industrial internal procurement and quality assurance departments in addition to the preferences given to farm milk of non-industrial areas. However, no such monitoring process ensuring quality and standard of raw milk exists in heavily populated cities where a vast majority rely on open street vended raw milk.

Among other trace elements, mean copper contents in fresh milk (0.159 mgL^{-1}) were slightly lower than the findings from Romania (Binghila et al., 2008) i.e. 0.170 mgL^{-1} however, very low concentration of copper (0.062 mgL^{-1}) has been reported by (Damisa, Lawal, & Mohammed, 2006). Studies carried out in other parts of Pakistan indicate lower level of copper (0.14-0.21 mgL^{-1}) and nickel (0.08 mgL^{-1}) (Hussain et al., 2010). Comparative study on micro-elemental levels of raw and branded milk revealed relatively higher concentration of Cu in raw milk (0.159 mgL^{-1}) as compared to UHT processed (0.024 mgL^{-1}) whilst Ni level were higher in branded milk (0.149 mgL^{-1}).

Comparative correlation of current study with the foregoing results on heavy metal prevalence in milk samples of Karachi - an industrial city of Pakistan (Mumtaz, Perveen, & Usmani, 2005) revealed deleteriously higher levels of Cu (0.227 - 0.293 mgL^{-1}) in fresh and UHT processed milk. Concentration of Mn (0.044-0.085 mgL^{-1}) was quite lower than observed in fresh milk collected from Multan city however Mn contents of UHT processed brands were lower than the results reported from Karachi. Zinc concentration in processed milk was surprisingly higher than observed in raw milk depicting a positive change in qualitative milk selection process of industry. Contrarily, heavily adulterated street vended milk of the city represented a marked reduction in average zinc contents i.e. 2.155 mgL^{-1}. One study showed commercial milk brands of the country to contain zinc in a range of 2.96-7.31 mgL^{-1}. Bovine milk has already been reported to carry zinc in a range of 0.29 - 4.96 mgL^{-1} (Licata et al., 2004) however, modified and organic farming system deploying micro and macro-elements supplemented feeding has also been reported to improve the normal essential elements level (Malbe et al., 2010). It has been seen in the last couple of year that prominent processed milk brands in Pakistan have either started their own progressive farming system or had invested in private sector to promote dairy farming with an objective to produce nutritionally enriched and safe milk for consumption in Pakistan.

Another recent study (Tajkarimi et al., 2008) confirmed average lead contents up to 0.230 mgL^{-1} in 54 pasteurized bovine milk samples. Higher level of lead in milk have also been reported from cow and buffalo farms of Egypt depicting lead contents in a range of 0.044 to 1.081 and 0.040 to 0.96 in cow and buffalo milk, respectively. Similarly, cadmium levels in buffalo and cow milk were found to prevail at 0.118 and 0.086mg/Kg. Concentrations of both metals were relatively lower than those obtained in the present study suggesting a variation in the conditions for feeding patterns, milk collection and processing (Enb et al., 2009).

Variation in elemental concentration in milk has also

Table 4. Mean Concentration of Electrolytes in Raw and UHT Processed Milk Samples

Type of Milk	Element	Spectrometry Method	Mean (mg/ml)	Standard Deviation	Range (Min-Max) (mg/ml)	Number of Samples
UHT	Ca	FFM	1.7	0.57	1.0-2.5	15
	K	FFM	13.2	0.76	12.5-14.5	15
	Na	FFM	8.7	1.255	7.5-10.5	15
Raw	Ca	FFM	2.063	0.845	2.0-4.0	24
	K	FFM	6.562	2.493	5.0-12.0	24
	Na	FFM	2.938	0.443	4.0-5.0	24

been attributed to the process of high temperature degradation during ultrahigh temperature treatment in addition to heterogeneity of milk derived from different source i.e. bovine or ovine (Tajkarimi et al., 2008). Similarly higher electrolytic elemental concentration in processed milk might be associated with milk treatment practices like application of disodium and dipotassium phosphate for pH stabilization during processing. In addition, trends of fortification and supplementation have also been adopted by UHT milk manufacturers to augment microelement concentration in processed milk.

Milk fortification is practiced by some national and international milk manufacturers as visible from the iron contents of branded milk carrying a mean concentration of 1.66 mgL^{-1} far higher than yielded from raw milk i.e. 1.03 mgL^{-1}. Mean elemental concentration of electrolytes (Ca, K, Na) in farm milk samples has been recently reported as 9.4mg/g, 13.9mg/g and 2.80mg/ml respectively (Herwig et al., 2011). Present study features lower values for calcium and potassium in raw milk however; sodium contents were significantly compatible with the reported results. Contrarily, processed milk was found to carry almost similar concentration of potassium as reported from Germany (Herwig et al., 2011). The upshots of the present study suggested that raw milk offered for sale in the local market of the Multan city carries higher electrolytes balance as compared to the UHT processed milk of various local brands. It would not be out of place to claim that a major segment of population in Pakistan especially young children and elderly are not taking adequate amount of calcium from UHT processed milk.

Slight contamination of food stuff with heavy metals is the major precursor of chronic intoxication leading to several deadly disorders that have been invasively propagated in the communities either established in industrial hubs or those relying on the fresh food commodities furnished from the contaminated lands. Such intoxications are often reported in the form of necrosis of liver and kidney, reproductive failure and encephalopathies. Contamination of milk with residual higher levels of heavy metals might have forked linkage with certain anomalies like feed of milking animals, processing operations and environmental conditions specifically in case of openly vended raw milk. Critically, most significant among the aforementioned factors are green pastures, drinking water and industrial feed that are normal dietary requirement of the milking animals. Some most serious results declared from the current study are more or less linked to similar certainties.

The higher levels of heavy metals specifically lead and cadmium observed in raw milk samples procured from the street venders reflect an overall unhygienic milk selling environment with no control over milk adulteration in Pakistan. Furthermore, industrial waste, judicious use of pesticides and motor vehicles leaded smoke is the genuine inlet of lead intoxication in food stuff. Indirect accumulation of heavy metals in animal body through contaminated feed and direct contamination of street vended raw milk through metals laden smoke of motor vehicles are thought to be the factors that need to be addressed in the region with higher incidence of food heavy metals contamination.

4. Conclusion

Higher incidence of accidental and deleterious environmental pollution has impacted some profound drastic effects on our food resources. Findings from the current study validate impact of systemic environmental pollution on potential food sources and derived products. Variable micro-element concentration in raw milk derived from the animals reared in cotton belt region of Multan could potentially help the milk processing industry to segregate the raw material for their production qualitatively in addition to build same counter procedures in order to minimize the levels of toxicants in raw and processed milk. Study further suggests conducting a country wise milk safety survey to locate and identify the regions with risks of maximum toxicity from environmental pollutants. Animal feed and water should be analytically targeted to determine the residual levels of pesticides and industrial sludge metal's translocation. Water and animal feed is always at the risk of heavy metal pollution (Codex, 2003) and demands such type of surveys to comprehend milking animal feed trace metal levels to be well in the safe limits.

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