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Chemical Composition of Raw Milk and Heavy Metals Behavior During Processing of Milk Products

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Abstract: Whole buffalo's and cow's milk as well as dairy products manufactured from them were analyzed for chemical composition and metal contents. Buffalo's milk showed higher levels of fat (4.9%), total protein (3.6%), total solids (13.4%) and ash (0.76%) than cow's milk (3.2, 3.2, 12.1 and 0.65%, respectively). Also, higher levels were detected in pasteurized and sterilized buffalo's milk. However, lactose content in cow's milk was higher (5.0%) than buffalo's milk (4.8%). Most of gross composition was found at higher levels in cream, butter, samna (ghee) and yoghurt from buffalo's milk as compared with cow's milk. Metals, i.e. iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) were detected in buffalo's milk at concentrations of 0.88, 0.201, 0.072 and 4.35 mg/kg milk and were higher than in cow's milk (0.572, 0.131, 0.047 and 2.828 mg/kg, respectively). Also cow's milk contained chromium (Cr), nickel (Ni), cobalt (Co) and tin (Sn), at lower levels. Harmful metals such as lead (Pb) and cadmium (Cd) were detected in the collected samples at low levels. Metals contents were concentrated in dairy products manufactured from buffalo's milk at higher levels as comparing to cow's milk.

Key words: Chemical composition, Buffalo, Cow, Milk, Metals, Cream, Yoghurt, Samna, Butter, Dairy Products

INTRODUCTION

Milk is considered as a nearly complete food since it is a good source for protein, fat and major minerals. Also, milk and milk products are main constituents of the daily diet, especially for vulnerable groups such as infants, school age children and old age [1]. Several studies have reported the distribution and occurrence of the essential components in various animal milks [2,3]. The reported data show that the component contents of selected milks vary considerably and that their composition appears to be affected by genetics, physical and environmental factors.

Milk and milk products are the most diversified of the natural foodstuffs in terms of composition, contains more than twenty different trace elements. Most of them are essential and very important such as copper, zinc, manganese and iron [4-6]. These metals are co-factors in many enzymes and play an important role in many physiological functions of man and animals. Lack of these metals causes disturbances and pathological conditions [7,8]. The amount of metals in uncontaminated milk is admittedly minute, but their contents may be significantly altered through manufacturing and packaging process as well as metals that may be contaminate different cattle's feed and environment such as lead, cadmium, chromium, nickel and cobalt could be excreted into milk at various levels [3,9] and causing serious problems.

Toxicity of metal is closely related to age, sex, route of exposure, level of intake, solubility, metal oxidation state, retention percentage, duration of exposure, frequency of intake, absorption rate and mechanisms/efficiency of excretion [10,11].

Pollution of the environment with metals such as lead is world-wide problem. Lead alkyl additives in petrol are combusted and emitted into the atmosphere and can be responsible for high concentration of lead in some vegetation, roadside, soil, air, water and plants [12]. Manufacturing processes, incineration of refuse and combustion of coal, are also the other sources contribute to lead in the atmosphere; hence it is not surprising that lead levels are highest in area of intense industrialization [13,14]. Lead is toxic to the blood and the nervous, urinary, gastric and genital systems. Furthermore, it is also implicated in causing carcinogenesis, mutagenesis

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and teratogenesis in experimental animals [15,16]. Accumulation of lead produces damaging effects in the hematopoetical, hematic, renal, gastrointestinal systems [17]. On the other hand, cadmium is also easily volatilized at the operating temperatures of common industrial processes, much of the cadmium in the atmosphere results from incineration of ferrous scrap and metallurg processes [18]. Cadmium is considered to be one of the most toxic metals. In addition, it is implicated in high blood pressure [19], prostate cancer, mutations and foetal (embryonic) death [15]. Chromium, nickel and cobalt are also toxic metals at higher levels which released to the environment. They originated from dumping industrial wastes in the rivers, as well as the application of phosphatic fertilizers [10].

The composition of the mineral fraction of milk and milk products has been frequently considered, but only a few published investigations deal with minor and trace elements, despite their importance in nutrition or in food [20-22].

The aim of this investigation was to evaluate buffalo's and cow's milk from animal farms in Egypt based on chemical composition. Therefore the study assesses the concentrations of metals in both buffalo's and cow's milk as well as the effect of the manufacturing on their levels.

MATERIALS AND METHODS

Materials

Milk: One hundred and twenty samples of raw milk (1kg each) representing 60 samples of each buffaloes and cows were obtained at random from animal farms in Egypt. The samples were collected during the period from 2006 to 2007. Each sample was packaged in polyethylene bags and immediately freezed at -20ºC until analyzed.

Bacterial Strains: Pure strains belonging to *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were obtained from Egyptian Microbial Collection (EMC) at Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt.

Heavy Metal Standards: Standard solutions of heavy metals i.e., iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), cobalt (Co) and tin (Sn) were provided, in 1000 ppm solution for each one, by Merck (Merck, Darmastadt, Germany).

Treatments and Milk Products Manufacture: Buffalo's and cow's raw milk were exposed to each of the following treatments and manufactured to some products:

Heat Processing:

Pasteurization: Milk was heated to 73ºC for 15 sec. and cooled to 10ºC.

Sterilization: Milk was sterilized at 121º C for 5 min. in an autoclave.

Yoghurt: Milk was heated to 80-82ºC for 20 min. and cooled to 40ºC. Milk was incubated with 2% starter (1:1 mixture of *S. thermophilus* and *L. bulgaricus*) at 40ºC for 3 hr. as described by the Egyptian Organization for Standardization [23]

Cream: Raw milk was warmed to 45ºC and cream was obtained by using a mechanical separator.

Butter: Butter was obtained by warming the milk and separated by a mechanical separator to obtain cream and churned by using churner to obtain butter according to the method of Eckles *et al.* [24].

Samna (Ghee): Samna was prepared from cream according to the method described by El-Sadek *et al.* [25].

Methods of Analysis

Chemical Analysis: Fat, total nitrogen (T.N) and titratable acidity, lactose, total solids (T.S) and ash were determined according to AOAC method, [26]. The pH value was measured by using Knick-Digital pH meter model 646.

Metals Content: Metals were extracted from the milk and milk products according to AOAC method [26]. The metals were measured by Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer 2380). Maximum absorbance was obtained by adjusting the Cathode lamps at specific slit and wave lengths. Fe was measured at 248.3 nm, Cu at 324.8 nm, Mn at 279.5 nm, Zn at 319.9 nm, Pb (217.0 nm), Cd (228.8 nm), Cr (357.9 nm), Ni (232.0 nm), Co (240.7 nm) and Sn (286.3 nm). The limit of detection (sensitivity) was 10 µg/kg for Pb, Cd, Ni, Co and Sn as well as 1.0 µg/kg for the other metals. The recovery of metals was studied by adding known amounts of standard solution to samples. The amounts added were selected, so that they would be

close to the amounts naturally found in milk and milk products under investigation. The recoveries in different samples were 96-103% for Cu, Mn and Zn and were 91-98% for Pb, Cd, Cr, Ni, Co and Sn. All of the obtained results were corrected according to the recovery percentage.

All glassware, were washed, before use, with distilled water, soaked in nitric acid (30%), then rinsed in redistilled water and air dried. The glassware kept in clean place, to avoid contamination.

RESULTS

Chemical Composition of Buffalo's and Cow's Milk: Results of chemical composition of buffalo's and cow's milk samples which were collected from animal farms in Egypt were shown in Table 1. Fat, T.P., ash and T.S. content were in buffalo's milk (4.9, 3.6, 0.76 and 13.4%, respectively) than those detected with cow's milk (3.2, 3.2, 0.65 and 12.1%, respectively). However, lactose content was higher in cow's milk (5.0%) than in buffalo's milk (4.8%). Acidity and pH values were approximately equals in both species.

Table 1: Chemical composition (%) of buffalo's and cow's milk samples from different animal farms.

Item	Buffalo's milk (Mean±SD)	Cow's milk (Mean±SD)
Acidity	0.16±0.09	0.16±0.08
pH value	6.70±1.25	6.60±1.10
Fat	4.90±0.86	3.20±0.95
T.P	3.60±0.95	3.20±0.85
Ash	0.76±0.14	0.65±0.25
T.S	13.40±1.50	12.10±1.80
Lactose	4.80±0.55	5.00±0.60

The levels of metals in buffalo's and cow's milk are show in Table 2. All the detected metals were higher in buffalo's milk than that in cow's milk. Zinc was found at the highest levels in both buffalo's and cow's milk (4.366 and 3.146 mg/kg, respectively), followed by iron (0.980 and 0.682 mg/kg, respectively) and copper (0.212 and 0.142 mg/kg, respectively). On the other hand, the other metals (Pb, Cd, Cr, Ni, Co and Sn) were detected in both milk samples in lower values.The levels of essential metals (Zn, Fe, Cu and Mn) were higher in the collected samples than those of non-essential metals (Pb, Cd and Sn).

Table 2: Metals content (mg/kg) of buffalo's and cow's raw milk samples from different animal forms

		Buffalo's milk		Cow's milk	
Metals		Mean±SD	Range	Mean±SD	Range
Iron	(Fe)	0.980±0.442	0.786-1.242	0.682±0.406	0.607-0.794
Copper	(Cu)	0.212±0.102	0.188-0.542	0.142±0.116	0.108-0.194
Manganese	(Mn)	0.076±0.044	0.051-0.916	0.056±0.038	0.048-0.084
Zinc	(Zn)	4.366±0.814	3.966-6.814	3.146±1.081	3.001-3.940
Lead	(Pb)	0.084±0.042	0.044-1.088	0.066±0.056	0.040-0.960
Cadmium	(Cd)	0.118±0.086	0.094-0.142	0.086±0.062	0.070- 0.112
Chromium	(Cr)	0.042±0.022	0.036-0.058	0.034±0.014	0.028-0.066
Nickel	(Ni)	0.006±0.010	0.003-0.009	0.004±0.002	0.002- 0.009
Cobalt	(Co)	0.008±0.010	0.003 -0.014	0.004±0.001	0.003-0.006
Tin	(Sn)	0.006±0.010	0.004-0.010	0.003±0.006	0.002-0.005

Table 3: Chemical composition (%) of buffalo's milk and milk products manufactured from it

	Buffalo's milk products						
Item	Raw milk	Pasteurized milk	Sterilized milk	Cream	Butter	Samna	Yoghurt
Acidity	0.16	0.17	0.17	0.20	0.163	-	0.96
pH-Value	6.70	6.70	6.65	6.50	6.65	-	4.71
Fat	4.90	5.80	5.50	50.00	79.40	99.20	5.60
T.P	3.60	3.80	3.92	6.20	0.70	0.10	4.10
Ash	0.76	0.90	0.78	0.40	0.25	0.04	1.23
T.S	13.40	14.90	14.62	58.90	82.50	99.50	13.20
Lactose	4.80	4.30	4.20	2.00	-	-	3.20

Table 4: Chemical composition (%) of cow's milk and milk products manufactured from it

	Cow's milk products						
Item	Raw milk	Pasteurized milk	Sterilized milk	Cream	Butter	Samna	Yoghurt
Acidity	0.16	0.17	0.18	0.19	0.165	-	0.92
pH-Value	6.60	6.70	6.68	6.70	6.45	-	4.43
Fat	3.20	3.10	3.00	42.00	78.90	98.70	2.70
T.P	3.20	3.20	3.30	5.40	0.65	0.25	3.20
Ash	0.65	0.78	0.74	0.68	0.20	0.06	1.05
T.S	12.10	11.80	11.90	53.20	81.50	99.0	10.40
Lactose	5.00	4.80	4.70	4.20	-	-	3.60

Behavior of Chemical Composition of Milk During Manufacture of Milk Products: The same trend recorded for raw milk was observed with milk pasteurization in both buffalo's and cow's milk (Tables 3 and 4).

Regarding to sterilized milk, data in the same tables showed similar trend as previous detected with raw and pasteurized milk. However, acidity and pH value were lower in buffalo's than cow's milk.

Cream from buffalo's milk was higher in acidity, fat, T.P. and T.S. contents and was lower in lactose, ash contents and pH value than cow's cream. Butter from buffalo's cream also indicated higher levels of fat, T.P., ash, T.S. and pH values as compared to cow's cream.

Buffalo's samna contained higher levels of fat (99.2%) and T.S. (99.5%) than cow's samna (98.7% and 99.0%, respectively).Yoghurt from buffalo's milk contained all the gross composition at higher levels than that recorded from cow's milk except lactose content, which was lower.

The distribution patterns of various metals in buffalo's and cow's milk products was recorded in Tables 5 and 6.

In butter, levels of metals were concentrated by 5.6-7.7 folds as compared to buffalo's milk and 1.4-1.6 folds as compared to buffalo's cream. On the other hand, these levels were concentrated by 5.9-9.0 folds as cow's milk and 1.3-1.5 folds as cow's cream. Samna contained also concentrated metals in both buffalo's and cow's butter. The concentrated factors ranged from 6.7-9.2 folds than that in initial buffalo's milk, (1.6-1.8 folds) than that in buffalo's cream and 1.1-1.2 folds than that in butter from buffalo's cream. The concentrated factors of metals in cow's samna were 6.7-10.5, 1.6-2.3 and 1.2-1.5 folds than that in raw milk, cream and butter, respectively.

Concerning yoghurt manufactured from buffalo's and cow's milk, results in Tables (5 and 6) indicated that all studied metals were detected in it but at lower levels than that the levels in raw milk. The reduction levels were ranged from (5.0 to 15%) in yoghurt from buffalo's milk and from 4.0 to 17.0% in yoghurt from cow's milk for Fe, Cu, Mn, Zn, Pb, Cd and Cr. However the reduction of Ni, Co and Sn were ranged from 25.0 to 50% in yoghurt from buffalo's milk and 50 to 100% in yoghurt from cow's milk.

Table 5: Concentration of metals in buffalo's milk and milk products manufactured from it

		Metals concentrations in buffalo's dairy products (mg/kg)				
Metals		Milk	Cream	Butter	Samna	Yoghurt
Iron	(Fe)	0.880	4.520	6.780	8.136	0.766
Copper	(Cu)	0.201	0.922	1.472	1.572	0.185
Manganese	(Mn)	0.072	0.360	0.486	0.583	0.060
Zinc	(Zn)	4.350	19.570	29.363	35.200	4.059
Lead	(Pb)	0.062	0.230	0.345	0.414	0.060
Cadmium	(Cd)	0.106	0.451	0.676	0.811	0.090
Chromium	(Cr)	0.040	0.180	0.270	0.324	0.038
Nickel	(Ni)	0.003	0.013	0.020	0.024	0.002
Cobalt	(Co)	0.004	0.018	0.027	0.032	0.003
Tin	(Sn)	0.002	0.009	0.013	0.016	0.001

Table 6: Concentration of metals in cow's milk and milk products manufactured from it

		Metals concentrations in cow's dairy products (mg/kg)				
Metals		Milk	Cream	Butter	Samna	Yoghurt
Iron	(Fe)	0.572	2.938	4.407	5.288	0.498
Copper	(Cu)	0.131	0.609	0.907	1.372	0.120
Manganese	(Mn)	0.047	0.234	0.316	0.379	0.039
Zinc	(Zn)	2.828	12.721	19.086	22.880	2.638
Lead	(Pb)	0.040	0.149	0.224	0.269	0.039
Cadmium	(Cd)	0.068	0.293	0.439	0.527	0.059
Chromium	(Cr)	0.026	0.117	0.176	0.211	0.025
Nickel	(Ni)	0.002	0.010	0.013	0.016	0.001
Cobalt	(Co)	0.002	0.010	0.018	0.021	0.001
Tin	(Sn)	0.001	0.005	0.008	0.010	ND

ND = Not Detected.

DISCUSSIONS

The results of chemical composition of buffalo's and cow's milk in this study were in agreement with that reported by Dabiza *et al.* [27]. On the other hand, buffalo's milk samples in this study were found to contain fat, lactose and T.P. at lower values than that found by Castagnetti *et al.* [28], who found fat, lactose and protein at higher levels (8.54; 5.12 and 4.5%, respectively). Also, fat and T.S in buffalo's milk in this study were lower than that detected by Kholif *et al.* [2] and Abou-Arab, [29], who reported that fat and T.P. contents were (6.0 and 5.9%, respectively) and (15.23 and 14.98%, respectively). In contrast, T.P in the present study was higher than that detected by Kholif *et al.* [2] (3.23% in buffalo's and 3.02% in cow's milk) and Abou-Arab [29] (3.41 in buffalo's milk) studies.

The results under investigation coincide with the results of El-Shabrawy and Hagrass [30], who showed that cow's milk contained lower levels of Fe, Cu and Zn, than that of buffalo's milk.

Comparing our results with the accepted upper limits [31], iron was detected at mean levels higher than the legally accepted limit (0.5 mg/kg). Also, mean value of lead was below the general statutory limit of 1 mg/kg set by The Lead in Food Regulations [32] as mentioned, but above the proposed European maximum limit of 20 µg/kg [33]. Cadmium as a toxic metal was detected at higher level than the permissible limit (0.03 mg/kg). Mean concentrations of heavy metals were comparable to the International Dairy Federation [34], limits of Fe, Cu, Mn, Zn, Pb and Cd were found to exceed in buffalo's milk. However Cu and Mn were found to exceed in cow's milk. The recorded limits of IDF [34] were 0.37, 0.10, 0.025, 3.28, 0.049 and 0.026 mg/kg milk for Fe, Cu, Mn, Zn, Pb and Cd, respectively.

Mean concentration of metals were compared with mean levels of metals detected in raw milk samples of Abou-Arab [22] which were collected from Cairo during the period of 1988 to 1990. The present study revealed that metals concentrations in our samples were below the earlier research. The author reported that Zn, Fe, Cu, Mn, Pb and Cd were 4.25, 0.95, 0.22, 0.06, 0.05 and 0.348 mg/kg, respectively. Results also revealed that, levels of metals under study were higher than levels recorded by Koops *et al.* [35]; Debeka and Mckenzie [36]. High levels of heavy metals in this study may be attributed to the high contamination of animal feed and water by such pollutants and could be excreted into milk at various levels [3,9] and also may be reached to milk through handling procedures. In this respect, several studies have been carried out to assess metal contents of milk from different areas [37, 38]. The first author reported that Zn, Cu, Pb and Cd were detected in cow's and buffalo's milk samples at levels ranged between 3.177-3.697 mg/l, 0.043-0.195 mg/l, 1.70-3.35 µg/l and 0.07-0.10 µg/l, respectively. However, the second author determined Zn, Cu, Fe, Cr, Pb, Cd, Co and Ni at levels ranged from 0.39-2.75, 0.07-0.67, 1.68 to 15.1, 0.005-0.030, 0.03-0.18, 0.004-0.009, 0.03-0.12 and 0.04-0.09 mg/l. Regarding to the investigation of Lante *et al.* [39], they reported that Zn (4.631 mg/l), Cu (0.0518 mg/l), Fe (0.290 mg/l), Mn (0.0291 mg/l), Cr (0.004 mg/l), Pb (5.23 µg/l) and Cd (0.40 µg/l) were detected at various levels. On the other hand, Caggiano *et al.* [40] reported that, Mn, Cr, Pb and Cd were detected at mean levels of 0.13, 0.20, 0.20 and 0.06 mg/kg cow's milk, respectively. Besides, the determination of lead in raw milk samples collected from milk tankers was recorded by Tajkarimi *et al.* [41] as 7.9 ng/l, with a range from 1-46 ng/ml.

Gross composition of raw milk (buffaloes and cows) was investigated as mentioned previously (Table1). The same trend for the gross composition of raw milk in buffaloes and cows milk was observed with milk pasteurization in species as was reported by El-Etriby *et al.* [42].

Regarding to sterilized milk, data in the same tables showed similar trend as previously detected with raw and pasteurized milk. However, acidity and pH value were lower in buffalo's than cow's milk. Results in this research for components of sterilized cow's milk were almost took the same trend which was reported by Mohammed *et al*. [43].

Based on the present findings, cream from buffalo's milk was higher in acidity, fat, T.P. and T.S. contents. While it was lower in lactose, ash contents and pH value than that in cow's cream. These results of butter from buffalo's cream were in agreement with that reported by Ismail *et al.* [44]. On the other hand, the results for yoghurt from buffalo's milk revealed that it contained all the gross composition in levels higher than that found for that from cow's milk except lactose content. These results were in accordance with that reported by El-Shibiny and El-Dein [45] and Ibrahim *et al.* [46] for buffalo's yoghurt, while for cow's yoghurt the present results were in agreement with that reported by Mehanna and Gonc [47] and Mehanna and Hefnawy [48].

Results for the distribution patterns of various metals in buffalo's and cow's milk indicated that buffalo's and cow's cream contained the same concentrations factors

for Fe, Cu, Mn, Zn, Pb and Cr, which contained 5.1, 4.6, 5.0, 4.5, 3.7, 4.3 and 4.5 folds, respectively, than that in raw milk. Similar results were obtained by Abou-Arab [22] who reported that, metals concentrated in cream.

Regarding to concentration of metals, the present results proved that metals were concentrated in butter and samna with levels more than that of buffalo's and cow's milk and cream. Similar results reported by Abou-Arab [22] who detected trace levels of metals in skim milk, butter milk and murta, this means that metals concentrated in cream, butter and samna.

Concerning yoghurt manufactured from buffalo's and cow's milk, results indicated that all studied metals were detected in the products, but at lower levels than that the levels in buffalo's and cow's raw milk. The higher levels of reduction due to the presence of these metals at lower concentrations and any small loss will rise the percentage of the reduction. Based on these findings, the reduction of metals may be due to the manufacture process and the fermentation by starter used.

It could be concluded that buffalo's milks (raw, pasteurized and sterilized) contained higher levels of fat, total protein, ash and total solids than cow's milks. In contrast, lactose content was detected at higher levels in cow's milk than that reported with buffalo's milks. Most of the gross composition was higher in cream, butter, samna and yoghurt from buffalo's milk than from cow's milk. Metals content concentrated in dairy products from buffalo's milk at higher levels than cow's milk.

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