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Evaluation of the Factors Influencing the Content and Retention of Selected Heavy Metals in Milk and Some Dairy Products

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ABSTRACT

The concentration and retention of some heavy metals in milk and milk products are closely dependent upon manufacturing process, ripening and storage. The present study deals with the assessment of selected trace elements such as Cd, Pb, Cu, Fe and Zn in milk as well as in fresh soft cheese (Domiati cheese and UF cheese) hard cheese, Yoghurt and fatty products (crem, butter and ghee). The evaluation of the influence of manufacturing process on the concentration ranges of trace elements in milk products were carried out using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). The results obtained showed that the retention% of heavy metals in the experimented cheese types (Domiati, UF and Ras cheese) showed some variation. The higher retention was obtained in UF cheese in Pb, Cd, Cu, and Zn, while lowest retention was obtained in fresh Ras cheese and also decreased during storage. The highest retention% was observed in Fe (12.76%) at the end of storage than other heavy metals in Ras cheese. The reduction levels obtained at the end of storage period were (17.04, 11.11, 10.6 and 15.8%) in yoghurt for Fe, Cu, Zn and Cr, respectively. While the reduction of Cd was 50% in yoghurt. Concerning Pb level in yoghurt no reduction was observed than that the level in raw milk. Further concentration of milk fat to butter and ghee decrease the retention% levels of all heavy metals than the retention% in cream out of starting amount in milk.

Key words: Heavy metals contamination, manufacturing process, ICP

INTRODUCTION

Milk products are a very important human nutrient since their consumption has increased in recent years.

The "purity" of milk affects the other dairy product which drives from it (Florea *et al*., 2006). There are correlation between environmental situation, manufacturing process and equipment and levels in raw milk and dairy products. The manufacturing process considerably influences element concentration both by chemical and physical treatments involved and by processing equipment employed that bring about the greatest variation in the element concentration (Coni *et al*., 1995).

The rationalization of production process and identification of quality markers for milk and milk products is of a great importance for the protection and evaluation of typical dairy products. Consequently, as safeguard, the first objective is to undertake a careful and through assessment of all mechanisms by manufacturing process can influence milk and milk products.

The concentration range of certain heavy metals in milk and cheese are dependent upon animal species and feeding time of year of sample collection, environmental condition and manufacturing process (Coni *et al*., 1996).

A previous investigation (El-Sadek *et al*., 1972) centerd on raw milk samples to elevate the concentration ranges of trace elements as affecting by environmental conditions and time year of 72 samples collection.

The main objectives of this study was to elucidate the behavior of certain milk contaminants heavy metals (Pb, Cd, Fe. Cu, Zn and Cr) during manufacturing process for cheese, yoghurt and fatty products compared with corresponding raw milk.

MATERIALS AND METHODS

Milk used in cheese making, Yogurt, cream, butter and Ghee: Fresh milk used in cheese making, Yogurt, cream, butter and Ghee were obtained from the herd of Faculty of Agriculture, Cairo University, Giza, Egypt.

Starter culture: Yoghurt culture was obtained from Chr. Hansen Lab., Copenhagen, Denmark (Yo- mix 495).

Rennet: Powder rennet was obtained from DSM Food specialties company, France.

Salt: Commercial sodium chloride was obtained from El-Nasr Company, Alexandria, Egypt.

Heavy metals Standards (Pb, Cd, Fe, Cu, Zn and Cr): were obtained from Sigma-Aldrich Company.

Analysis of heavy metals: Heavy metals level (Pb, Cd, Fe, Cu, Zn and Cr) in milk and milk products samples were analyzed as the following:

All reagents were of analytical reagent grade unless otherwise stated. Double-deionized water was used for all dilutions. HNO_3 and H_2O_2 were of super pure quality. Samples (0.5 g) were digested with 7 mL of HNO_3 (65%) and 1 mL of H_2O_2 (30%) in Advanced Microwave Digestion for 31 min and diluted to 100 mL with deionized water. A blank digest was carried out in the same way (digestion conditions for microwave system were: 2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, 8 min for 550 W, vent: 8 min, respectively). This procedure was preferred because of its higher accuracy with respect to both time and recovery values. The recovery values were nearly quantitative (>95%) for the above-mentioned digestion method.

Analysis of trace elements in milk samples was by an Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) using iCAP 6000 Series; Thermo Scientific (Germany) was used for the analysis of the studied trace elements in milk and milk products samples.

Cheese processing

Fresh domiati cheese: Domiati cheese is very popular amongst Egyptians who consume it either fresh or ripened by pickling after 3 months of storage. It is considered as cheese with a very high salt compared to other soft types (up to 10%) where salt is added directly to the milk before renneting. Its composition according to the Egyptian standards should not exceed 60% for moisture content and 9% for salt content. A minimum limit of 40% is prescript for Fat/Dry Mutter (FDM).

Additionally, the cheese should meet the Egyptian standards for absence of pathogens and harmful additives. Domiati cheese was manufactured according to Fahmi and Sharara (1950).

Fresh UF cheese: Ultrafiltration is a membrane separation process which is carried out under pressure using a semi-permeable membrane made out of a high polymer substance or cellulose acetate. The milk constituents that pass through the membrane are referred to as permeate and the materials that do not pass through the membrane are known as retentate. Thus in theory, the application of Ultrafiltration in the cheese industry resembles the traditional concept of cheese making in which the milk fat and proteins are concentrated by means of whey separation.

Ras cheese: Ras cheese is the main Egyptian hard cheese variety manufactured on the industrial scale. It is very similar to the Greeck "kefalotyri" cheese.

Ras cheese was manufactured according to the method of Hofi *et al.* (1970) was adopted for the manufacture of Ras cheese.

Yoghurt processing: Yoghurt was manufactured according to the method described by the Egyptian Organization for Standardization (EOS, 1993).

Fatty products processing: Cream prepared by warming the milk (7.1% fat) to 45°C and separating in conventional separator. Cream was churned after cooling using churner to obtain butter according to Eckles *et al.*(1951). Then Ghee was prepared from butter according to the method described by El-Sadek *et al.* (1972).

Statistical analysis: The two-way analysis of variance (ANOVA) and factor factorial were performed by running the MSTAT-C package (ver. 2.10, Michigan state university, USA) on a personal computer. The same program was used to analyze factorial analysis of variance completely randomized design. The statistical significance of the data determined using $p \leq 0.05$ (Snedecor and Cochran, 1976).

All experiments were carried out in triplicates and each analysis in duplicates and the mean values were tabulated.

RESULTS AND DISCUSSION

Cheese: The concentration ranges of certain trace elements in cheese are dependent upon milk and manufacturing process. The evaluation of the influence of manufacturing process on the concentration ranges of trace elements in cheese production were carried out by ICP. Data obtained in this work refer to three complete cycles of cheese production.

Heavy metals residues in domiati cheese: Table 1 showed the changes and distribution of heavy metals between cheese and whey. Most of metals as Pb, Fe, Cu, Zn and Cr have been concentrated (0.112, 5.341, 0.775, 1.535 and 0.305 mg kg^{-1}, respectively) in the cheese than in whey (0.096, 2.88, 0.598, 1.05 and 0.179 mg kg^{-1}). While Cd showed another pathway during Domiati cheese manufacture it concentrated in the whey (0.004 mg kg^{-1}) than cheese (0.002 mg kg^{-1}).

The retention of heavy metals in Domiati cheese showed some variations, more favorable retention results were obtained for Fe (29.8%) and Cr (29.7%) than for Zn, Cu and Pb

Table 1: Heavy metals residues and retention% in milk and related dairy product (Domiati cheese)

	Residue (mg kg^{-1})					
	Toxic metals			Essential metals		
Product	Pb	Cd	Fe	Cu	Zn	Cr
Raw milk	0.099±0.04	0.004±0.000	3.587±0.270	0.676±0.076	1.323±0.844	0.205±0.004
Cheese	0.112±0.011	0.002±0.000	5.341±1.794	0.775±0.080	1.535±0.357	0.305±0.400
Whey	0.096±0.000	0.004±0.000	2.876±0.313	0.598±0.107	1.054±1.142	0.179±0.007
Retention%						
Cheese	22.6	10	29.8	22.9	23.2	29.7

Values are as Mean±SD. LSD value, Cd: 0.001, Fe: 2.230, Cu: 0.198, Zn: 0.590, Cr: 0.103. Pb:0.017; SD: Standard deviation of the mean

Table 2: Concentration of heavy metals residues (mg kg^{-1}) and retention % in milk and related dairy product (UF cheese).

	Reside (mg kg^{-1})					
	Toxic metals			Essential metals		
Product	Pb	Cd	Fe	Cu	Zn	Cr
Raw milk	0.099±0.04	0.004±0.000	3.587±0.270	0.676±0.076	1.323±0.844	0.205±0.004
Retentate	0.105±0.032	0.004±0.004	3.247±0.309	0.710±0.132	1.580±1.069	0.166±0.025
Permeate	0.079±0.012	0.006±0.002	3.025±0.029	0.698±0.265	1.261±1.480	0.191±0.032
Cheese	0.088±0.005	0.002±0.000	2.895±0.321	0.732±0.053	1.401±0.997	0.167±0.011
Retention%						
Cheese	23	11.9	19.2	25.7	25.6	19.3

Values are as Mean±SD. LSD: Cd: 0.001, Fe :0.534, Cu: 0.042, Zn: 0.381, Cr: 0.112. Pb: 0.029, SD: Standard deviation of the mean

(23.2, 22.9, 22.6 %), respectively . Very low retention for Cd was obtained in Domiati cheese (Table 1). This may be ascribed to the fact that the elements of the former group are preferentially bound to caseins and fat and consequently shift mostly to the curds. Also the salting process causes the loss of these elements for osmosis (Fresno *et al*., 1995).

These results were in agreement with results reported by Yuzbasi *et al*. (2003) who found in a survey that the concentration of Pb, Cd, Fe, Cu and Zn in Kasar cheese samples were (86.0, 1.8 μg kg^{-1}, 4.2 mg kg^{-1}, 0.7 mg kg^{-1} and 37.7 μg kg^{-1}), respectively.

These results were higher in Zn and Fe levels than Kebary *et al*. (1992) who reported that the concentration of Zn, Fe and Cu in Domiati cheese were (0.38, 0.08 and 1.00 mg kg^{-1}), respectively. The results were lower than that of Mohammed (2009) who found that the heavy metals concentration of Domiati cheese samples was (0.014, 1.42, 38.59, 0.4 and 26.45 mg kg^{-1}) for Cd, Cu, Fe, Pb and Zn, respectively.

Heavy metals residues in UF cheese: Table 2 indicated that not all trace elements have the same permeability, since Pb, Cu, Fe and Zn were concentrated in retentate (UFR), while Cd and Cr displayed highest concentration in permeate (UFP). Cadmium concentrations did not vary greatly between the different final products suggesting that this compound was more closely

associated with the soluble fraction and probably equally distributed between caseins and components of low molecular mass (Anastasio *et al*., 2006).

Also, the results indicated that UFR retention% of Pb, Fe, Cu and Zn were considerably higher (25.3,21.525 and 28.4%), than the UFP retention% (19.03, 20.1, 24.5 and 22.75%) of starting amount of milk of Pb, Fe, Cu and Zn, respectively. While the UFR retention of Cd and Cr were lower (23.8 and 19.2%), than the UFP retention% (35.7 and 22.1), respectively.

Further more the UF cheese retained about (23, 11.9, 19.2 25.7, 25.6 and 19.3%) of starting amount of milk of Pb, Cd, Fe, Cu, Zn and Cr, respectively.

These results tend to show, that the use of UF-technique in manufacture of dairy products can led to reduction of contaminants such as Cd, Fe and Cr but Cu, Pb and Zn were not effectively removed by the membrane used in the experiments.

These results agree with the data reported by Fayed *et al*. (1995) who reported that the permeability of Fe, Cu, Zn or Pb was very low; as at CF 3 the concentrate contained about 91, 53, 91 or 63%1, respectively, of these metals. While the final reduction level in Fe, Cu, Zn or Ph was 13, 63, 16 or 50%, respectively by The 2x diafiltration. Also, the results agree with the data of Fukuwatari *et al*. (1982) who found that Fe, Zn, Cu and Mn elements in milk were concentrated in the retentate. On the other hand, Green *et al*. (1984) claimed that the retention of zinc, iron or copper depends on the proportion bound to casein micelles and that the amounts relative to casein usually decrease with increase in CF.

It is not- worthy to mention the strong pumping to which milk is exposed during the UF-process in addition to the elevated temperature used (50°C) may be a cause for reduction in fat globule size and damage in fat globule membrane (Green *et al*., 1984).

Heavy metals residues in Ras cheese: Table 3 showed the changes of heavy metals residues during Ras cheese ripening period.

All heavy metals residues in fresh Ras cheese decreased gradually till the end of storage period. Also it noticeable that the retention% of Cd at the end of storage period was not detected (nd). Cd was bounded to the Casein and lipids and released from the curd as consequences of acidification that causes the demineralization of casein micelles (Coni *et al*., 1996). While Cr, Pb, Cu and Zn

Table 3: Heavy metals residues and retention % in milk and related dairy product (Ras cheese) during ripening

	Reside (mg kg^{-1})					
	Toxic metals			Essential metals		
Product	Pb	Cd	Fe	Cu	Zn	Cr
Milk	0.114±0.110	0.004±0.001	3.330±0.659	0.480±0.121	1.321±0.231	0.192±0.14
Ras cheese						
Fresh	0.122±0.021	0.002±0.002	5.521±1.524	0.551±0.221	1.125±0.357	0.325±0.321
1 month	0.101±0.002	ND	4.52±0.121	0.480±0.005	1.006±0.002	0.160±0.011
3 months	0.08±0.002	ND	4.25±0.221	0.310±0.001	0.83±0.003	0.140±0.001
Retention%						
Ras cheese before ripening	10.7	5	16.58	11.48	8.5	16.9
Ras cheese after ripening	7.018	ND	12.76	6.46	6.28	7.29

Values are as Mean±SD. LSD value: Pb: 0.038, Cd: 0.001, Fe: 1.034, Cu: 0.256, Zn: 0.279, Cr: 0.132. SD: Standard deviation of the mean. ND: Means below the limit of detection of ICP

retention were (7.29, 7.018, 6.46 and 6.28%), respectively. The highest retention% was observed in Fe (12.76%) at the end of storage than other heavy metals in Ras cheese.

These results agree with El-Kenany *et al.* (2001) who recorded that the Cd concentration in cheese decreased during storage to 0.55 ppm after 45 days and till the end of storage season. And the chromium concentration decreased gradually during storage and reduced in cheese to 64% at the end of storage period and copper reduced by 28.6% and Iron 26.9%.

The results were markedly lower than the results reported by Mohammed (2009) who found that the heavy metals concentration of fresh Ras cheese samples was (0.011, 0.082, 25.15, 1.313 and 19.58 mg kg^{-1}) of Cd, Cr, Cu, Fe, Pb and Zn, respectively.

And also these results were lower than the results reported by Coni *et al.* (1995) who found that Cd, Cr, Cu, Fe, Pb and Zn content of cheese curd before molding were (0.0565, 0.458, 10.15, μg kg^{-1} 7.17, 0.0822 and 41.17 mg kg^{-1}), respectively.

The retention% of heavy metals in the experimented cheese types (Domiati, UF and Ras cheese) showed some variation. The higher retention was obtained in UF cheese in Pb, Cd, Cu and Zn, while lowest retention was obtained in fresh Ras cheese and also decreased during storage.

It also observed that the highest retention was found for Fe and Cr, in Domiati cheese and Ras cheese, while in UF cheese the highest retention was found for Cu and Zn. whereas, Cd showed the lowest retention in all cheeses.

Thus it can be concluded that, about 70-80% reduction in heavy metals concentration in cheese were found depending on the type of cheese. Also, it is obvious that the hard cheese displayed the highest reduction of heavy metals risk and increases the safety of these products.

The results showed different patterns of heavy metal contents in milk and cheese samples. The chemical and physical properties of the manufacturing process may thus influence the metal concentrations.

Heavy metals residues in set yoghurt: Table 4 indicated that metals were detected in low levels than that in raw milk and also it was observed that the reduction of most of the heavy metals was occurred in the first 5 days of storage. However, by prolonging the storage period to 10 days, there was a slight decrease in Fe, Cu and Cr levels.

The reduction levels obtained at the end of storage period were (17.04, 11.11, 10.6 and 15.8%) in yoghurt for Fe, Cu, Zn and Cr, respectively. While the reduction of Cd was 50% in yoghurt. Concerning Pb level in yoghurt no reduction was observed than that the level in raw milk.

Table 4: Heavy metals residues and retention % in milk and related dairy product (Yoghurt) during storage

	Reside (mg kg^{-1})					
	Toxic metals			Essential metals		
Product	Pb	Cd	Fe	Cu	Zn	Cr
Milk	0.04±0.001	0.004±000	5.4±0.002	0.9±0.002	1.79±0.002	0.19±0.001
Yoghurt						
5 days	0.04±0.001	0.002±0.00	4.58±0.002	0.9±0.001	1.60±0.002	0.17±0.001
10 days	0.04±0.001	0.002±0.001	4.48±0.004	0.8±0.00	1.60± 0.003	0.16±0.001
Reduction%						
At the end of storage period	00	50	17.1	11.11	10.6	15.8

Values are as Mean±SD. LSD value: Cd: 0.003, Fe: 1.211, Cu: 0.0.02, Zn: 0.201, Cr: 0.040, Pb: 0.031. SD: standard deviation of the mean

Table 5: Heavy metals residues and retention % in milk and related dairy product (fatty product)

	Residues (mg kg^{-1})					
	Toxic metals			Essential metals		
Product	Pb	Cd	Fe	Cu	Zn	Cr
Milk	0.07±0.0002	0.002±000	5.47±0.0017	0.72±0.0006	0.89±0.0001	0.44±0.0006
Cream	0.11±0.0003	0.004±000	6.73±0.0019	1.26±0.0006	1.12±0.0001	0.46±0.0007
Butter	0.07±0.0001	0.002±0.0001	4.56±0.002	0.4±0.0005	0.47±0.0001	0.44±0.001
Ghee	0.06±0.003	ND	4.45±0.002	0.3±0.0003	0.35±0.0001	0.41±0.001
Retention%						
Cream	13.09	16.66	10.25	14.58	10.4	8.71
Butter	7.3	7.4	6.14	4.09	3.89	7.38
Ghee	4.22	ND	4	2.05	1.93	4.58

Values are as Mean±SD. LSD value: Pb: 0.051, Cd: 0.021, Fe: 2.421, Cu: 0.931, Zn: 1.231, Cr: 0.0521. ND: below the limit of detection of ICP; SD: standard deviation of the mean

The higher levels of reduction might be due to the presence of these metals at lower concentration and any small loss will rise the percentage of the reduction. Based on these findings, the reduction of metals may be due to manufacture process and fermentation by starter used.

Compared with the values described in the literature for heavy metals in yoghurt, the reduction percentage levels found in this study were higher values than Enb *et al.* (2009) who reported that the reduction% were (12.95, 8.96, 6.69, 3.23, 15.09 and 5.04%) yoghurt from buffaloes milk for Fe, Cu, Zn, Pb, Cd and Cr, respectively. And also lower reduction% than that described by Bordajandi *et al.* (2004) who reported that the reduction levels of Cu, Zn, Cd and Pb being (79.7, 22.4, 100 and 37.0%), respectively.

Heavy metals residues levels in cream, butter and Ghee: The distribution patterns of heavy metals in raw milk, cream, butter and ghee were recorded in Table 5. Small amount of heavy metals are distributed between cream and separated milk in proportion to the curd nitrogen but on completely centrifuging the fat a higher concentration of metals occurs in the cream, showing adsorption of complex proteinate at the fat globule surface.

The results showed that the levels of heavy metals in cream increased than in initial raw milk, and also the results showed that the concentration factors ranged from 1.2-2 fold in cream levels than in initial raw milk.

In general the concentrations obtained in this study for milk, cream, butter and ghee samples analyzed showed Fe as the element presenting the highest levels, followed by Zn, Cu, Cr, Pb and Cd. The lowest values in milk, Cream, butter and ghee, respectively.

The retention of heavy metals in cream, butter and ghee analyzed showed some variation as affected by manufacturing process. Concerning with cream, the highest retention% was observed in Cu followed by Cd, Pb and Cr being 14.58, 16.7, 13.1 and 8.71%, respectively of starting amount of milk, while Zn and Fe showed the lowest retention.

It obvious that further concentration of milk fat to butter and ghee decrease the retention% levels of all heavy metals than the retention% in cream out of starting amount in milk. Cr, Pb, Cd, and Fe showed the highest retention% (7.38, 7.3, 7.4 and 6.14, respectively, while Cu, Zn displayed the lowest retention% (3.89 and 4.09) in butter.

Furthermore, ghee showed the lowest levels of heavy metals in fatty dairy products, the retention% ranged between (n.d-4.%).

From an overall point of view, it could be concluded that the highest reduction of heavy metals were observed in ghee than butter and cream.

The mode of combination and distribution of trace heavy metals in dairy products. by adsorption of heavy metals on fat- globule surface, which holds alike in water in oil as well as oil in water emulsion, that heavy metals associated with the portion nitrogen of butter milk (Davies, 1933).

Similar results were obtained by Enb *et al.* (2009) who detected the concentration factors of Fe, Cu, Mn, Zn, Pb and Cr in buffalos and cows cream 5.1, 4.6, 5.0, 4.5, 3.7, 4.3 and 4.5 fold, respectively that in raw milk.

Though the different studies made evident, that the applying of some good agricultural practices can lead to the elimination of important fluctuation of concentration of Zinc, Copper and iron ions, which were found in identical reports in the raw material and in the finished product (Huszti *et al.*, 2006).

CONCLUSION

This work provides important information on safety and quality standards of milk and milk products and constitutes a noticeable step forward for sound and effective action in the safeguard of food safety and consequently, human health.

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