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# Distribution of Contaminated Heavy Metals in Milk

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Milk and most of the dairy products are also important parts of human diet. These products are likely to be exposed to heavy metals contamination. The present study determined distribution of added copper, iron and zinc to bovine milk. In different ratio of copper, iron and zinc solution was added to bovine milk and incubated. Milk samples were fractionated into fat and skim milk by centrifugation. Then, casein was separated by acid precipitation at pH 4.5 followed by centrifugation. Copper, iron and zinc concentrations in fat, casein and whey fractions were determined by atomic absorption spectrometry. Most of the copper, iron and zinc were recovered in the skim milk, average in the all of dose 76.38, 84.44 and 85.07 %, respectively whereas only 23.62, 15,56, 14.93 % of the these metals were associated to the lipid fraction. While copper, iron and zinc addition increases in milk, ratio of copper were bound casein and whey, ratio of iron were bound casein and ratio of zinc were bound to the whey increases.

**Key Words: Copper, Iron, Zinc, Heavy metals.**

## INTRODUCTION

Milk and dairy products are the fundamental food for people. They are composed of specific proteins, fats designed to be easily digested, minerals, vitamins and other components that may have an important role. Although milk and most of the dairy products are also important parts of human diet, these products are likely to be exposed to heavy metals contamination[1-3].

The toxic metal content of milk and dairy products is due to several factors-in particular-environmental conditions [from heavy metals natural presence in the environment, as consequence of agricultural practices (*e.g.*, application of fertilizers), human activities (*e.g.*, pollution from car exhausts, mining) or as a result of industrial emissions], the manufacturing process and the possible contamination during several steps of the manufacturing processes[4-8].

Certain ions, sodium, potassium and chloride exist largely in the ionized state in the aqueous compartment of milk. The other ionic species, such as $Ca^{2+}$, $Mg^{2+}$, $Zn^{2+}$, $Cu^{2+}$, $Fe^{3+}$, $CO_3^{2-}$, $PO_4^{3-}$ and citrate ions, are distributed among the structural compartments and proteins components in a highly specific manner[9,10].

Metals can bind to functional groups of proteins, such as imidazole, sulphydryl, carboxyl, amino and peptide groups. Two possible molecular mechanisms for enzyme-related metal toxicity have been identified: (a) the toxic metal displaces a benefical metal from the active site of the enzyme; (b) the toxic metal binds to a deactivating site on the molecule[11]. Many factors effect the association of cations with milk proteins, especially casein that the major class of protein in most milk species. Factors include the nature and concentration of the added ion (previously, attention was mainly paid to the effect of calcium) and the composition of the milk serum (ionic composition, pH, presence or absence of whey proteins)[12].

The aim of this work is to detect the distribution of some heavy metals in milk and their variation during the transition from raw materials to the end products.

## EXPERIMENTAL

Fresh cow milk was supplied from Kirgülü Dairy Products Ltd. in Van. Copper, iron and zinc solutions were provided by Accustandard (USA).

**Determination of copper, iron and zinc:** Copper, iron and zinc were determined by atomic absorption spectrometry (ATI Unicam-929). The determinations were carried out at 324.8, 248.3 and 213.9 nm for copper, iron and zinc, respectively. Standard copper, iron and zinc solutions were freshly prepared from a 1000 ppm stock solution and a linear calibration curve was used. The quantify these metals, samples were solubilized by dry ashing 550 °C in a furnace[13].

**Distribution of copper, iron and zinc among fat, casein and whey from bovine milk:** 0.1, 0.2, 0.4, 0.6, 0.8 ppm of copper and iron and 1.74, 3.5, 4, 4.5, 5 ppm of zinc solution was added to 50 mL of bovine milk and incubated at 4 °C for 24 h. After incubation, milk samples were fractionated into fat and skim milk by centrifugation at 2000 g at 4 °C for 24 h for 20 min. Then, casein was separated by acid precipitation at pH 4.5 followed by centrifugation at 4000 g for 20 min. To eliminate the whey remaining in the casein fraction, the pellet was washed with distilled water and centifuged at 4000 g for 20 min. Copper, iron and zinc concentrations in fat, casein and whey fractions were determined by atomic absorption spectrometry (ATI Unicam-929). The amount of these metals in water obtained after washing the casein was also measured and added to those obtained for whey.

## RESULTS AND DISCUSSION

Results obtained from the study of copper, iron and zinc distribution in bovine milk are shown in Table-1. Most of the copper, iron and zinc were recovered in the skim milk, average in the all of dose 76.38, 84.44, 85.07 %, respectively whereas only 23.62, 15,56, 14.93 % of the these metals were associated to the lipid fraction.

**Distribution of copper in bovine milk:** Copper is strongly bound to organic matter (particularly albumin, metallothionein, transcuprein as with proteins and low -molecular-weight ligands)[14-16].

TABLE-1
DISTRIBUTION OF COPPER, IRON AND ZINC IN BOVINE MILK FRACTIONS

| | Dose (ppm) | Cu (%) | Fe (%) | Dose (ppm) | Zn (%) |
|---|---|---|---|---|---|
| Skimmed milk | Control | 77.46 | 84.34 | Control | 82.4 |
| | 0.1 | 68.17 | 81.66 | 1.74 | 82.4 |
| | 0.2 | 67.17 | 82.64 | 3.5 | 86.37 |
| | 0.4 | 77.15 | 78.83 | 4 | 87.32 |
| | 0.6 | 83.39 | 91.51 | 4.5 | 85.67 |
| | 0.8 | 84.95 | 87.68 | 5 | 86.27 |
| | Average | 76.38 | 84.44 | Average | 85.07 |
| Fat | Control | 22.54 | 15.66 | Control | 17.61 |
| | 0.1 | 31.83 | 18.34 | 1.74 | 17.61 |
| | 0.2 | 32.82 | 17.36 | 3.5 | 13.63 |
| | 0.4 | 22.85 | 21.17 | 4 | 12.68 |
| | 0.6 | 16.61 | 8.49 | 4.5 | 14.33 |
| | 0.8 | 15.05 | 12.32 | 5 | 13.73 |
| | Average | 23.61 | 15.55 | Average | 14.93 |
| Acid precipitation | | | | | |
| Casein | Control | 64.16 | 57.98 | Control | 42.29 |
| | 0.1 | 67.66 | 68.45 | 1.74 | 43.86 |
| | 0.2 | 47.61 | 75.58 | 3.5 | 48.22 |
| | 0.4 | 50.05 | 71.04 | 4 | 37.19 |
| | 0.6 | 48.34 | 73.84 | 4.5 | 38.90 |
| | 0.8 | 49.09 | 80.09 | 5 | 32.90 |
| | Average | 54.48 | 71.16 | Average | 40.56 |
| Whey | Control | 35.84 | 42.02 | Control | 57.71 |
| | 0.1 | 32.34 | 31.55 | 1.74 | 56.14 |
| | 0.2 | 52.39 | 24.42 | 3.5 | 51.78 |
| | 0.4 | 49.95 | 28.96 | 4 | 62.81 |
| | 0.6 | 51.66 | 26.16 | 4.5 | 61.10 |
| | 0.8 | 50.91 | 19.91 | 5 | 67.10 |
| | Average | 45.52 | 28.84 | Average | 59.44 |

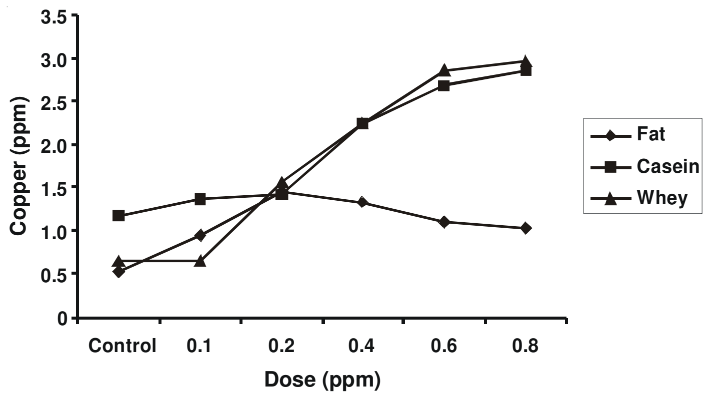


Fig. 1. Distribution of copper among fat, casein and whey from bovine milk

The concentration of Cu in all fractions increased with increasing 0.2 ppm level of Cu addition. When copper was added to milk in the concentration range of 0.4, 0.6 and 0.8 ppm, more of copper were bound to the casein and whey fractions. King and Williams[17] also reported that the casein and whey fractions were about equal in copper concentration/unit of protein. Copper is mainly distributed between the cationic and the 'casein-bound' fraction, being probably associated with some enzymes or other proteins[18].

**Distribution of iron in bovine milk:** Table-1 indicates the percentages of iron in the skimmed milk, fat, casein and whey. The percentages of iron in casein were higher than in fat and whey. Thus, when iron was added to milk in the concentration range of 0.1, 0.2, 0.4, 0.6, 0.8 ppm more of iron were bound to the casein fraction. About 84.44 % of the iron is bound to skim milk while 15.55 % is bound to fat. In skim milk, 60.17 % of the iron is bound to casein, 24.26 % is in the whey. Therefore Gaucheron[19] reported that about 24 % of the iron is bound to casein while 29 % is bound to whey proteins and 32 % is associated with a low-molecular-weight fraction. In skim milk, 50-65 % of the iron is bound to casein, 18-33 % is in the whey proteins fraction and 15-33 % is in the non-protein fraction. Early researchers[20] orginally concluded that the binding of added or natural iron was nonionic and that iron would bind to fat globule membranes. However, King *et al.*[21] reported that although most of the natural iron in milk was bound to the fat globule, added iron was not. Later studies[22,23] found that iron was bound mostly to milk proteins, particularly phosphorylated caseins. The strength of iron binding to caseinophosphopeptides (CPP) is about 100 times greater than that of calcium and others cations[24]. Hekmat and Mcmahon[22] have reported that the relative affinity of $Fe^{3+}$ to milk proteins is, $\alpha_{s1}$-casein > β-casein > bovine serum albumin > κ-casein > β-lactoglobulin > α-lactoalbumin. In general, those milk proteins that contain more phosphoryl serine groups have the greatest iron-binding affinity; although carboxyl groups (of Asp and Glu) can also bind iron. For the caseins (including $\alpha_{s2}$-casein) both the phosphoryl serines and carboxyl groups can bind iron. In contrast, the whey proteins (bovine serum albumin, β-lactoglobulin and α-lactoalbumin) are not phosphorylated and only have a low affinity for iron.

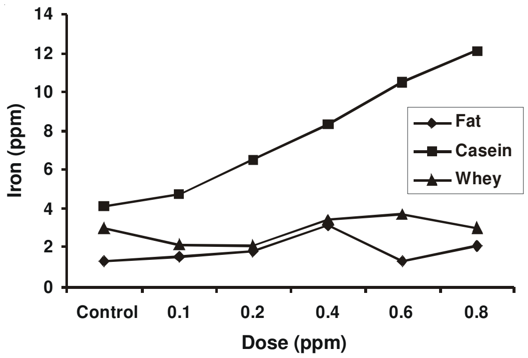


Fig. 2. Distribution of iron among fat, casein and whey from bovine milk

It can be seen from Fig. 2 while ratio of the iron added to milk increases, ratio of iron were bound to the casein fraction increases but in contrast ratio of iron were bound to the whey decreases.

**Distribution of zinc in bovine milk:** Table-1 indicates that most of the zinc (85 %) is found in skimmed milk while about 15 % of the zinc is in the fat.

It can be seen from Fig. 3 and Table-1, while zinc addition increases in milk, ratio of zinc were bound to the whey increases. Addition of 3.5 ppm of zinc resulted in a decrease in zinc in the casein fraction and an increase in the whey. Zinc distribution in the milks studied was similar to that described in previous reports[25]. Miquel and Fare[26] also reported that zinc is absorbed more efficiently from aqueous solitions than solid meals in food matrix.

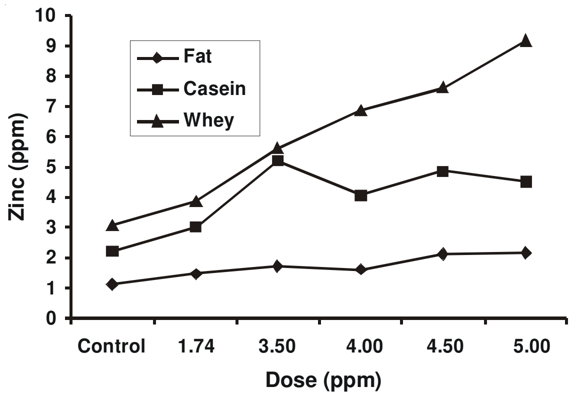


Fig. 3. Distribution of zinc among fat, casein and whey from bovine milk

Zinc in cow's milk is primarily bound to casein (≈ 90-95 %) and only small amounts are linked to whey proteins and low molecular weight ligands in serum[27]. When the pH was lowered, there were changes in zinc distribution. Zinc moved from casein into the whey, probably due to changes in casein conformation, decreasing its capacity to bind zinc[25]. The solubility of zinc decreased as the pH increased[28].

Singh *et al.*[29] documented that non-micellar zinc levels increased when the pH of milk was reduced before ultracentrifugation so that *ca.* 95 % of the zinc was non-sedimentable at pH 4.6. Within the casein micelles, one-third of the zinc is loosely bound to casein phosphoserine residues, whereas two-thirds are more tightly bound to colloidal calcium phosphate. Most of the zinc directly bound to casein is readily removed when pH decrease to low values.

## Conclusion

The results of this study showed that while ratio of the iron and zinc added to milk increases, ratio of iron were bound to the casein fraction increases and ratio of zinc were bound to the whey increases. Therefore more of copper were bound to the casein and whey fractions. Physical parameters (concentration and pH) affected of copper, iron and zinc ions on the casein and whey proteins. Metals contaminated to milk are preferentially removed from the casein micelles or curd during cheese manufacturing

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