

Effects of Natural Chelating Agents on the Solubility of Some Physiologically Important Mineral Elements in Oat Bran and Oat Flakes

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ABSTRACT

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The solubility of mineral elements from oat bran and flake samples was studied by a method using equilibrium dialysis after enzymatic digestion of starch and proteins. The effects of six potential chelating agents common in food were tested on the solubility of mineral elements. The minerals studied were calcium, magnesium, iron, manganese, zinc and potassium, and the chelating agents were citric, lactic, malic, and ascorbic acids, glucose and xylitol. The mineral elements were tightly bound to the dietary fiber of the samples. Bran fiber bound even the zinc and calcium contributed through the enzymes used. Adding citric, malic, or lactic acids increased

the solubility of the mineral elements studied, except for potassium which was easily dialyzable as such. Iron was insoluble in all situations. Citric acid was the most efficient chelating agent in solubilizing the mineral elements. The effect of malic and lactic acids on the solubility of minerals was small. No effect was found with glucose, ascorbic acid, and xylitol. Thus, the intestinal availability of mineral elements may be affected by dietary hydroxy acids such as citric and malic acids in high dietary fiber diets.

Intestinal absorption on mineral elements may be thought of consisting of two main phases: passive diffusion from the digestive chyme to the gut surface and often active absorption through the intestinal epithelium. The extent of passive diffusion is mainly determined by physicochemical factors: solubility product, ion exchange capacity of food macromolecules, mass action, and chelate formation. The chyme is an extremely complex matrix in which the physicochemical conditions change continuously with diet. Consequently, simplified models may give only tentative indication of the real factors affecting the bioavailability of nutrients.

Dietary fibers are known to bind with nutritionally significant minerals. Components such as cellulose, hemicellulose, pectins, other polysaccharides, and lignin may form insoluble complexes with mineral elements and thus reduce bioavailability of minerals (Rendleman 1982, Hallberg et al 1986, Persson et al 1987, Idouraine et al 1996). Earlier studies reported that the availability of Ca, Fe, and Zn from cereal foods is very poor (Camire and Clydesdale 1982, Rendleman 1982, Rendleman and Grobe 1982, Maha Lakshmi and Sumathi 1997) and that the affinity of dietary fiber for different mineral elements varies (Idouraine et al 1996, Maha Lakshmi and Sumathi 1997). Rendleman and Grobe (1982) found that the affinity of wheat bran for Zn is about three times greater than that for Ca. Also, the ability of different cereals to bind minerals varies (Anglani 1998). It is proposed that fiber has specific sites for binding Ca or the sites might have greater affinity for Ca than other minerals (Idouraine et al 1996). Pectin binds to Zn strongly, and binding capacity of lignin for Fe and Ca is high (Rendleman 1982, Torre et al 1991). Also, the different composition of fiber components and associated compounds such as phytic and oxalic acid may cause the specific binding capacity (Nair et al 1987, Idouraine et al 1996). However, natural chelating agents may play a key role in the solubilization of food minerals and trace elements (Miller et al 1981, Hallberg et al 1986, Reinhold et al 1986, Hazell and Johnson, 1987).

The mineral binding capacity of dietary fiber has been studied mainly using isolated and carefully purified fiber fractions and components (Nair et al 1987, Persson et al 1991, Idouraine et al 1996). In the present study, we used oat bran and oat flake samples without prior isolation of fiber or fiber components. Our goal in this study was to clarify the effects of six potential chelating agents common in foods on the solubility of K, Ca, Mg, Fe, Zn, and Mn in fiber-rich foods after enzymatic treatments of the sample.

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MATERIALS AND METHODS

The oat bran was a commercial product of Melia Oy (Raisio Group, Nokia, Finland). All the bran samples used were from the same batch. The oat flake samples were purchased from eight food stores in the Helsinki area and pooled on an equal weight basis to make one sample for analysis. The contents of mineral elements of the samples were determined by flame atomic absorption spectrometry (AAS) (Perkin Elmer 5100, Norwalk, CT) using an air-acetylene flame. The minerals studied were Ca, Mg, Fe, Mn, Zn, and K. For K, Mg, Mn, and Zn determinations, wet-digestion with 10 mL of concentrated HNO₃ was used; for Ca and Fe, the samples were dry-digested. Lanthane solution (0.09%) was used as an ionization buffer in Ca, K, and Mg determinations. Fat, dietary fiber, and protein content in the samples used were as specified by the manufacturers (Table I).

The samples were treated according to the method of Asp et al (1983) for dietary fiber with some modifications. In this method (Fig. 1), the chelating agents were added to the samples, after which the starch and protein of the samples were digested enzymatically. The samples were then dialyzed and the soluble minerals were analyzed from the dialyzate. Insoluble minerals bound by fiber components of the samples remained in the dialysis residue.

Samples were ground (Cyclotec 1093, Tecator AB, Höganäs, Sweden) to a particle size <0.5 mm. Ground sample (5 g) was weighed into a 100-mL flask. Purified water (50 mL, MilliQ, Millipore Corporation, Bedford, MA) and chelating agent were added. An exception was ascorbic acid which was added after the enzymatic procedure to avoid its destruction during the heat treatment of the samples for starch hydrolysis. The chelating agents were

TABLE I
Mineral Element, Dietary Fiber, Protein, and Fat Content of Samples

| | Oat Bran | Oat Flakes |
|-------------------------------|------------|------------|
| Mineral elements ^a | | |
| Ca | 81 ± 0.8 | 50 ± 4 |
| Fe | 7.5 ± 1.0 | 5.5 ± 0.2 |
| K | 630 ± 12.0 | 397 ± 1 |
| Mg | 231 ± 8.3 | 154 ± 1 |
| Mn | 5.5 ± 1.9 | 5.3 ± 0.4 |
| Zn | 4.6 ± 0.2 | 3.9 ± 0.1 |
| Dietary fiber ^{b,c} | 17 | 11 ± 0.4 |
| Protein ^{b,c} | 17 | 14 ± 0.7 |
| Fat ^{b,c} | 7.5 | 7 ± 0.3 |

^a Values are mg/100 g, mean ± standard deviation.

^b Values are g/100 g, mean ± standard deviation.

^c Based on manufacturer's specifications.

citric, malic, lactic, and ascorbic acid, glucose, and xylitol. The concentrations used were 0, 0.5, 1.0, 1.5, and 3.0% of the amount of bran or flakes except ascorbic acid, which was added at 0, 20, 40, 60, and 100 mg corresponding to the amount of ascorbic acid in 0–5 oranges. The mixture of sample and chelating agent was kept in a boiling water bath with horizontal agitation for 15 min to gelatinize the starch. After adding 0.5 mL of Termamyl 300 L (Novo Nordisk A/S, Bagsvaerd, Denmark), the mixture was further incubated in a boiling water bath for 1 hr, 45 min. The presence of any unhydrolyzed starch was checked with I₂/KI-solution, and an additional 0.15 mL of the enzyme was added if necessary. Thus, the amount of Termamyl was kept as low as possible to minimize contamination with mineral elements originating from the enzyme. The pH of the reaction mixture was adjusted to 1.5 with 1M HCl. After addition of 0.5 g of Pepsine (0.7 FIP-U/mg, EC 3.4.23.1, Merck, Darmstadt, Germany), the mixture was incubated at 40°C for 60 min. The pH was adjusted to 6.8 with 1M NaOH, and 0.5 g of Pancreatin (8 X U.S.P. Sigma Chemical Co., St. Louis, MO) was added and the sample was incubated for an additional 60 min at 40°C. The samples were dialyzed (MW cutoff 3,500) against 600 mL of purified water overnight at room temperature. The concentrations of mineral elements in the dialyzate were determined by flame atomic absorption spectrometry (Perkin Elmer 5100, Norwalk, CT) as described previously for oat bran and flakes. For K, Mg, Mn, and Zn determinations, 25 mL of the dialyzate was digested; for Ca and Fe, 50 mL

of the dialyzate was digested. All treatments of the samples were made in triplicate and the determinations of mineral elements were made in duplicate.

The contents of mineral elements in enzymes were determined as described above. The high mineral content of the enzymes used created a problem in the processing of the data. Therefore, the amounts of dialyzable minerals (background level) of all enzymes used were determined from the same amount of enzymes and chelating agent as in the determination of the oat sample itself but without any substrate. The mineral elements of oat bran and flakes were considered to be soluble when their concentration in the dialyzate exceeded the background level. Thus, the reported results describe the solubility of the minerals native in the samples. Negative values (Figs. 2–5) indicate the amount of mineral element retained by the dietary fiber from enzymes used in the procedure.

The accuracy and the precision of the method was tested using NBS 1567a wheat flour reference material (National Bureau of Standards, Gaithersburg, MD) (Table II) and one unofficial homemade reference solution containing known amounts of K, Mg, Mn, and Zn (Table III). Data were tested statistically using analysis of variance (ANOVA).

RESULTS AND DISCUSSION

This study was a part of a project dealing with the dietary fiber composition of oats. To investigate the mineral binding capacity of fiber constituents, oat bran and oat flakes were chosen as sample materials to represent two kinds of commercial milled products with vastly different mineral contents. The mineral element concentration of oat bran is much higher than that of oat flakes, the former being mainly used in animal feeds, while the latter is the main form of oats in human diets. The types of samples were restricted to these two to keep the procedure simple and give adequate information of the overall interrelation of minerals and fiber.

Solubility of Minerals from Oat Bran and Flakes Without Chelating Agent

In the method used in this work, all other components of the sample except the dietary fiber were digested. Thus, dialyzable minerals originated not only from fiber components but also from proteins, some amino acids, and other compounds present in oat bran or oat flakes. They were considered to represent the available fraction of mineral elements of the samples.

The studied mineral elements, except potassium, were tightly bound to oat bran (Figs. 2–5, 0% added chelating agent). This may mean that they are also poorly available in physiological conditions. Monovalent potassium was easily (≈80%) dialyzable without any addition of chelating agent. Divalent cations Mn and Mg were only ≈5 and 20% dialyzable, respectively. The concentrations of Ca and

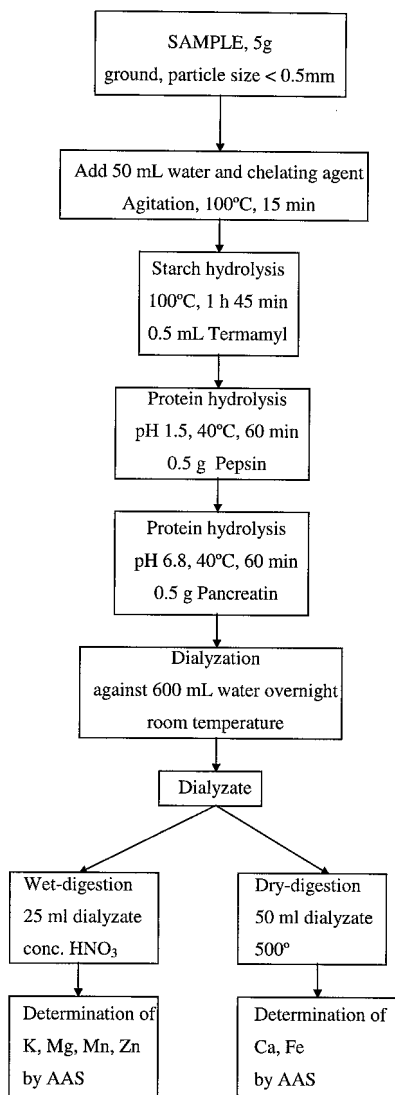


Fig. 1. Main steps in digestion of samples and determination of mineral elements.

TABLE II
Analysis of NBS 1567a Wheat Flour Reference Material

| Element | N | Present Study | Certified Value |
|---------|----|----------------------|----------------------|
| Ca | 30 | 0.0197 ± 0.0021% w/w | 0.0191 ± 0.0004% w/w |
| Fe | 33 | 15.1 ± 2.1 g/kg | 14.1 ± 0.5 g/kg |
| K | 5 | 0.131 ± 0.001% w/w | 0.133 ± 0.003% w/w |
| Mg | 5 | 0.034 ± 0.002 g/kg | 0.040 ± 0.002 g/kg |
| Mn | 5 | 9.1 ± 0.2 µg/kg | 9.4 ± 0.9 µg/kg |
| P | 11 | 0.111 ± 0.014% w/w | 0.134 ± 0.006% w/w |
| Zn | 5 | 11.3 ± 0.4 µg/kg | 11.6 ± 0.4 µg/kg |

TABLE III
Analysis of a Noncertified Control

| Element | N | Analyzed | Weighted Amount |
|---------|----|-----------------|-----------------|
| K | 21 | 39.4 ± 0.9 mg/L | 40 mg/L |
| Mg | 20 | 10.1 ± 0.2 mg/L | 10 mg/L |
| Mn | 20 | 98.8 ± 4.6 µg/L | 100 µg/L |
| Zn | 20 | 203 ± 13 µg/L | 200 µg/L |

Zn in the dialyate were below the background level (Figs. 2–5). This suggests that soluble Zn and Ca originating from the enzymes used were bound to the oat bran. The concentration of iron was almost zero in the dialyates, which indicates that all iron of the oat bran sample was in the insoluble form and that the bran fiber did not bind extra iron originating from enzymes.

Comparison of oat bran and oat flakes showed that the mineral binding capacity was greater for bran. All the studied mineral elements were clearly more soluble in the oat flake sample (Figs. 6–7, 0% added chelating agent) than in the bran sample (Figs. 2–5). The solubility of potassium in flakes was >99%, and that of Mn and Mg was ≈10 and 40%, respectively. These values are twice as high as those from oat bran. In oat flakes, Ca and Zn were poorly soluble, but their solubility was still above the background level, unlike the oat bran sample.

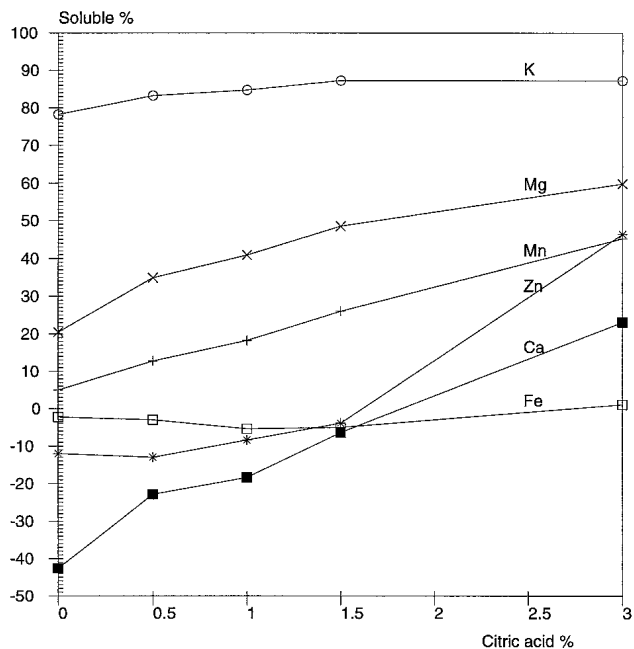


Fig. 2. Effects of citric acid on solubility of mineral elements in oat bran.

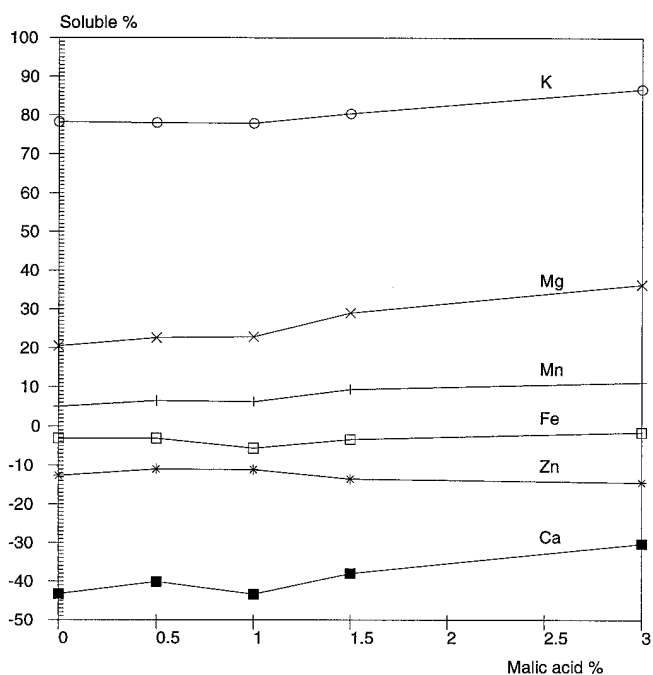


Fig. 3. Effects of malic acid on solubility of mineral elements in oat bran.

The dietary fiber of oats seemed to bind minerals strongly. The higher binding capacity of the oat bran sample compared with that of the oat flakes is probably due to the higher dietary fiber content of the bran (Table I). The different binding capacity of bran and flour has been found in other cereals (Sandström 1987, Maha Lakshimi and Sumathi 1997). Also, the selective capacity of dietary fiber to bind Ca, Fe, and Zn was reported earlier (Rendleman 1982, Rendleman and Grobe 1982, Claye et al 1996, Idouraine et al 1996, Maha Lakshimi and Sumathi 1997). Thus, our findings are in good agreement with the earlier results. In our studies, the solubility of Mn and Mg was also poor. Therefore, the availability of those elements may be very poor from fiber-rich cereal foods. The only soluble element was potassium, and a high fiber content of diet does not appear to affect its availability.

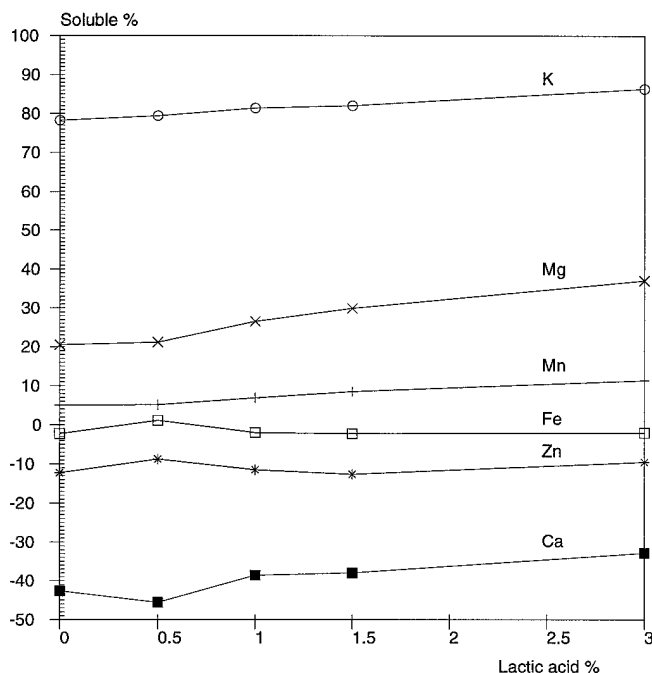


Fig. 4. Effects of lactic acid on solubility of mineral elements in oat bran.

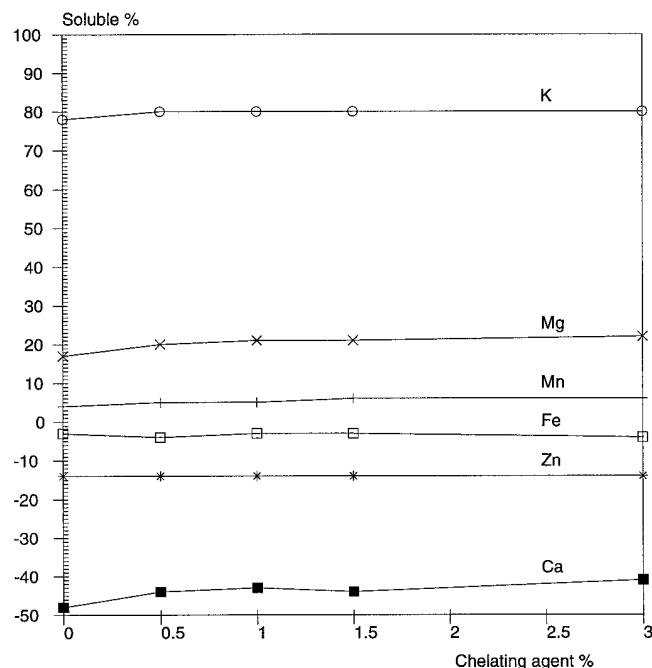


Fig. 5. Combined effects of ascorbic acid, glucose, and xylitol on solubility of mineral elements in oat bran sample.

Solubility of Minerals from Oat Bran and Oat Flakes with Chelating Agent

In the present study, the addition of a chelating agent generally increased the solubility of the elements in both oat samples (Figs 2–7). The solubility of minerals increased with the concentration of the chelating agent. Iron and potassium were exceptions. Only minor effects on the solubility of iron and no effects on the solubility of potassium were found with the chelating agents tested in this study.

Citric acid was the most efficient chelating agent. The solubilities of Ca, Zn, Mg, and Mn were increased significantly ($P = 0.003$) when citric acid was added to the bran or flake sample. When the highest amount (3%) of citric acid used in this study was added to the oat bran sample, the solubility of Mg and Mn increased 20–60% and 5–45%, respectively (Fig. 2). An increase in the solubility of Ca and Zn was also seen with all concentrations of citric acid, but the solubility exceeded the background level only with the highest concentration. The effect was very poor on iron in the oat bran sample. The concentration of Fe in the dialyzate only slightly exceeded the background level when 3% of citric acid was used. Citric acid had an effect on the solubilities of studied mineral elements of oat flakes (Fig. 6) similar to that of oat bran.

Malic and lactic acid increased the solubility of minerals significantly, although less than citric acid (Figs 3, 4, and 7). Malic acid significantly increased ($P = 0.0015$) the solubility of Mg (20–35% in oat bran and 40–50% in oat flakes) and also affected the solubilities of Mn and Ca ($P = 0.0206$) in oat bran sample (Fig. 3). The same trend was seen with the solubilities of Mn and Ca in the oat flake sample (Fig. 7). The influence of lactic acid on the solubilities of mineral elements is shown in Fig. 4. The differences in solubility were significant ($P = 0.0025$) for Mg and Mn in the oat bran sample, but lactic acid did not affect the other minerals. The other compounds studied as potential chelating agents were glucose, xylitol, and ascorbic acid. Figure 5 combines the results obtained with these compounds and shows that they had no effects on the solubility of any mineral elements determined.

The carboxylic group as a structural element in citric, lactic, and malic acids seems to be the most important part of the molecule which may affect the solubility of mineral elements. The carboxylic group is able to form more or less stable complex compounds with most of the metal ions and thus could release the mineral elements

from dietary fiber. The hydroxyl groups in the structure of glucose, xylitol, and ascorbic acid do not have this tendency for complex formation with metal ions. The present results show that they have no effect on the solubility of mineral elements in oat bran and oat flakes.

The stability constants ($\log K$) of citrate, malate, and lactate complexes measured in water solutions with ionic strength $I = 0.1$ are given in Table IV. These constants for complexes with mineral elements studied in this work show that citric acid can form complexes with Fe(+III), Fe(+II), Zn, Mn, Ca and Mg. The increase in solubility of the mineral elements in this study was mainly in line with the stability constant values of citric acid. The exception was Fe whose solubility did not increase.

The stability constant values of complexes of malic and lactic acids are lower than those of citric acid with the minerals studied. In the present study, malic and lactic acids had only moderate effects on the solubility of the elements studied (Figs. 3, 4, and 7). However, the effect was greatest on Mg ($P = 0.002$ with malic acid, $P = 0.005$ with lactic acid). The stability constant values do not support this result. It is clear that the effect of chelating agents on the solubility of mineral elements in dietary fiber is not explainable by stability constant values of complexes alone. The stability constant values presented in Table IV are measured in water solutions with low ionic strength. They are highly dependent on the conditions and are thus useful only in evaluation of the ability to form complexes.

None of the studied chelating agents increased the solubility of iron in oat bran or flakes, or had only a moderate effect on it; all Fe remained insoluble (Figs. 2–7). According to the stability constant values, citric, malic, and ascorbic acids should have affected the solubility of Fe because they form the strongest chelates with it in pure solutions (Table IV). To confirm the unexpected result, the Fe contents were determined from dialysis residues and all of the iron was recovered. This result indicates that the fiber may bind iron so strongly that it is not released by any of the studied chelating agents.

According to the results of earlier studies, several organic acids can affect the solubility of iron. Even quite dilute ascorbic acid solution can release Fe completely from whole sorghum flour, bran, maize fiber, and neutral detergent fiber samples (Reinhold et al 1986, Maha Lakshmi and Sumathi 1997). However, ascorbic acid had no effect or only a minor effect on Fe retention in saline solutions or

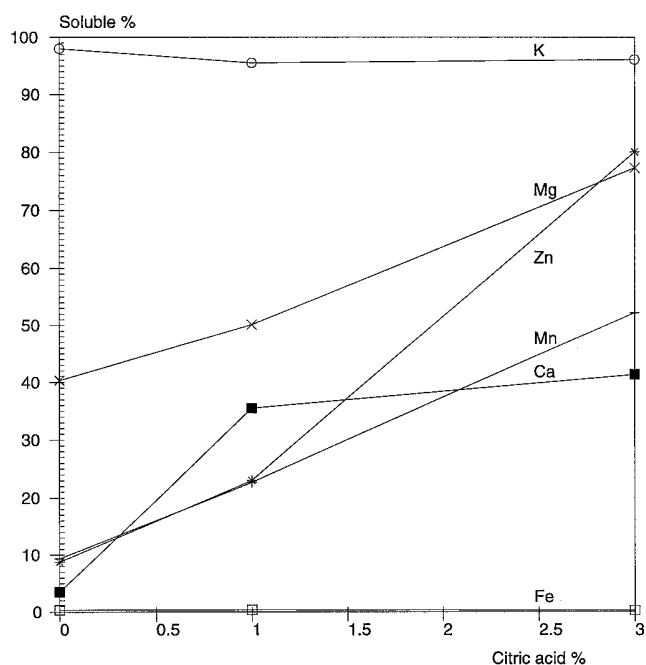


Fig. 6. Effects of citric acid on solubility of mineral elements in oat flakes.

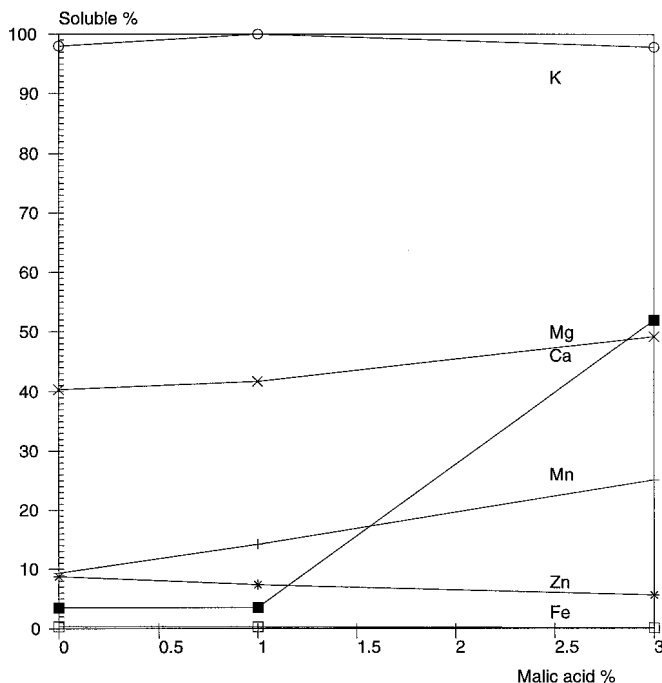


Fig. 7. Effects of malic acid on solubility of mineral elements in oat flakes.

TABLE IV
Stability Constants of Used Chelating Agents^a

| | K | Ca | Mg | Zn | Mn | Fe ²⁺ | Fe ³⁺ |
|---------------|-----------------|-----|-----|------------------|------------------|------------------|------------------|
| Citric acid | 0.6 | 3.5 | 3.4 | 4.7 | 3.8 | 4.8 | 11.2 |
| Lactic acid | na ^b | 1.1 | 0.9 | 1.8 | 1.2 | na | na |
| Malic acid | 0.2 | 1.9 | 1.7 | 2.9 ^c | 2.2 | 2.6 ^c | 7.1 ^c |
| Ascorbic acid | na | 0.2 | na | 1.0 ^d | 1.1 ^d | 0.2 ^e | 6.3 |

^a According to National Institute of Standards and Technology (NIST) Critically Selected Stability Constants of Metal Complexes, Vers. 4.0, 1997 ($t = 25^{\circ}\text{C}$, $I = 0.1$).

^b Not available.

^c $t = 20^{\circ}\text{C}$, $I = 0.1$.

^d $t = 25^{\circ}\text{C}$, $I = 0.0$.

^e $t = 25^{\circ}\text{C}$, $I = 3.0$.

solubility from diet samples (van Dyck et al 1996). Ascorbic acid also prevents the fiber-Fe binding formation when it was added to a mixture of Fe-solution and bran (Miller et al 1981, Camire and Clydesdale 1982). Fruit juices, ascorbic, citric, and malic acids increased Fe diffusibility from wheat flour. In whole wheat flour, the effect was slower (Hazell and Johnson 1987). In this study, however, no effects of organic acids on the solubility of Fe were seen.

CONCLUSIONS

It is evident that in human diets the amount and availability of mineral elements is highly dependent on dietary choices. Sugar, fats, and other refined products are dietary diluting agents with respect to minerals and trace elements. Diets composed of a varied range of non- or low-fractioned foods in a balanced way contain adequate amounts of both mineral elements and natural chelating agents and no fortification is needed. In animal production, where fortification is a common practice, the bioavailability of minerals may be improved by the use of proper chelators.

According to the results of this study, the solubility of mineral elements from high dietary fiber oat samples was very poor. The higher fiber content of the oat bran sample bound the mineral elements tighter than the oat flake sample with a lower fiber content. The solubility of mineral elements can be increased with chelating agents. The carboxylic groups seemed to be the most important part of the molecule in forming soluble complex compounds. The hydroxyl group alone did not seem to affect the solubility as strongly. It may be possible to increase the availability of mineral elements when foodstuffs containing citric acid, for instance fruit products, are eaten together with high dietary fiber oat foods. Supplementing animal feeds with citric acid may also increase the absorption of minerals because of its ability to increase the solubility of some mineral elements.

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