

# *In vitro* Extractability of Calcium, Iron, and Zinc in Finger Millet and Kidney Beans During Processing

P.R.S. MAMIRO, J. VAN CAMP, S.M. MWIKYA, A. HUYGHEBAERT

**ABSTRACT:** Finger millet (*Eleusine coracana*) and kidney beans (*Phaseolus vulgaris*) were processed by soaking, germination, autoclaving, and fermentation for incorporation into a complementary food for children. Extractability of calcium, iron, and zinc were determined by *in vitro* HCl-Pepsin and Pepsin-Pancreatin methods after each processing step. Germination significantly increased the *in vitro* extractability of these minerals, while soaking, autoclaving and fermentation showed a smaller or insignificant effect. Iron extractability was low in germinated, autoclaved and fermented millet, as determined by the pepsin-pancreatin method, but increased 6.8 times with addition of vitamin C. Phytic acid was reduced by 85 and 66% in finger millet and kidney beans, respectively, during the overall processing. These results show that various processing methods, especially germination, increase mineral extractability. Addition of vitamin C and mango could be used to enhance mineral extractabilities, thereby helping to alleviate micronutrient deficiencies in populations subsisting on these foods.

**Keywords:** mineral, extractability, millet, beans, pepsin

## Introduction

A NUMBER OF INORGANIC MINERALS are important either in the structure or functioning of the body and must therefore be provided by the various components that make up the diet. Cereals and legumes individually or as composite, are extensively used to prepare complementary foods in developing countries (Uwaegbute 1991; Kingamkono and others 1995; Towo and Tatala 1998). Minerals from plant sources, particularly those from plant seeds, are less bio-accessible than those from animal sources due, in part to phytic acid, tannins, and fiber present in the plants (Moeljopawiro and others 1988; WHO 1998). These anti-nutritional factors chelate dietary minerals in the gastrointestinal tract reducing their bio-accessibility and bioavailability (Frolich 1995). Processing techniques such as soaking, germination, and fermentation have been found to reduce significantly the levels of phytates and tannins by exogenous and endogenous enzymes formed during processing (Mosha and Svanberg 1990; Lorri and Svanberg 1995; WHO 1998). Honke and others. (1998) found that germination, which is often used to prepare legumes for consumption, causes considerable degradation of inositol phosphate (IP<sub>6</sub>), and hence increases the bio-accessibility of trace elements. A study by Sripriya and others. (1997) revealed that germination was more effective in increasing the ex-

tractability of trace elements like copper, zinc, and manganese while fermentation was more effective in increasing the extractabilities of calcium, phosphorus, and iron from finger millet. Kazanas and Fields (1981) did not find any significant changes in total ash content of sorghum due to fermentation. However a study by Chompreeda and Fields (1984) found that specific minerals such as phosphorus and iron were more extractable from corn meal after fermentation. Food processing and preparation may therefore influence both the content and form of the anti-nutritional factors thus in turn modulating mineral bioavailability (Svanberg and Sandberg 1988; Frolich 1995).

Ascorbic acid has been shown to enhance iron bioavailability from meals. A study by Hallberg (1981) on the effect of adding small amounts of ascorbic acid to composite whole meals has shown that the effect of ascorbic acid is significant. Pyke (1986) reports that since ascorbic acid enhances the bioavailability of iron, supplementary amounts of ascorbic acid can be useful in the treatment of iron deficiency anaemia. Provision of Ca, Fe, and Zn in children's diets is important, as deficiency of these minerals has affected many children in developing countries. According to Thu and others. (1999) micronutrient deficiencies remain common in preschool children in developing countries. Iron deficiency anemia is

one of the common nutritional problems affecting millions of people in both developing and developed countries (Yip 1994; Ziegler and Fomon 1996). According to Lönnnerdal (2000), marginal zinc deficiency and suboptimal zinc status are now recognized in many population groups in both less developed and industrialized countries. Inhibitors of zinc absorption are hereby considered as an important causative factor. Children fed on diets deficient in calcium can develop rickets (Martinez and others 1998), which is a disease common in most of the developing countries.

The objectives of this study were 2-fold. Firstly, an evaluation was made on how the various processing steps, when carried out consecutively and for periods practical for processing weaning foods, influenced the *in vitro* extractability of calcium, iron, and zinc of the ingredients. Secondly, the effect of addition of vitamin C and mango puree on the *in vitro* extractability of calcium, iron, and zinc were evaluated.

## Materials and Methods

### Raw materials and preliminary handling

Brown speckled kidney bean seeds (*Phaseolus vulgaris* var. Rose Coco) and brown finger millet (*Eleusine coracana* L. Gaertner) were brought from Tanzania (1999 harvest). They were

sorted by removing extraneous material and damaged seeds and washed with distilled water, spread on trays, and allowed to dry at 37 °C for 24 hr.

A portion of clean and dry seeds were milled with an attrition mill and sieved repeatedly until all material passed through a 200 µm sieve (no bran was removed). The flour was sealed into plastic bags and stored at -18 °C until analysis.

### Processing

#### Soaking

Finger millet and kidney bean samples were weighed into large plastic petri dishes and soaked in deionized distilled water in the ratio 1:2 w/v. Finger millet and kidney beans were soaked for 3 and 8 hr respectively, in a ventilated room at 30 °C. A portion of the soaked seeds were dried in a ventilated room at 37 °C for 48 hr and then milled into fine flour (passing through 200-µm sieve).

#### Germination

A portion of the soaked seeds were put in petri dishes and covered with perforated aluminium foil. They were kept in the dark at 30 °C for 48 hr to germinate. Germination and fermentation, when carried out for extended periods may result in spoilage or excessive dry matter losses resulting from the respiring seeds or fermenting microorganisms. The germination time was set to 48 hr, since in a previous research (Mbithi-Mwikya and others 2000) it was observed that germination of finger millet beyond 48 hr resulted in excessive dry matter loss, without corresponding nutritional gains. Germination was stopped by removing the samples and freezing them immediately to -18 °C until further processing. The frozen samples were dried in a ventilated room at 37 °C for 48 hr and then milled into fine flour (200 mesh).

#### Fermentation

*Lactobacillus salivarius* subsp. *salivarius* (LMG 9477<sup>T</sup>), obtained in lyophilized form from the BCCN/LMG Bacteria Collection Center of Ghent University, was used for the fermentation of both kidney beans and finger millet. The lyophilized microorganisms were incubated for 48 hr at 30 °C in MRS broth in order to be activated.

A starter culture was prepared for millet and beans before inoculating the samples. 50 g of millet and beans were separately added into 500 mL of dis-

**Table 1—Total Calcium, Iron, Zinc and Phytic Acid Content in Finger Millet and Kidney beans during processing (on 100 g DM)<sup>1</sup>.**

Process	Calcium (mg)		Iron (mg)		Zinc (mg)		Phytic Acid (%)	
	Mean	sd	Mean	sd	Mean	sd	Mean	sd
<b>Finger millet</b>								
Raw	232.91 <sup>a</sup>	0.84	5.48 <sup>a</sup>	0.22	2.05 <sup>a</sup>	0.01	1.31 <sup>a</sup>	0.04
Soaked	232.29 <sup>a</sup>	0.24	5.43 <sup>a</sup>	0.12	2.03 <sup>a</sup>	0.02	1.25 <sup>a</sup>	0.01
Germinated	232.75 <sup>a</sup>	0.63	5.61 <sup>a</sup>	0.34	2.06 <sup>a</sup>	0.02	0.63 <sup>b</sup>	0.04
Autoclaved	233.35 <sup>a</sup>	0.36	5.55 <sup>a</sup>	0.14	2.04 <sup>a</sup>	0.04	0.60 <sup>b</sup>	0.03
Fermented	233.10 <sup>a</sup>	0.14	5.68 <sup>a</sup>	0.14	2.08 <sup>a</sup>	0.01	0.20 <sup>c</sup>	0.03
<b>Kidney Beans</b>								
Raw	92.81 <sup>a</sup>	0.82	9.83 <sup>a</sup>	0.44	3.28 <sup>a</sup>	0.08	1.34 <sup>a</sup>	0.01
Soaked	91.39 <sup>a</sup>	0.39	9.61 <sup>a</sup>	0.42	3.27 <sup>a</sup>	0.06	1.34 <sup>a</sup>	0.05
Germinated	91.62 <sup>a</sup>	0.48	9.90 <sup>a</sup>	0.34	3.28 <sup>a</sup>	0.03	1.09 <sup>b</sup>	0.02
Autoclaved	91.20 <sup>a</sup>	1.15	10.22 <sup>a</sup>	0.29	3.24 <sup>a</sup>	0.03	0.96 <sup>c</sup>	0.06
Fermented	91.53 <sup>a</sup>	0.25	9.90 <sup>a</sup>	0.46	3.21 <sup>a</sup>	0.12	0.45 <sup>d</sup>	0.02

<sup>1</sup>values are means and one standard deviation of 3 replicates. For finger millet and kidney beans, values with the same superscript in the same column are not significantly different ( $\alpha < 0.01$ ).

tilled water. The suspension was autoclaved at 121 °C for 20 min, cooled and inoculated with the activated *Lactobacillus salivarius* at 10% v/v. The starter culture had 10<sup>5</sup>-10<sup>6</sup> cfu/g (Total plate count on MRS agar).

Kidney beans and finger millet slurries were prepared by mixing the flour with water (10% w/v). The suspension was autoclaved at 121 °C for 20 min and allowed to cool. It was then inoculated with the starter culture at 10% (v/v) and allowed to ferment for 48 hr at 30 °C. This fermentation time was chosen on the basis of a pre-study, which indicated that most increases in titratable acidity (AOAC No. 922.28, 1995) occurred during the first 48 hr of the fermentation, and that longer fermentation times resulted in high losses in dry matter. The fermented samples were sealed in a plastic bag and stored at -18 °C until analysis.

#### Laboratory analysis

**Total minerals:** Total mineral content of the samples after the various processing stages was carried out by AOAC method No 968.08 (AOAC, 1995). Total iron and zinc was determined by atomic absorption spectrophotometry (AOAC method 970.12, AOAC, 1995) while total calcium was determined by flame photometry according to AOAC method 963.13, AOAC, 1995). This experiment was conducted in triplicate.

**HCl- pepsin and pepsin-pancreatin mineral extractability:** Extraction of Ca, Fe and Zn by HCl and pepsin (HCl-P) was carried out by a method described by Kumar and Chauhan (1993). For the pepsin-pancreatin (P-P) method, extraction of the minerals was carried out as described by Miller et al. (1981). The amounts of Ca, Fe and Zn were deter-

mined by AOAC (1995) as previously explained. The experiment was carried out in triplicate.

**Mineral extractability after addition of vitamin C and mango puree :** In 2 g flour samples of raw, soaked, germinated, autoclaved and fermented finger millet and kidney beans, 0.3 milligram of vitamin C was added. One g of mango puree containing 0.3 mg vitamin C was added to separate 2-g samples of raw, soaked, germinated, autoclaved, and fermented finger millet and kidney beans. The pepsin-pancreatin digestion method was used for mineral extraction as described previously. The extracted Ca, Fe, and Zn were evaluated by AOAC (1995) as described previously. This experiment was carried out in triplicate.

**Analysis of phytates:** A method for the rapid determination of phytates developed by Haugh and Lantzsch (1983) was used. This experiment was carried out in triplicate.

**Statistical analysis:** All analyses were conducted in triplicate and calculations were based on 100-g dry matter. An analysis of variance of the results was done at 99% confidence interval ( $\alpha < 0.01$ ) using Tukeys Honestly Significant Difference. This analysis was done using Microsoft excel (2000) for Windows computer software.

## Results and Discussion

### Total minerals

Total calcium, iron, and zinc in raw finger millet and kidney beans were 232.91, 5.48, 2.05, and 92.81, 9.83, 3.28 mg/100 g DM respectively. These values did not change significantly during processing (Table 1).

### Calcium extractability

In finger millet, calcium extractability both by the HCl-pepsin (HCl-P) and pepsin-pancreatin (P-P) method increased during soaking (4.1 and 12.6%) and germination (20.7 and 16.5%) (Table 2). Autoclaving and fermentation, however, only showed moderate changes in calcium extractability (HCl-P) in finger millet. However, the P-P method further increased calcium extractability during autoclaving by 7.9% and during fermentation by 7.0% (Table 2). In kidney beans, significant increases by the HCl-P method were observed during soaking and germination, with only minor changes in extraction during autoclaving and fermentation. With the P-P method, Ca extractability increased continuously during processing, with the major increase being observed after germination (21.6%) (Table 3). The HCl-P method consistently showed higher values than those from the P-P method in finger millet and kidney beans. Ca extractability increased by 26.7 and 44.0% in finger millet and 56.2 and 39.5% in kidney beans for the HCl-P and P-P methods, respectively, during the entire processing.

Addition of vitamin C and mango significantly increased Ca extractability during the soaking and germination steps in both foods. However, after autoclaving and fermentation, the levels of extraction remained constant. Addition of vitamin C raised the extractability to almost identical values to those obtained by the HCl-P method. After addition of mango, the trend of extractability increase (in finger millet and kidney beans) was parallel to that of HCl-P and P-P with vitamin C, but consistently lower by 6.0 to 10.2%, except for raw kidney beans which gave similar values with both methods. Overall, samples fortified with vitamin C and mango increased in their Ca extractability by 22.8 and 22.5% in finger millet and 50.5 and 39.1% in kidney beans, respectively, during processing.

### Iron extractability

Iron extractability in soaked finger millet was not enhanced by vitamin C and mango addition and was not affected by the method of determination (Table 2). This extractability, when determined by P-P method, increased slightly during germination (4.7%) and later declined significantly (6.2%) during autoclaving and stayed low after fermentation, at a level not significantly different from that of the raw sample. The HCl-P method however, showed a

**Table 2—*In vitro* extractability (in %) of calcium, iron, and zinc in finger millet during processing as determined by the HCl-pepsin (HCl-P) and pepsin-pancreatin (P-P) method<sup>1</sup>.**

Process	Finger millet (HCl-P)		Finger millet (P-P)		Finger millet (P-P) + vitamin C		Finger millet (P-P) + mango	
	Mean	sd	Mean	sd	Mean	sd	Mean	sd
<b>Calcium</b>								
Raw	70.87 <sup>k</sup>	0.23	38.02 <sup>a</sup>	1.19	71.94 <sup>f</sup>	0.08	64.90 <sup>i</sup>	0.43
Soaked	75.02 <sup>l</sup>	0.25	50.62 <sup>b</sup>	1.81	76.71 <sup>g</sup>	0.43	66.17 <sup>j</sup>	0.78
Germinated	95.70 <sup>m</sup>	0.55	67.08 <sup>c</sup>	0.93	94.30 <sup>h</sup>	0.35	85.15 <sup>k</sup>	0.79
Autoclaved	96.14 <sup>m,n</sup>	0.61	74.98 <sup>d</sup>	0.57	93.09 <sup>h</sup>	0.24	86.10 <sup>l</sup>	0.43
Fermented	97.58 <sup>n</sup>	0.71	82.00 <sup>e</sup>	0.72	94.69 <sup>h</sup>	0.71	87.39 <sup>l</sup>	0.68
<b>Iron</b>								
Raw	5.06 <sup>a</sup>	0.18	4.49 <sup>a</sup>	0.22	5.64 <sup>e</sup>	0.14	4.81 <sup>a</sup>	0.17
Soaked	6.01 <sup>b</sup>	0.10	5.88 <sup>b</sup>	0.49	6.81 <sup>b</sup>	0.27	6.16 <sup>b</sup>	0.41
Germinated	29.91 <sup>f</sup>	1.82	10.57 <sup>c</sup>	0.81	31.20 <sup>f</sup>	1.99	29.02 <sup>f</sup>	2.00
Autoclaved	42.99 <sup>g</sup>	0.90	4.40 <sup>a</sup>	0.33	33.43 <sup>f</sup>	0.90	29.97 <sup>f</sup>	0.94
Fermented	51.98 <sup>h</sup>	1.35	4.98 <sup>d</sup>	0.05	33.87 <sup>f</sup>	2.02	29.19 <sup>f</sup>	1.39
<b>Zinc</b>								
Raw	50.60 <sup>e</sup>	0.24	30.24 <sup>a</sup>	1.59	50.51 <sup>e</sup>	0.41	46.71 <sup>h</sup>	0.48
Soaked	54.16 <sup>f</sup>	0.50	33.94 <sup>a</sup>	1.03	54.66 <sup>f</sup>	0.26	50.19 <sup>i</sup>	0.40
Germinated	78.41 <sup>g</sup>	0.58	39.20 <sup>b</sup>	1.30	77.19 <sup>g</sup>	2.55	69.98 <sup>i</sup>	0.48
Autoclaved	76.01 <sup>g</sup>	1.31	47.34 <sup>c</sup>	0.92	75.41 <sup>g</sup>	2.92	69.40 <sup>g,j</sup>	1.47
Fermented	81.93 <sup>k</sup>	0.37	51.12 <sup>d</sup>	0.66	78.55 <sup>g,k</sup>	2.02	69.19 <sup>i</sup>	0.67

<sup>1</sup>Values are means and 1 standard deviation of 3 replicates. For each mineral, values with the same superscript in the same row or column are not significantly different ( $\alpha < 0.01$ )

rapid and significant increase in iron extractability in finger millet throughout the rest of the processing steps with an overall increase of 46.9%.

In kidney beans, iron extractability increased consistently during processing, with major changes occurring during germination (Table 3). Overall increases in this extractability were 48.9 and 20.8 % by HCl-P and P-P methods, respectively. Addition of Vitamin C and mango in finger millet significantly increased iron extractability during germination by 25.5% and 24.2%, respectively (Table 2). For kidney beans, these increases were 21.7% and 26.2%, respectively (Table 3). However in both finger millet and kidney beans, iron extractability after addition of vitamin C and mango was not affected by both autoclaving and fermentation.

### Zinc extractability

Values of zinc extractability in both finger millet and kidney beans were lower when determined by the P-P than the HCl-P method. In finger millet, zinc extractability with the HCl-P method improved with germination (24.2%) but remained constant during autoclaving. It slightly increased (6.0%) during fermentation. With the P-P method, zinc extractability increased gradually with processing with an overall increase of 20.9% (Table 2). In kidney beans, zinc extractability with the HCl-P method increased with processing. The largest

increase was during germination (24.8%). Zinc extractability with the P-P method also increased with processing by an overall 36.4% in kidney beans (Table 3).

Addition of either vitamin C or mango in finger millet showed a significant increase during germination by 22.5 and 19.8% respectively, and then maintaining constancy during autoclaving and fermentation (Table 2). In germinated kidney beans, zinc extraction increased by 24.3 and 27.5%, respectively, after addition of vitamin C and mango (Table 3).

### Phytates

There was a marked reduction in phytic acid content in both kidney beans and finger millet during processing (Table 1). For the finger millet samples, the main decreases were observed after germination and fermentation where it decreased by 49.2 and 66.5%, respectively. Phytic acid decreased in finger millet by 84.7% overall during processing. In kidney beans, the overall decrease was 66.5% with the main decrease occurring after fermentation (53.1%).

### Discussion

ANTHONY AND CHANDRA (1998) found 313.1, 6.53, and 2.02 mg/100 g dry matter for Ca, Fe, and Zn in finger millet. Contents of calcium, iron, and zinc in red kidney beans (*Phaseolus vulgar-*

**Table 3—In vitro extractability (in %) of calcium, iron, and zinc in kidney beans during processing as determined by the HCl-pepsin (HCl-P) and the pepsin pancreatin (P-P) method<sup>1</sup>.**

Process	Kidney beans (HCl-P)		Kidney beans (P-P)		Kidney beans (P-P) + vitamin C		Kidney beans (P-P) + mango	
	Mean	sd	Mean	sd	Mean	sd	Mean	sd
<b>Calcium</b>								
Raw	36.82 <sup>j</sup>	0.49	32.75 <sup>a</sup>	0.54	40.85 <sup>f</sup>	1.15	39.80 <sup>f</sup>	0.27
Soaked	52.00 <sup>k</sup>	0.39	35.70 <sup>b</sup>	0.71	48.14 <sup>g</sup>	2.02	43.75 <sup>g</sup>	1.15
Germinated	88.33 <sup>l</sup>	0.93	57.34 <sup>c</sup>	0.59	91.07 <sup>h</sup>	0.89	78.39 <sup>i</sup>	0.48
Autoclaved	90.49 <sup>h,l</sup>	1.67	60.53 <sup>d</sup>	0.44	90.31 <sup>h</sup>	1.60	78.26 <sup>i</sup>	0.78
Fermented	92.95 <sup>h</sup>	0.62	72.25 <sup>e</sup>	0.41	91.30 <sup>h</sup>	1.50	78.87 <sup>i</sup>	1.77
<b>Iron</b>								
Raw	11.06 <sup>j</sup>	0.31	2.20 <sup>a</sup>	0.20	9.31 <sup>e</sup>	0.39	6.28 <sup>h</sup>	0.54
Soaked	15.05 <sup>f</sup>	0.91	3.57 <sup>b</sup>	0.12	15.6 <sup>f</sup>	0.81	6.80 <sup>h</sup>	0.62
Germinated	38.50 <sup>g</sup>	1.13	17.11 <sup>c</sup>	0.31	37.31 <sup>g</sup>	1.21	32.97 <sup>i</sup>	0.99
Autoclaved	42.51 <sup>k</sup>	0.77	15.89 <sup>c</sup>	0.83	36.67 <sup>g</sup>	1.16	31.22 <sup>i</sup>	0.92
Fermented	60.10 <sup>l</sup>	3.05	23.05 <sup>d</sup>	0.92	37.66 <sup>g</sup>	1.81	34.05 <sup>g,i</sup>	1.98
<b>Zinc</b>								
Raw	30.56 <sup>f</sup>	1.22	23.00 <sup>a</sup>	1.37	31.61 <sup>f</sup>	0.79	30.65 <sup>f</sup>	0.75
Soaked	37.89 <sup>k</sup>	0.45	31.10 <sup>b</sup>	0.10	34.19 <sup>g</sup>	0.68	31.56 <sup>b,f</sup>	0.40
Germinated	62.71 <sup>l</sup>	2.78	45.56 <sup>c</sup>	0.80	58.54 <sup>h</sup>	0.61	59.04 <sup>i,h</sup>	1.22
Autoclaved	68.64 <sup>h,m</sup>	2.62	52.15 <sup>d</sup>	1.32	58.91 <sup>h</sup>	0.03	61.36 <sup>i</sup>	0.42
Fermented	75.21 <sup>m</sup>	3.32	59.44 <sup>e</sup>	1.20	65.96 <sup>j</sup>	2.88	68.51 <sup>j,m</sup>	2.08

<sup>1</sup>Values are means and 1 standard deviation of 3 replicates. For each mineral, values with the same superscript in the same row or column are not significantly different ( $\alpha < 0.01$ )

is) were observed at 131, 5.16, and 3.1 mg/100 g dry matter. These values are in agreement with those observed in our study (Table 1). Since no biosynthesis or degradation of minerals is expected during processing, loss in mineral composition could occur mainly through leaching. In our study, solutions leached during germination were collected at the bottom of the petri dishes and incorporated into the sample thereafter. Changes in mineral composition can also occur due to apparent effects caused by loss of other nutrients. The values presented in our study were corrected for dry matter loss during processing. Germination did not alter the concentrations of Ca, Fe, and Zn in pearl millet (Kumar and Chauhan 1993). Kazanas and Fields (1981) did not find any significant change in total ash content of sorghum due to fermentation.

Germination of pearl millet at 30 °C for 48 h enhanced the HCl extractability of Ca and Fe by 100% or more. During the same period, Zn extractability increased by about 50% (Kumar and Chauhan, 1993). Divalent cations may be present as mineral-phytate chelates in ungerminated grains, which may explain the lower extractability of those minerals in HCl. Hydrolytic diminution of phytic acid in finger millet during germination has been observed by Mbithi-Mwikya and others (2000), who

observed that phytic acid values decreased from 0.35 in the raw sample to 0.02g/100g dry matter after 96 h germination. Chrompreeda and Fields (1984), observed a 17.2% decrease in HCl extractable iron after autoclaving soybeans (121 °C, 30 min). Iron extractability in finger millet doubled after 48 h of fermentation at 30 °C (Anthony and Chandra 1998). During the same period, Ca and Zn extractability increased by 20 and 30.5%, respectively.

According to Anthony and Chandra (1998), HCl extractability of minerals and trace elements under simulated gastric conditions, is an indicator for their bioaccessibility. However, the digestion of minerals is mainly in the duodenum where some of the non-heme iron and other minerals are absorbed (Hallberg 1981; Oski 1993). In the stomach, the pH is between 1 and 3 due to the presence of HCl in the gastric secretions while in the duodenum, the pH is alkaline in the range of 7.5 and 8.0 (Rao and Prabhavathi, 1978; Miller and others 1981). Extractability of minerals and trace elements after a pepsin-pancreatin digestion process simulates the events in the 2 key areas of the gastrointestinal tract. This is important in the sense that minerals in the 2-pH systems behave differently. In acidic medium of the stomach minerals are soluble since they combine with HCl to form chlorides (Hallberg 1981; Miller and others 1981). As the pH

is raised further (from 1.2 to 7.5) to simulate duodenal conditions, solubility decreases due to formation of hydroxides, which precipitate out and are unavailable for absorption (Rao and Prabhavathi 1978). It was evident from the mineral digestions at the various processing stages that the HCl-P (acidic) method had the highest extractability compared to P-P (alkaline), probably due to mineral precipitation.

The effect of vitamin C was very significant, with the addition of 0.3 mg to each sample digested from pH 1.2 to 7.5 (Tables 2 and 3). Ascorbic acid has been shown to markedly increase the bioavailability of non-heme iron and other minerals (Hallberg 1981; Skikne 1988). Danisova and others (1994) report that ascorbic acid which was not present in dry lentil seeds reached measurable values of 20 mg/100 g in germinated samples. This might also explain a higher mineral extractability during germination. In a study by Hallberg (1981), the addition of 25 or 50 mg of ascorbic acid to a Southern Asian type of meal composed of rice, cooked vegetables, and curry increased the bioavailability of non-heme iron 50 to 90% respectively. Hallberg (1981) also indicated that 150 g of papaya containing 66 mg of ascorbic acid, when added to a Thai rice meal increased the non-heme iron bioavailability 3 to 4 times. When orange juice containing 70 mg ascorbic acid was added to A Continental breakfast with coffee, bioavailability of non-heme iron increased by 2.5 times. It has been shown that vitamin C enhances the bioavailability of non-heme iron, probably by preventing the precipitation of ferric iron when the pH rises in the small intestine, either by keeping iron in a reduced form and/or by forming a ligand with ferric ions. In this way an appreciable amount of iron remains soluble and thus available for absorption (Hallberg 1981). Likewise, addition of 1 g of mango puree containing 0.3 mg vitamin C improved extractability of calcium, iron, and zinc. This might also be due to the presence of vitamin C and other substances contained in mango puree. Addition of mango might also have contributed to an increase in total dietary fiber content of the sample. Since dietary fiber is negatively correlated to mineral extractability (Frolich 1995), this might explain the observed decrease in Ca, Fe, and Zn extractability of samples added with mango when compared with those

added with pure vitamin C. According to Oski (1993), orange juice doubles the bioavailability of non-heme iron whereas tea decreases bioavailability by 75%. Skikne (1988) reports that a dose of 1000 mg ascorbic acid has been shown to increase the bioavailability of non-heme iron from a semi-purified meal 10-fold from 0.7% to 7.1%. This study, however, has also demonstrated that vitamin C improves mineral extractability.

The amount of phytic acid had a significant effect on the observed mineral extractability. Wolters and others (1993), investigating the relationship between *in vitro* availability of minerals and food composition with a mathematical model, reported phytic acid as having a strong negative effect on the calculated availability of Ca, Fe, and Zn and to a lesser effect on Mg availability. Traditional household techniques such as soaking and germination followed by fermentation have been shown to reduce phytates (Svanberg and Sandberg 1988). Honke and others (1998) observed a 15 and 78% reduction in inositol phosphate (IP6) after 48 and 172 h, respectively, of germination in faba beans. According to Lopez and others (1983), phytic acid was reduced by 65% after 5 d of fermenting corn meal samples. Chompreeda and Fields (1984), observed that autoclaving (121 °C for 30 min) of soybean meal and natural lactic acid fermentation of corn meal reduced phytic acid by 17.5% and 77.8%, respectively. A study by Sharma and Khetarpaul (1998) showed that a fermented rice-legume mixture had a reduced phytic acid content and increased Ca and Fe availability, compared to control products containing unfermented blends. According to Surtadi and Buckle (1985), phytic acid is significant from a nutritional point of view because of its ability to form stable chelates or phytates. The ability to chelate multivalent metal ions can result in very insoluble salts that are poorly absorbed from the gastrointestinal tract (Haug and Lantzsch 1983). Numerous studies have indicated that phytic acid reduces the bioavailability of dietary magnesium, calcium, zinc, and iron in monogastric animals (Lorenz 1983; Chompreeda and Fields 1984; Honke and others 1998). Heaney and others (1991) showed that soybean calcium was bioavailable with efficiencies roughly comparable to milk calcium, although the phytate content influenced its bioavailability.

### Conclusion

GERMINATION INFLUENCED MINERAL Extractability the most, with soaking, autoclaving and fermentation showing only slight or insignificant changes. Zinc extractability was lower compared to that for calcium, while the effect of processing on iron extractability as measured by the pepsin-pancreatin method was limited or absent. However, addition of vitamin C or mango puree to the ingredients showed a significant increase in mineral extractability at all processing stages. Values obtained by the HCl-pepsin method for calcium, iron and zinc extractability were consistently higher than those by the pepsin-pancreatin method. In addition, this *in vitro* study has demonstrated that a decrease in phytates was accompanied by greater mineral extractability.

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Authors are with the Dept. of Food Technology and Nutrition, Faculty of Agricultural and Applied Biological Sciences, Gent University, Coupure Links 653, B-9000 Gent, Belgium. Direct inquiries to author Mamiro (E-mail: petermamiro@suanet.ac.tz) (Author Van Camp's e-mail: john.vancamp@rug.ac.be).