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The effect of multi-mineral mix (Fe, Zn, Ca and Cu) on magnesium binding to starchy legumes under simulated gastrointestinal conditions

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Abstract

The present in vitro study on starchy legumes and their Mg bioavailability, covers the factors: cooking, dietary fibre, phytic acid and the competitive binding of other minerals. Mg-binding capacity of food legumes (butter beans, broad beans and lentils) in the raw, cooked and fibre-rich fraction (FRF) forms with Mg addition alone and with Ca, Fe, Zn and Cu (at concentrations based on the recommended daily allowance) was investigated in conditions simulating the small intestine. The three legumes in the raw and cooked forms showed more significant ($P < 0.01$) Mg-binding when Mg was added with Ca, Fe, Zn and Cu than with Mg addition alone. The FRFs were found to bind significantly ($P < 0.01$) more Mg than the raw and cooked forms. Cooking significantly decreased ($P < 0.01$) the Mg-binding capacity of raw butter beans, broad beans and lentils at separate Mg addition or with Ca, Fe, Zn and Cu.

1. Introduction

Minerals from plant foods, have been reported to be less utilized by man than those from animal sources (O'Dell, 1969). Endogenous factors, such as the accompanying chelating agents in plant foods and exogenous conditions, such as competitive binding of other minerals, affect the mineral absorption or availability.

Reviewing the effect of fibre sources (cereals and legumes) on mineral binding, Torre, Rodriguez and Saura-Calixto (1991) found that it is very difficult to draw definite conclusions regarding the relation between the composition of the different products and the mineral binding observed. In effect, the polysaccharides in these plant foods are associated with many other substances as proteins, polyphenolic compounds, phytate, etc which can modify mineral binding by dietary fibre. It was reported that mineral bioavailability may be influenced negatively by dietary fibre, phytic acid, oxalic acid, tannins and flavanoids (Platt & Clydesdale, 1987; Elhardallou & Walker, 1992; Hallberg, Rossander & Skanberg, 1987; Liebman & Doane, 1989; Wolters, Schrender et al., 1993; Brune, Rossnder & Hallberg, 1989). It may also be influenced positively by ascorbic acid, citric acid, lactose and fructose, (Elhardallou & Walker; Hazell & Johnson, 1987; Schuette, Knowles & Ford, 1989; Holbrook, Smith & Reiser, 1989).

There is no standard in vitro mineral-binding methodology, yet the reported methods attempt to mimic the human digestion and absorption processes. A majority of in vitro studies have evaluated the fibre constituents which affect mineral absorption, on the basis of the percent of mineral bound under pH conditions simulating those of the duodenum or the small intestine. Camire and Clydesdale (1981) used phthalate sodium hydroxide buffer to attain pH 5, pH 6 and tris-maleate (0.1 M) for pH 7; Thompson and Weber (1979); used 0.1 M phosphate buffer for pH 6.8 and Reinhold, Garcia, and Garzon (1981) used glucose-saline solution with pH (6.45) adjustment by sodium hydroxide or hydrochloric acid (0.1 or 0.01 M). However, Camire and Clydesdale using pH 5, 6 and 7, at 30°C, with rotation for 24 h, investigating in vitro Fe-binding to different celluloses (wheat bran, Solka floc, CMC and Sigma cellulose), found the maximum Fe-binding at pH 7: 13.0, 69.5, 47.7 and 38.2%, respectively. They also found lignin to bind a substantial amount of Mg, Ca, Zn and Fe.

In this investigation, as in others (Elhardallou & Walker, 1992, 1993, 1995), we use tris-maleate buffer for pH 7. The fibre-rich fraction, FRF, of studied legumes

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was prepared in conditions simulating the small intestine, where mineral absorption takes place. The non-starch polysaccharides (soluble and insoluble) and phytic acid of the legumes studied and their FRFs were analyzed.

Various components of dietary fibre, such as cellulose, hemicellulose, pectin and lignin, have been proposed as mineral binding factors. The number and strength of binding sites of lignin, cellulose and pectin have been studied (Platt & Clydesdale, 1987). However, the literature has conflicting data on divalent cation binding to plant food materials and their fibres. Phytate has long been reported to affect mineral metabolism. Torre, Rodriguez and Saura-Calixto (1991), reviewing effects of dietary fibre and phytic acid on mineral bioavailability, reported a slight negative influence of phytic acid on Mg bioavailability.

The purpose of this study is to investigate the in vitro binding capacity of Mg to raw and cooked starchy legumes (and their FRFs), with Mg addition alone and in presence of a mineral mixture, at pH 7 and temperature 37°C (simulating physiological conditions at which most minerals are absorbed).

The mineral concentration levels (100, 100, 5, 2 and 1 ppm for Mg, Ca, Fe, Zn and Cu, respectively), were based on half the recommended daily amount (RDA) for Fe and Cu and less than that for Mg, Ca and Zn. In fact, the endogenous level of Mg and Ca was high in the legumes studied. However, the RDA, for an adult man, for Mg, Ca, Zn and Fe is reported to be 350, 800, 15 and 10 mg/day, respectively (Food and Nutrition Board of the National Research Academy of Sciences, USA, 1980, see Pike & Brown, 1984). For Cu, the RDA (for an adult man) is 2–3 mg/day (WHO, see Pike & Brown, 1984).

2. Materials and methods

2.1. Legume samples and model fibres

Mature and healthy seeds of butter beans (*Phaseolus lunatus* L.) were supplied by Whiteworths; broad beans (*Vicia faba* L. *minor*) from the Agricultural Research Corporation (Sudan). The lentils (*Lens culinaris* L.) were purchased from the local market, Reading. Solka floc, crystalline cellulose (Johnson Jergenson Wetter, UK) and carboxylmethylcellulose (CMC) (Whatman, Maidstone, Kent, UK) were included as model fibres. The two fibres are characterized by small amounts of endogenous minerals.

2.2. Cooked sample preparation

Seeds of each sample were cleaned and soaked in distilled water (1:5 w/v ratio), at room temperature overnight (16 h). A few crystals of thymol were added to hinder microbial growth. The swollen seeds and unabsorbed water were autoclaved (Autoclave Sterilizing Equipment Company, Mainsfield, Nottinghamshire, UK) at 121 °C, 15 psi (103.4 kN/m^2) for 1.25 h. The cooked material was then cooled, blended and spread together with rinsing distilled water on trays. Trays were transferred to a freeze-drier (Vickers Armstrong, South Marston Ltd, Swindon, UK) and left 48 h with a shelf heat of 22°C. The freeze-dried cooked legumes were then finely ground using the pin disk mill (Simon Handling Engineers; now Satake-Robinson, Stockport, UK) to pass a 60 mesh sieve (1.6 mm^2).

2.3. Preparation of buffer solution (Gomori's buffer)

The intestinal conditions for the mineral-binding experiments were simulated with a Tris-maleate buffer solution at pH 7 (Camire & Clydesdale, 1981) and an incubation period of 24 h at 37°C with moderate shaking. The buffer solution was prepared by adding 24 ml 0.2 M NaOH to 25 ml of 0.02 M Tris-maleate (made by dissolving 24.2 g Trizma base, Sigma, and 19.0 g maleic anhydride in 1L deionized water) and diluting to 100 ml.

2.4. Fibre-rich fraction (FRF) preparation

Pancreatin (Grade 4 from porcine pancreas, Sigma, Poole, Dorset, UK), was used for a double digestive technique at 37°C. The pancreatin was first suspended in phosphate buffer, pH 7 (3 g in 27 ml) well stirred using a magnetic stirrer for 15 min and centrifuged at 10 000 rpm for 20 min. The residue was similarly treated and the supernatants were used as the pancreatic solution. Forty ml of the latter were added to 100 g cooked legume flour, the volume was brought up to 1 l using Gomorri's buffer (pH 7). A few crystals of thymol were added to stop microbial growth and stirred overnight at 37°C using a shaker (Phillip Harris International, Lichfield, UK). After centrifugation, the residue was subjected to a second digestion, as the end products (e.g. maltose) were suggested to cause enzyme inhibition (Lakin, 1960).

The slurry was then centrifuged and the remaining FRF was placed in forced air oven at 35°C overnight, then placed in a vacuum oven (Phillip Harris International, Lichfield, UK) at 35°C overnight to complete drying. The dry material was then ground using the pin disk mill (Sumon Handling Engineers; now Stake-Robinson, Stockport, UK) to pass a 20 mesh and left for further drying under vacuum overnight.

2.5. Proximate analysis including endogenous minerals

The moisture and ash contents were determined using AACC (1983) methods. Protein content was determined

using the Leco FP228 (Leco Instrument Ltd, Cheshire, UK) nitrogen analyzer and multiplying the nitrogen % by the factor 6.25.

The endogenous Mg, Ca, Fe, Zn and Cu were determined using the AACC (1983) method. The Atomic Absorption Spectrophotometer, AAS, (Unicam SP9, Pye Unicam, Cambridge, UK) was used to measure Mg, Ca, Fe, Zn and Cu at wavelengths of 285.2, 422.7, 248.3, 213.9 and 324.8 nm, respectively, with corresponding lamp currents (mA) of 4, 8–10, 12–15, 8–10 and 4–5. The flame condition was stoichiometric for Mg, Ca, Fe and Zn and Lean-stoichiometric for Cu.

2.6. Non-starch polysaccharide analysis

The Englyst method (Englyst, Wiggins & Cummings, 1982) was used for the determination of non-starch polysaccharides (NSP) of the investigated raw legumes and their FRFs.

2.7. Phytic acid determination

The method of Latta and Eskin (1980) was followed with some modification, to analyze phytic acid. For extraction of phytate, the method of Chang, Schwinner and Burr (1977), using trichloroacetic acid and warming at 60°C, was followed.

2.8. Mineral stock solutions

Mineral stock solutions (Mg, 10 000 ppm, Ca, 10 000 ppm; Fe, 500 ppm; Zn 200 ppm and Cu 100 ppm) were made by adding 10.141 g $MgSO_4 \cdot 7H_2O$, 3.6677 g $CaCO_3 \cdot 2H_2O$, 0.2489 g $FeSO_4 \cdot 7H_2O$, 0.0880 g $ZnS)_4 \cdot 7H_2O$ and 0.0393 g $CuSO_4 \cdot 5H_2O$ each to 1 ml of concentrated HCl and diluting to 100 ml; so that 1 ml of the mineral solution could give the required concentration in 100 ml buffer solution. Mineral concentrations in the stock solutions were checked by AAS against BDH Certified Standard solutions.

2.9. Atomic absorption standards

A set of standards in the range of 0.6–3, 4–20, 1–5, 0.4–2 and 0.2–1 ppm for Mg, Ca, Fe, Zn and Cu, respectively, were prepared by dilution of stock solutions; to give reasonable absorption readings with the AAS.

2.10. Mg, Ca, Fe, Zn and Cu adsorption isotherms

To determine the extent of saturation of the binding sites, binding tests were run for each mineral. From stock mineral concentrations, the volumes equivalent to 50, 100, 150 and 200 ppm for Mg and for Ca; 2.5, 5.0, 7.5 and 10 ppm for Fe, 1, 2, 3 and 4 ppm for Zn and 0.4, 0.8, 1.2 and 1.6 ppm for Cu, were added each to 1 g (on dry basis) raw broad beans and the volume completed to 100 ml using Gomori's buffer. Each experiment was completed as described for the exogenous Mg-binding test. The data were plotted as adsorption isotherms.

2.11. Buffer-soluble endogenous Mg

The method used by Camire and Clydesdale (1981) was followed with some modifications. Raw or cooked legume flour (1 g on dry basis) was weighed into a 250 ml Erlenmeyer flask, and 99 ml Gomori's buffer solution were added and stirred. The pH was adjusted to 7 (± 0.05) with 0.05 N NaOH where necessary. The flasks were covered using Nescafilm and/or cling film and incubated at 37°C for 24 h with moderate shaking (Phillip Harris International, Lichfield, UK). Solutions were filtered through Whatman No. 1 filter paper (7 cm) in a Buchner funnel with vacuum aspiration: filtrates were placed in glass sample bottles and kept at 2°C for analysis. For the FRF, the amount equivalent to that in 1 g (db) cooked legume flour (butter beans, 0.2445 g; broad beans. 0.3296 g and lentils 0.2770 g) was weighed using an analytical Mettler balance (Mettler Instrument, Grifensee, Zurich, Switzerland) and placed in a 250 Erlenmeyer flask. Gomori's buffer (99.5) ml was added and the whole treated in a similar manner to the cooked flour. The buffer-soluble endogenous Mg in the presence of Ca, Fe, Zn and Cu at 100, 5, 2, and 1 ppm, respectively, was determined similarly, considering the volume of added minerals (4 ml) and NaOH needed for the pH adjustment. A blank of a 100 ml buffer solution was treated similarly. The buffer-soluble endogenous Mg was measured using the AAS (Unicam SP9, Pye Unicam, Cambridge, UK) as described in the endogenous mineral test.

2.12. Exogenous Mg-binding: after the addition of Mg separately, or with Ca, Fe, Zn and Cu

Each test was carried out in a 250 ml conical Erlenmeyer flask in which was placed 1 g (db) of the raw or cooked legume, Solka floc or CMC or the FRF equivalent to that in 1 g (db) of cooked legume flour. For Mg added alone, 1 ml Mg stock solution was added and the volume completed to 100 ml using Gomori's buffer. For added mineral(s) other than Mg, 1 ml of each stock mineral solution was added and (1.0–3.5) ml 0.2 M NaOH were added within the 100 ml volume, to keep the pH at 7 ± 0.05.

For each test, the Mg bound (the average of three replicates) was calculated by subtracting the mean of Mg in the supernatant from the total added amount (100 ppm) and buffer-soluble endogenous amounts. Values in ppm were equivalent to mg/10 g of the raw and cooked samples or ing/equivalent FRF; as the volume used in analysis was 100 ml and weights were 1 g and its equivalent FRF:

$$\text{Percent of Mg bound} = \frac{\text{The amount of Mg bound}}{\text{Amount of added Mg+buffer}-\text{soluble endogenous Mg}} \times 100$$

2.13. Statistical treatment

Analysis of variance was carried out on Mg-binding data. Significance was accepted at $P < 0.01$. However, the (SE) standard error of difference was applied, particularly to show the trends that were slightly dissimilar.

3. Results and discussion

The means of three replicates for the moisture content of raw butter beans, broad beans and lentils were found to be 10.9, 6.5 and 8.8%, respectively; the protein contents were 24.6, 33.9 and 28.5%, respectively, while the ash contents were 4.6, 4.1 and 3.1%, respectively. These results were in agreement with those found in the literature.

The FRF preparation method recovered an insoluble residue for butter beans, broad beans and lentils (32.1, 38.1 and 27.1 g/100 g, dry weight, respectively) containing a complex mixture of non-starch polysaccharides, protein, lignin and starch.

The means of three replicates for endogenous Mg in raw butter beans, broad beans and lentils were found to be 138, 154 and 108 mg/100 g (db), respectively (Table 1). The proportion of endogenous Mg present in the FRF of the three legumes studied was found to be low (Table 1), comprising 5–9% only of the total endogenous Mg. Values in the literature vary considerably and those found in this study fit into the broad range of values reported.

Using the Englyst method, the total NSP of raw butter beans, broad beans and lentils was found to be 17.0, 18.1 and 14.1%, respectively, with the corresponding total NSP of 32.1, 38.1 and 27.1% for their FRFs. The insoluble NSP fractions were found to be 9.9, 14.0 and 10.2% for raw butter beans, broad beans and lentils, respectively, with corresponding NSP fractions of 25.5, 29.4 and 21.8% for their FRFs. The three legumes showed high arabinose content, 2.2–6.1 and 4.5–6.4 g/100 g, for the raw and FRF forms, respectively (Table 2). The uronic acid content of raw butter beans, broad beans and lentils were found to be 2.1, 3.0 and 3.3% respectively.

Phytic acid contents of raw butter beans, broad beans and lentils were found to be 0.96, 1.50 and 0.86%, respectively (Table 3), all within the range reported in the literature, with 0.11, 0.42 and 0.13% phytic acid content for the corresponding FRFs.

For the Mg adsorption isotherm, Mg concentrations of 50, 100 and 150 ppm presented a linear relationship to the bound Mg, indicating the potential of the Mg (100 ppm)-treated legumes for extra Mg-binding sites. However, at a Mg concentration of 200 ppm, the percentage of bound Mg fell (showing the classic Langmuir curve), suggesting saturation of binding sites and perhaps some weakly bound Mg. For the Fe, Zn and Cu adsorption isotherm tests, the amount of each mineral bound was found to be directly proportional to the amount of mineral in the supernatant (showing the classic Nernstian curve). At no point over the range of concentrations chosen did the mineral binding reach saturation. For the Ca adsorption isotherm, Ca con-

Table 1
The means (±SD) endogenous Mg, Fe, Zn, Ca and Cu in raw butter beans, broad beans and lentils and their fibre-rich fractions (FRF) with the percent of the FRF contribution and in Solka floc and carboxymethyl-cellulose (CMC) (mg/100 g)[a]

Sample	Mean endogenous mineral (mg/100 g)				
(1 g on dry basis)	Mg	Fe	Zn	Ca	Cu
Butter beans					
Raw	138 ± 0.95	6.8 ± 0.12	4.4 ± 0.12	106 ± 0.65	0.32 ± 0.07
FRF	28.6 ± 0.77 (5.1)	18.1 ± 0.07 (65.1)	3.0 ± 0.26 (16.8)	97.7 ± 0.62 (22.5)	1.11 ± 0.02 (84.8)
Broad beans					
Raw	154 ± 1.05	11.0 ± 0.32	5.8 ± 0.27	167 ± 1.06	0.91 ± 0.07
FRF	42.4 ± 0.77 (9.0)	23.6 ± 0.52 (70.9)	2.9 ± 0.36 (16.5)	173 ± 0.61 (34.1)	1.40 ± 0.01 (50.7)
Lentils					
Raw	108 ± 0.55	12.2 ± 0.41	4.8 ± 0.10	150 ± 1.03	0.91 ± 0.07
FR	34.9 ± 0.71 (9.0)	39.2 ± 0.66 (89.0)	6.2 ± 0.36 (35.8)	163 ± 0.65 (30.1)	3.00 ± 0.20 (91.3)
Solka floc	12.0 ± 0.45	11.3 ± 0.40	0.5 ± 0.07	18.8 ± 0.20	0.22 ± 0.00
CMC	4.5 ± 0.07	7.4 ± 0.07	0.4 ± 0.07	4.3 ± 0.30	0.20 ± 0.00

[a] The FRF (g) equivalent to 100 g (dry wt) of butter beans, broad beans and lentils was 24.5, 33.0 and 27.7, respectively. The endogenous mineral of the FRF as per cent of that in the raw legume is shown within parentheses. The figures are the means of three individual replicates. Cooked legumes were expected to have the same endogenous mineral concentration as the raw form as neither soaking nor cooking water was discarded during preparation.

Table 2
Non-starch polysaccharide (NSP) in raw butter beans, broad beans and lentils and their fibre-rich fractions (FRF) (on dry basis)

Total (g/100g)		Composition (g/100g)						
		Fuc[a]	Ara	Xyl	Man	Gal	Glu	U.Ac
Butter beans								
Raw								
Soluble NSP	7.1	–	3.4	0.9	0.7	0.2	0.7	1.2
Insoluble NSP	9.9	–	2.7	1.4	0.3	0.3	4.3	0.9
Total NSP	17.0	–	6.1	2.3	1.0	0.5	5.0	2.1
FRF								
Soluble NSP	6.6	t	2.2	0.9	0.8	0.3	1.4	1.0
Insoluble NSP	25.5	t	4.2	4.3	0.3	0.8	14.6	1.3
Total NSP	32.1	t	6.4	5.2	1.1	1.1	16.0	2.3
Broad beans								
Raw								
Soluble NSP	4.1	t	1.2	0.3	t	1.2	0.8	0.6
Insoluble NSP	14.0	t	1.8	2.5	t	0.6	6.7	2.4
Total NSP	18.1	t	3.0	2.8	t	1.8	7.5	3.0
FRF								
Soluble NSP	8.7	t	1.9	1.3	t	0.6	2.1	2.8
Insoluble NSP	29.4	0.2	2.9	4.5	t	1.3	19.2	1.3
Total NSP	38.1	0.2	4.8	5.8	t	1.9	21.3	4.1
Lentils								
Raw								
Soluble NSP	3.9	t	1.2	0.4	t	0.1	0.9	1.3
Insoluble NSP	10.2	t	1.0	1.4	t	0.2	5.6	2.0
Total NSP	14.1	t	2.2	1.8	t	0.3	6.5	3.3
FRF								
Soluble NSP	5.3	t	1.8	0.7	t	0.2	1.1	1.5
Insoluble NSP	21.8	t	2.7	3.4	t	1.0	12.4	2.3
Total NSP	27.1	t	4.5	4.1	t	1.2	13.5	3.8

[a] Fuc = fucose; Ara = arabinose; Xyl = xylose; Man = mannose; Gal = galactose; Glu = glucose; U.Ac = uronic acid. Note: rhamnose was not detected. The amount of glucose represents primarily the cellulose content.

Table 3
The mean (±SD) phytic acid content of the raw and cooked legumes (butter beans, broad beans and lentils) and their fibre-rich fractions (FRF)[a]

Sample	Phytic acid (%)		
(1 g on dry oasis)	Raw	Cooked	FRF
Butter beans	0.96 ± 0.02	0.48 ± 0.02	0.11 ± 0.01
Broad beans	1.50 + 0.01	1.33 ± 0.03	0.42 ± 0.01
Lentils	0.60 + 0.02	0.34 ± 0.01	0.13 ± 0.01

[a] The figures are the means of three individual replicates. The FRF (g) equivalent to 100 g (dry) of butter beans, broad beans and lentils is 24.5, 33.0 and 27.7, respectively.

centrations of 50, 100 and 150 ppm presented a linear relationship to the bound Ca. At a Ca concentration of 200 ppm, the percentage of bound Ca fell (showing the classic Langmuir curve). Mg, Fe, Zn, Ca and Cu adsorption isotherms indicated that mineral additions of 100, 5, 2, 100 and 1 ppm, respectively, would be appropriate since they are within the linear portion of the adsorption isotherms.

The buffer solubility of the Mg of the three legumes was found to lie in the range 57–74% for the raw forms, 51–92% for the FRF and even higher for the cooked forms (81–96%) (Table 4). Meiners et al. (1976), investigating the content of nine minerals of raw and cooked mature dry legumes, including lentils, noted measurable amounts of all minerals in the cooking water. In water drained from cooked large lima beans (*Phaseolus lunatus*), they found a Mg content of 48.3 mg/100 g. Reduction in phytate content during cooking (and the relative protein solubilization) may release or solubilize more Mg. This is notable particularly for tested butter beans (Tables 3 and 4).

For Solka floc and CMC, the buffer-soluble endogenous Mg was found to constitute 47 and 27%, respectively (Table 4), with no buffer-soluble Mg in the presence of mineral mixture.

Mg-binding is notably high for the raw and cooked legumes and in the range 63.7–75.3% and higher for the FRFs (80.4–83.7%) (Table 5, Fig. 1). The investigated model fibres showed no Mg-binding with Mg added separately or with mineral mixture. Persson, Nair, Frolich, Nyman, and Asp (1987), studying the ability of soluble fractions in wheat bran, whole grain wheat

Table 4
The mean (±SD, in ppm) and per cent of the buffer-soluble endogenous Mg of raw and cooked legumes (butter beans, broad beans and lentils) and their equivalent fibre-rich fractions (FRF) and of Solka floc and carboxymethlycellulose (CMC) and the mean (±SD, in ppm) of buffer-soluble endogenous Mg after the addition of Fe, Zn, Ca and Cu[a]

Sample (on dry basis)	Mean buffer-soluble endogenous Mg	Per cent of the buffer-soluble endogenous Mg	Mean buffer-soluble endogenous Mg after the addition of Fe, Zn, Ca and Cu[b]
Butter beans			
Raw (10 g)	10.2 ± 0.24	74	7.52
Cooked (10 g)	13.2 ± 0.30	96	8.05
FRF (2.445 g)	0.57 ± 0.04	81	0.66
Broad beans			
Raw (10 g)	9.56 ± 0.21	62	7.08
Cooked (10 g)	12.6 ± 0.27	82	7.96
FRF (3.296 g)	1.29 ± 0.10	92	1.10
Lentils			
Raw (10 g)	6.19 ± 0.14	57	6.00
Cooked (10 g)	9.10 ± 0.20	84	7.42
FRF (2.77 g)	0.50 ± 0.03	52	0.71
Solka floc	0.56 ± 0.03	47	0.00
CMC	0.12 ± 0.00	27	0.00

[a] To simulate the small intestine, Gomori's buffer (pH 7) was used with incubation at 37°C and moderate agitation for 24 h. Values in ppm are equivalent to mg/10 g or mg/FRF equivalent of 10 g; 1 g sample (or its FRF equivalent) was analysed in 100 ml.

$$\%\ \text{of the buffer soluble Mg} = \frac{\text{Buffer} - \text{soluble endogenous Mg (ppm)}}{\text{Endogenous Mg content in mg/10 g (Table1)}} \times 100.$$

[b] Fe, Zn, Ca and Cu were added in 5, 2, 100 and 1 ppm amounts, respectively, in a mineral binding experiment under conditios simulating the small intestine.

Table 5
The mean (±SD, in ppm) and per cent of Mg (added and buffer-soluble endogenous amounts) bound by the raw and cooked legumes (butter beans, broad beans and lentils) and their equivalent fibre-rich fraction (FRF), and by Solka floc and carboxymethylcellulose (CMC) with Mg and Mg + Fe + Zn + Ca + Cu additions under conditions simulating the small intestinal tract[a]

Sample (on dry basis)	The bound Mg, by legumes, FRFs, Solka floc or CMC with the addition of Mg separately or in combination	
	Added Mg (100 ppm)	Mg, Fe, Zn, Ca and Cu (100, 5, 2, 100 and 1 ppm, respectively).
Butter beans (2 SE[b] = 0.588)		
Raw (10 g)	73.9 ± 0.40 (67.0)	76.1 ± 0.40 (59.1)
Cooked (10 g)	72.1 + 0.41 (63.7)	73.7 ± 0.38 (65.1)
FRF (2.445 g)	84.1 ± 0.34 (83.6)	82.7 ± 0.30 (82.2)
Broad beans (2 SE = 0.588)		
Raw (10 g)	72.8 ± 0.38 (66.5)	79.8 ± 0.38 (75.1)
Cooked (10 g)	71.3 ± 0.40 (63.3)	78.0 ± 0.40 (69.2)
FRF(3.296 g)	84.8 ± 0.35 (83.7)	83.0 ± 0.33 (82.5)
Lentils (2 SE = 0.588)		
Raw (10 g)	78.0 ± 0.42 (73.5)	80.0 ± 0.41 (75.3)
Cooked (10 g)	76.4 ± 0.37 (70.1)	78.3 ± 0.39 (71.8)
FRF (2.77 g)	81.7 ± 0.29 (81.3)	80.8 ± 0.31 (80.4)
Solka floc (10 g)	0.00	0.00
CMC (10 g)	0.00	0.00

[a] The Mg bound (the average of three replicates) was calculated by subtracting the mean of Mg in the supernatant from the total added amount (100 ppm) and buffer-soluble endogenous amounts. Values in ppm were equivalent to mg/10 g of the raw and cooked samples or mg/equivalent FRF; as the volume used in analysis was 100 ml and weights were 1 g and its equivalent FRF.

$$\%\ \text{of Mg bound} = \frac{\text{The amount of Mg bound}}{\text{Amount of added Mg} + \text{buffer soluble endogenous Mg (Table 3)}} \times 100.$$

[b] SE: Standard Error of difference

bread dough and cellulose, to bind copper, zinc and cadmium, found the soluble fibres to interact strongly with metal ions, whereas the binding of cellulose was negligible.

The high Mg-binding values of the legumes studied partly agree with the finding of Idourene, Hassani, Claye and Weber (1995), that fibre sources from legumes (including peanuts and soy flour) seem to bind

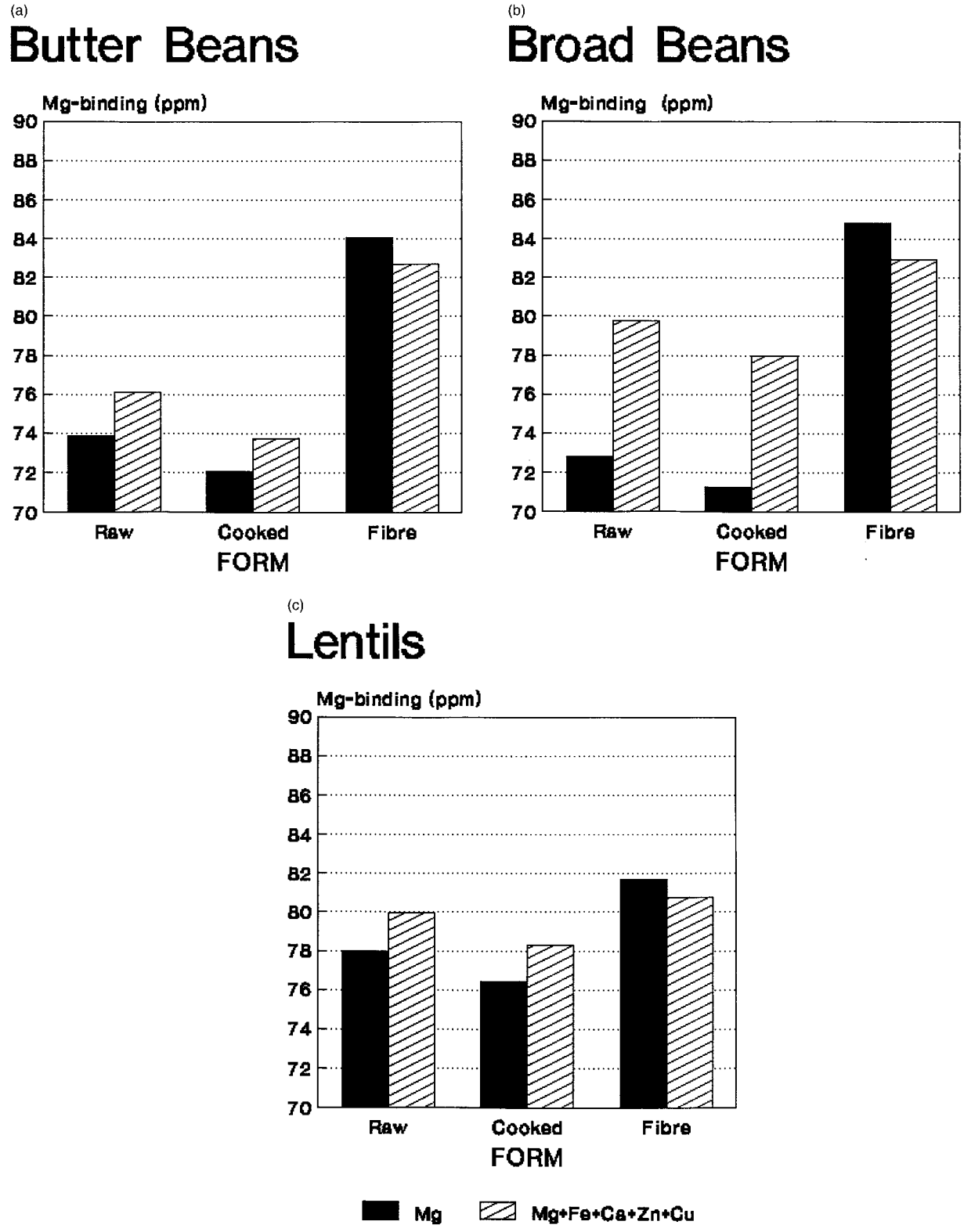

Fig. 1. The mean Mg-binding values of raw and cooked legumes and their equivalent fibre-rich fractions (FRF) in the presence of Mg alone or with Ca, Fe, Zn and Cu under simulated human small intestinal conditions.;

more Mg than those of cereals (barley fibre, wheat bran and corn bran), with very low binding to cellulose (source of cellulose: ICN Pharmaceuticals Inc., Staten Island, NY). The binding does not appear to be directly related to either the protein content or the phytic acid level of these fibre sources (Weber, Kohlhepp, Idouraine & Ochos, 1993). In reference to Tables 3 and 5, Mg-binding data do not appear to correspond to the phytic acid content. Clydesdale and Camire (1983), investigating the effect of the loss of phytic acid in defatted soy flour on Mg, Ca, Fe and Zn, showed that Mg-binding did not correlate with the presence of phytic acid. Wolters, Diepenmat, Hermas and Voragen (1993), investigating the relationship between in vitro availability of minerals and food composition (making a mathematical model), reported phytic acid as having a strong negative effect on calculated availability of Ca, Fe and Zn and a lesser effect on Mg availability (i.e. lesser Mg-binding).

The decrease in phytate content of broad beans, butter beans and lentils, after cooking was 11.3, 50.0 and 60.5%, respectively (Table 3). Mannan, Hussein, Ali and Igbal (1987) found cooking lentils to cause a 76% reduction in phytate level. However, the small decrease in broad beans phytate, after cooking, may be attributed to the formation of insoluble phytate complexes in relatively a larger amounts. Griffiths (1982), reported that phytate can form insoluble complexes with Ca and Mg. Broad beans have higher Ca, Mg and phytic acid contents (Tables 1 and 3). The high decrease in phytate content of lentils, after cooking, may be due to the presence of a lesser number of insoluble phytate complexes, thus providing more ready binding sites. This can affirm the higher Mg-binding % for raw lentils (Table 5) compared to other tested legumes.

From the data presented, it can be seen that there was a significantly ($P < 0.01$) higher Mg binding by FRF compared with the raw and cooked forms. In the literature, little information was found on Mg-binding to legumes, under conditions similar to those of this study. However, Mg-binding of the raw and cooked legumes increased slightly when Mg was added in combination with other minerals (indicating little extra interaction), while it decreased for the FRFs (Table 5, Fig. 1).

From the ANOVA results of exogenous Mg binding to legumes, it was noted that the main factors (species, form {treatment} and mineral addition), and their 2- and 3-way interactions each had a significant effect on Mg-binding ($P < 0.01$).

The SE of difference for the pooled means of bound Mg (ppm) (Table 5) was found to be 0.294. The differences in Mg-binding figures are generally significant with the following exceptions:

1. There is no significant difference (> 2SE, that is < 0.588) in Mg-binding (in the presence of Mg, Fe, Zn, Ca and Cu) for butter beans and broad beans in the fibre form, broad beans and lentils in the raw form or broad beans and lentils in the cooked form.
2. For each of the two legumes, butter beans and lentils, there is no significant difference (< 2SE) in Mg-binding for the raw form in the presence of Mg alone and for the cooked form in the presence of Mg with other minerals.
3. There is no significant difference in Mg-binding for: (i) butter beans in the raw form in the presence of Mg with other minerals and lentils in the cooked form in the presence of Mg alone; (ii) broad beans in the cooked form in the presence of Mg with other minerals, and lentils in the raw form in the presence of Mg alone.

4. Conclusions

The following conclusions can be drawn:

1. Overall, with a significant difference (> 2SE), the Mg-binding order of the legumes studied was: lentils > broad beans > butter beans.
2. Overall, more Mg was bound to the FRF and statistical difference between Mg-binding of the raw and cooked forms was apparent: FRF > > raw > cooked.
3. Overall, more Mg was bound with the Mg added in the presence of Fe, Ca, Zn and Cu, than when added separately.

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