Food Chemistry 46 (1993) 43–48

Binding of Zn by three starchy legumes in the presence of Zn alone or with Fe, Ca, Mg and Cu

Sirelkhatim B. Elhardallou* & Ann F. Walker

Department of Food Science and Technology, University of Reading, Reading, Berkshire, UK

(Received 2 August 1991; revised version received and accepted 21 November 1991)

Zn-binding capacity of butter beans, broad beans and lentils in the raw, cooked and fibre-rich fraction (FRF) forms; with Zn addition alone, and with Fe, Ca, Mg and Cu (at concentrations based on the recommended daily allowance) was investigated in conditions simulating the small intestine. The FRFs gave the highest Zn-binding levels with separate Zn addition, and the lowest with Zn addition in combination with other minerals. Overall, the raw and cooked forms were found to bind significantly ($P < 0{\cdot}01$) more Zn than the FRFs. Also more significant, ($P < 0{\cdot}01$), Zn-binding was noted when Zn was added together with Fe, Ca, Mg and Cu than with Zn addition alone.

INTRODUCTION

Mineral availability to man, particularly from plant foods, is known to be affected by the accompanying dietary fibre and phytates. The competitive binding of other minerals is similarly reported to affect mineral-binding. Evans and Hahn (1974) showed that Cu and Cd reduced the uptake of Zn by rats. Similarly, in a study on the interaction of Fe and Zn in the human intestine by Solomons and Jacob (1981) using $FeSO_4.7H_2O$ and $ZnSO_4.7H_2O$ as Fe and Zn sources, a ratio of Fe:Zn of 1:1 slightly inhibited Zn absorption, while Fe:Zn ratios of 2:1 and 3:1 substantially inhibited Zn uptake. O'dell (1969) reported a synergistic binding effect of phytates for zinc and calcium and zinc and copper.

MATERIALS AND METHODS

Legume samples and model fibres

Mature and healthy seeds of butter beans (*Phaseolus lunatus* L.), were supplied by Whitworths; broad beans (*Vicia faba* L., minor), from the Agricultural Research Corporation, Shambat (Sudan), and lentils (*Lens culinaris* L.), purchased from the local market, Reading.

Solka floc, crystalline cellulose, (Johnson Jorgensen Wetter, UK) and carboxymethylcellulose (CMC) (Whatman) were included as model fibres for cellulose and hemicellulose (heterogeneous) present in legumes. CMC contains a definite number of carboxymethyl groups and its mineral-binding saturation level is related to the number of free carboxyl groups. One advantage of the two models chosen, other than the high fibre content, was that they contain only small amounts of endogenous minerals.

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 at 121°C, 15 psi (103·4 KN/m^2) for 1·25 h. The whole cooked material was then cooled, blended and spread together with rinsing distilled water on trays. The latter were transferred to a freeze-drier and left 48 h with a shelf heat of 22°C. The freeze-dried cooked legumes were then finely ground to pass a 60 mesh (1·67 mm^2) sieve.

FRF preparation

Pancreatin (Grade 4 from porcine pancreas, Sigma), was used for a double digestion 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 pancreatin solution. A portion (40 ml) of the latter was added to 100 g cooked legume flour, the volume was made up to 1 litre using Gomori's buffer (pH 7); a few crystals of thymol were added to stop microbial growth and stirred overnight at 37°C. After centrifugation, the residue was similarly digested. The slurry was then centrifuged and the remaining

* Present address: Department of Food Science and Technology, University of Gezira, PO Box 20, Wad Medani, Sudan.

Food Chemistry 0308-8146/92/$05.00 © 1992 Elsevier Science Publishers Ltd, England. Printed in Great Britain

FRF was placed in a forced air oven at 35°C overnight, then placed in a vacuum oven at 35°C overnight to complete drying, ground to pass the 20 mesh sieve and further dried under vacuum overnight.

Proximate analysis including endogenous minerals

The moisture and ash contents were determined using the AACC (1983) method. Protein % was determined using the Leco FP228 nitrogen analyser and multiplying the nitrogen % by the factor 6·25. The endogenous Zn, Fe, Ca, Mg and Cu were determined using the AACC (1983) method.

Phytic acid determination

The method of Latta and Eskin (1980) was followed with some modifications. For extraction of phytate the method of Chang *et al.* (1977), using trichloroacetic acid and warming at 60°C, was used.

Preparation of buffer solution (Gomori's buffer)

tris-Maleate solution (0·02M, 25 ml) (made by dissolving 24·2 g Trizma base, Sigma and 19·6 maleic anhydride in 1 litre deionized water) and 24 ml 0·2M NaOH were diluted to 100 ml.

Mineral concentrations

The salts: $ZnSO_4.7H_2O$, $FeSO_4.7H_2O$, $CaCO_3.2H_2O$, $MgSO_4.7H_2O$ and $CuSO_4.5H_2O$ were taken as mineral sources. The stock solution of each metal was made by dissolving an appropriate amount in 1 ml of conc. HCl and diluting to 100 ml so that 1 ml of the mineral solution could give the required concentration in 100 ml buffer solution.

Atomic absorption standards

A working standard was prepared from Zn stock solution to cover the concentration ranges expected in the filtrates of the Zn-binding tests and give reasonable absorption readings with the atomic absorption spectrophotometer (AAS). The stock mineral concentrations used were checked against diluted aliquots from the BDH Certified Atomic Absorption Reference Solution for each mineral.

Zn adsorption isotherm

Zn concentrations, lower and higher than that selected for Zn-binding tests, were added to raw broad beans.

Buffer-soluble endogenous Zn

The method used by Camire and Clydesdale (1981) was followed with some modifications. Cooked legume flour (1 g, on dry basis) was accurately weighed into a 250 ml Erlenmeyer flask and 99 ml of Gomori's buffer solution were added with stirring. The pH was adjusted to 7 (± 0·05) with 0·5 M NaOH where necessary. The flasks were tightly covered using Nescafilm and/or cling film and incubated at 37°C for 24 h with moderate shaking. Solutions were filtered through a Whatman No. 1 filter paper (diameter 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 (on dry basis) cooked legume flour (butter beans, 0·2445 g; broad beans, 0·3296 g and lentils, 0·277 g) were accurately weighed 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. A blank of 100 ml buffer solution was treated similarly.

The buffer-soluble endogenous Zn was measured using the AAS.

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

The mineral-binding experiments were carried out in conditions simulating the small intestine, using 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 concentrations of the minerals added were standardized throughout. The levels used were based on approximately half the Recommended Daily Amount (RDA) for Zn and Fe (DHSS, 1979) and Cu (FAO/WHO), for an adult man, and on less than that for Ca and Mg as the endogenous level of each was high in the legumes studied.

Each test was carried out in a 250-ml conical Erlenmeyer flask in which was placed 1 g (on dry basis) of the raw or cooked legume, Solka floc or CMC or the FRF equivalent to that in 1 g (on dry basis) cooked legume flour. For Zn added alone, 1 ml of Zn stock solution was added and the volume made up to 100 ml using Gomori's buffer. For added mineral(s) other than Zn, 1 ml of each stock mineral solution was added and 1·0–3·5 ml 0·2M NaOH were added within the 100 ml volume, to keep the pH at 7 ± 0·05. Three replicates were carried out for each test.

Apparatus

The AAS, Unicam SP9, was used to measure Zn under 213·9 nm, 8–10 mA lamp current and stoichiometric flame conditions.

Statistical treatment

The analysis of variance was carried out for Zn-binding data. However, the standard error of difference (SE) was applied particularly in the case of trends that were slightly dissimilar.

RESULTS

The means of three replicates for the moisture content of raw butter beans, broad beans and lentils were

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

Sample (1 g on dry basis)	Mean endogenous mineral (mg/100 g)				
	Zn	Fe	Ca	Mg	Cu
Butter beans					
Raw	4·4 ± 0·12	6·8 ± 0·16	106·0 ± 0·65	138·1 ± 0·95	0·32 ± 0·07
FRF	3·0 ± 0·26	18·1 ± 0·70	97·7 ± 0·62	28·6 ± 0·77	1·11 ± 0·02
	(16·8)	(65·1)	(22·5)	(5·1)	(84·8)
Broad beans					
Raw	5·8 ± 0·27	11·0 ± 0·32	167·0 ± 1·06	154·5 ± 1·08	0·91 ± 0·07
FRF	2·9 ± 0·36	23·6 ± 0·52	172·6 ± 0·61	42·4 ± 0·77	1·40 ± 0·01
	(16·5)	(70·9)	(34·1)	(9·0)	(50·7)
Lentils					
Raw	4·8 ± 0·1	12·2 ± 0·41	150·5 ± 1·03	108·0 ± 0·55	0·91 ± 0·07
FRF	6·2 ± 0·36	39·2 ± 0·66	163·3 ± 0·65	34·9 ± 0·71	3·00 ± 0·20
	(35·8)	(89·0)	(30·1)	(9·0)	(91·3)
Solka floc	0·5 ± 0·07	11·3 ± 0·40	18·8 ± 0·20	12·0 ± 0·45	0·22 ± 0·00
CMC	0·4 ± 0·07	7·4 ± 0·07	4·3 ± 0·30	4·5 ± 0·07	0·20 ± 0·00

[a] The FRF (in g) equivalent to 100 g (dry wt) of butter beans, broad beans and lentils is 24·45, 32·96 and 27·70, respectively. The endogenous mineral of the FRF as % of that in the raw legume is shown within parentheses. The figures are the mean of 3 individual replicates. Cooked legumes were expected to have the same endogenous mineral concentrations of the raw form as neither soaking nor cooking water was discarded during preparation.

found to be 10·9, 6·5 and 8·8 %, respectively; the protein contents were 24·6, 33·9 and 28·5%, respectively and the ash contents 4·6, 4·1 and 3·1%, respectively.

The FRF preparation method recovered an insoluble residue containing a complex mixture of non-starch polysaccharides (32·1, 38·1 and 27·1 g/100 g on dry basis for butter beans, broad beans and lentils, respectively), protein, lignin and starch.

The endogenous Zn, Fe, Ca, Mg and Cu contents of the raw and FRFs are presented in Table 1. The phytic acid contents of the investigated legumes in the raw, cooked and FRF forms are shown in Table 2.

The moisture, protein, ash, mineral values and phytic acid content (particularly for the raw forms) were found to be in fair agreement with those in the literature.

The buffer-soluble endogenous Zn amounts for the studied legumes and model fibres, under conditions simulating the small intestine, are shown in Table 3. The endogenous Zn of the raw and cooked forms gave the range of 66–98 % buffer solubility compared with 42–60% for the FRFs and the model fibres. In fact, during the preparation of the FRF, substantial amounts of Zn were dissolved.

The adsorption isotherm test (using raw broad beans) showed that Zn concentrations of 1, 2, 3 and 4 ppm fall within the Nernstian curve, indicating the potentiality for extra binding sites. This reflects the suitability of Zn concentration (2 ppm) applied for Zn-binding experiments. The mean values of Zn (with separate Zn addition or with Fe, Ca, Mg and Cu)

Table 2. The mean (± SD) phytic acid content of the raw and cooked legumes (butter beans, broad beans and lentils) and their fibre-rich fractions (FRF)

Sample	Phytic acid (%)		
	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	0·86 ± 0·02	0·34 ± 0·01	0·13 ± 0·01

Table 3. The mean (± SD, in ppm) and % of the buffer-soluble endogenous Zn of raw and cooked legumes (butter beans, broad beans and lentils) and their fibre rich fractions (FRF) and of Solka floc and carboxymethylcellulose (CMC)[a]

Sample (on dry basis)	Mean buffer-soluble endogenous Zn	% of the buffer-soluble endogenous Zn
Butter beans		
Raw (10 g)	0·43 ± 0·01	(98)
Cooked (10 g)	0·40 ± 0·01	(90)
FRF (2·445 g)	0·03 ± 0·01	(42)
Broad beans		
Raw (10 g)	0·43 ± 0·02	(74)
Cooked (10 g)	0·38 ± 0·01	(66)
FRF (3·296 g)	0·14 ± 0·01	(47)
Lentils		
Raw (10 g)	0·43 ± 0·01	(90)
Cooked (10 g)	0·42 ± 0·02	(88)
FRF (2·77 g)	0·10 ± 0·01	(59)
Solka floc (10 g)	0·03 ± 0·01	(60)
CMC (10 g)	0·02 ± 0·01	(50)

[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 as 1 g sample (or its FRF equivalent) was analysed in 100 ml. % of the buffer-soluble endogenous

$$\text{Zn} = \frac{\text{Buffer-soluble endogenous Zn (ppm)}}{\text{Endogenous Zn content in mg/10 g (Table 1)}} \times 100$$

Table 4. The mean (± SD, in ppm) and % of Zn (added and buffer-soluble endogenous amounts) bound by the raw and cooked legumes (butter beans, broad beans and lentils) and their equivalent fibre rich fractions, (FRF), and by Solka Floc and carboxymethylcellulose (CMC) with Zn and Zn + Fe + Ca + Mg + Cu additions under conditions simulating the human intestinal tract[a]

Sample (on dry basis)	The bound Zn (and its %[b]) by legumes, FRFs, Solka floc or CMC with the addition of Zn separately or in combination	
	Added Zn (2 ppm)	Added Zn, Fe, Ca, Mg and Cu (2, 5, 10, 100 and 1 ppm respectively)
Butter beans		
Raw (10 g)	0·87 ± 0·01 (35·8)	2·18 ± 0·04 (89·7)
Cooked (10 g)	1·17 ± 0·01 (48·8)	1·95 ± 0·03 (81·3)
FRF (2·445 g)	1·21 ± 0·02 (59·6)	0·66 ± 0·02 (32·5)
Broad beans		
Raw (10 g)	1·23 ± 0·01 (50·6)	2·20 ± 0·02 (90·5)
Cooked (10 g)	1·32 ± 0·01 (55·5)	1·82 ± 0·04 (75·6)
FRF (3·296 g)	1·39 ± 0·04 (65·0)	1·32 ± 0·02 (61·7)
Lentils		
Raw (10 g)	1·03 ± 0·01 (42·4)	2·23 ± 0·02 (91·8)
Cooked (10 g)	1·13 ± 0·03 (46·7)	1·88 ± 0·03 (77·7)
FRF (2·77 g)	1·50 ± 0·01 (71·4)	1·10 ± 0·02 (52·4)
Solka floc (10 g)	0·30 ± 0·02 (14·8)	0·00
CMC (10 g)	0·31 ± 0·02 (15·3)	0·03 ± 0·01

[a] The Zn bound (the average of 3 replicates) was calculated by subtracting the mean of Zn in the supernatant from the total added (2 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 the weights were 1 g and its equivalent FRF.

[b] $$\% \text{ of Zn bound} = \frac{\text{The amount of Zn bound}}{\text{Amount of added Zn + buffer—soluble endogenous Zn (Table 3)}} \times 100$$

bound are presented in Table 4. The amounts of Zn bound were calculated by subtracting the Zn detected in the supernatant from the total Zn (added Zn and available buffer-soluble endogenous Zn).

From the ANOVA of the Zn binding data, the significance of the effects (particularly for the addition and form factors) was noted and illustrated in Fig. 1.

DISCUSSION

The following conclusions were drawn.

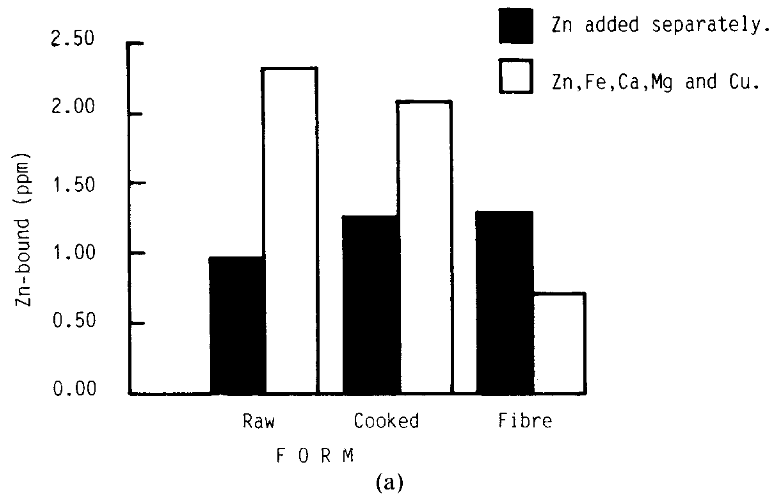
(1) In total, a greater amount of Zn was bound significantly to broad beans and lentils than butter beans. This can be attributed partly to the FRF of broad beans and lentils and its relatively higher phytic acid content (Table 2). In the raw form, proteins, which are comparatively higher in broad beans (and lentils), provide considerable binding sites. On the other hand, after cooking and protein denaturing, the complex formation with the released phytic acid and other minerals may account for a great part of the Zn-binding.

There was no significant difference (< 2SE) in the Zn-binding capacity of broad beans and lentils.

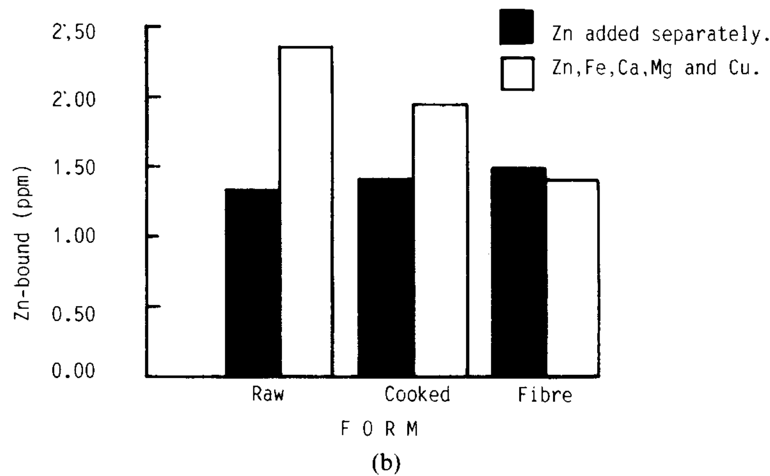
Maddaiah *et al.* (1964) found that Zn forms stable complexes with phytate *in vitro*. In agreement with this, Weingartner *et al.* (1979), investigating the effect of legume bran on the bioavailability of Zn from a soy flour-based rat diet, found the addition of legume bran, unlike wheat bran, to have no effect on the availability of Zn. This was attributed to the presence of phytates in legumes in the cotyledon but not in the hull (Walker, 1982).

(2) Overall, more Zn was significantly bound to the raw and cooked forms than the FRF. However, there was no significant difference (< 2 SE) in the binding capacity of the raw and cooked forms.

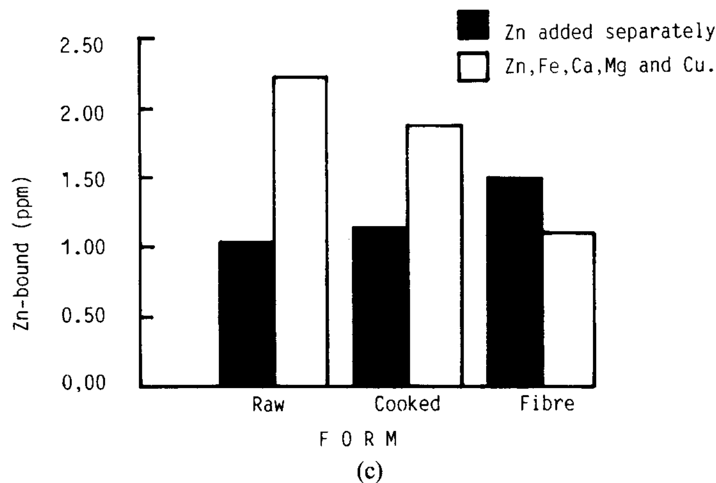
In fact, considering the separate Zn addition, Zn-binding of the legume forms was found to be in the order FRF > cooked > raw, and in the order raw > cooked > FRF with Zn addition in combination with Fe, Ca, Mg and Cu. Zn-binding, with separate Zn addition, seems to be enhanced by cooking. In agreement with higher Zn-binding after cooking, Clydesdale and Camire (1983) reported a significant increase in Zn-binding (0·075 mg/g) by defatted soy flour at pH 6·8 after incubation at 30°C, 24 h, because of boiling. However, the binding may be attributed primarily to the Zn–phytate complex formation, as phytates are released on cooking. In this study, the relatively lower Zn-binding by the FRFs, which have more binding sites and are of low phytate content, confirms this.



(a)



(b)



(c)

Fig. 1. The mean Zn-binding values of raw and cooked legumes ((a) butter beans, (b) broad beans and (c) lentils) and their equivalent fibre-rich fractions (fibre) with Zn and Zn + Fe + Ca + Mg + Cu additions under conditions simulating the human intestinal tract.

(3) Zn added in combination with Fe, Ca, Mg and Cu clearly resulted (in total) in a higher binding capacity of the legumes studied, than the Zn added separately.

As noted in Table 4, Zn-binding of the raw and cooked legumes was markedly higher when added in combination with the Fe, Ca, Mg and Cu. For the FRFs, the low phytic acid content could explain the opposite effect of the competing (added) minerals. Instead of participating in complex formation (with phytic acid), other minerals may occupy some binding sites on dietary fibre, thus reducing Zn-binding.

The model fibres, cellulose and CMC, gave low levels for Zn-binding (Table 4) with relatively more binding for Zn added separately. Thompson and Weber (1979),

investigating the endogenous Cu, Zn and Fe of six fibres including soy bran and cellulose (at pH 6·8 and incubation for 2 h in a water bath at 40°C with shaking), suggested the cellulose binding capacity for zinc to be lower than that of hemicellulose. They also added, the lower the protein content of fibre, the lower the zinc remaining bound, indicating that Zn-binding was due partly to protein.

However, binding of added zinc (source: zinc sulphate heptahydrate) to the NDF and ADF of cooked pinto beans, *in vitro* at pH 6·5 ± 0·05, was found to be almost at the same levels (Lee & Garcia-Lopez, 1985). Investigating the binding of Zn by wheat bran (1 g of bran in 100 ml zinc chloride solution, 1 ppm, at pH 6·41 and temperature 37°C), Rendleman and Grobe (1982) reported the binding of 98% of added Zn ions. They found that the bran water-soluble components, mainly phytate, have about 39% of the total binding power, while the acid-treated water-soluble fraction has 60–70%. The combination of cellulose, starch, hemicellulose and pectin was not greater than 10%.

The effect of addition of 5 minerals (including Zn) on cellulose and CMC, as shown by the FRFs, considerably lowers the Zn-binding capacity. This may suggest a role for dietary fibre in the Zn-binding of FRF. As for the raw and cooked legumes, protein and phytates, respectively, may be more important in affecting Zn-binding.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the valuable assistance of the Department of Statistics, University of Reading, particularly Prof. R. Mead.

REFERENCES

American Association of Cereal Chemists (AACC) (1983). *Approved Methods of the American Association of Cereal Chemists*, 8th edn St Paul MN, USA.

Camire, A. L. & Clydesdale, F. M. (1981). Effect of pH and heat treatment on the binding of Ca, Mg, Zn and Fe to wheat bran and fractions of dietary fibre. *J. Food Sci.*, **46**, 548–51.

Chang, R., Schwinner, S. & Burr, N. K. (1977). Phytate: removal from whole dry beans by enzymatic hydrolysis and diffusion. *J. Food Sci.*, **42**(4), 1098–101.

Clydesdale, F. M. & Camire, A. L. (1983). Effect of pH and heat on the binding of Fe, Ca, Mg and Zn and the loss of phytic acid in soy flour, *J. Food Sci.*, **48**, 1272–83.

Department of Health and Social Security (DHSS). Committee on Medical Aspects of Food Policy (COMA). (1979). *Recommended Daily Amounts of Food, Energy and Nutrients for Groups of People in the UK*, Report on Health and Social Subjects No. 15, HMSO, London.

Evans, G. W. & Hahn, C. J. (1974). Copper and zinc binding components in rat intestine. In *Advances in Experimental Medicines and Biology*, vol. 48, ed. H. Friedman. Plenum Press, New York.

Latta, M. & Eskin, M. (1980). A simple and rapid colorimetric method for phytate determination. *J. Agric. Food Chem.*, **28**, 1313–5.

Lee, K. & Garcia-Lopez, J. S. (1985). Iron, zinc, copper and magnesium binding by cooked pinto bean (*Phaseolus vulgaris*), neutral and acid detergent fibre. *J. Food Sci.*, **50**, 651–73.

Maddaiah, V. T., Kurnic, A. A. & Reid, B. L. (1964). Phytic acid studies. *Proc. Soc. Exp. Biol. Med.*, **115**, 391–3.

O'dell, B. L. (1969). Effect of dietary components upon zinc availability: a review with original data. *Am. J. Clin. Nutr.*, **22**, 1315–22.

Rendleman, J. A. & Grobe, C. A. (1982). Cereal complexes: binding of zinc by bran and components of bran. *Cereal Chem.*, **59**(4), 310–7.

Solomons, N. W. & Jacob, R. A. (1981). Studies on the bioavailability of Zn in humans: effects of heme and nonheme iron on the absorption of Zn. *Am. J. Clin. Nutr.*, **34**, 475–82.

Thomspon, S. A. & Weber, C. W. (1979). Influence of pH on the binding of copper, zinc, and iron in six fibre sources. *J. Food Sci.*, **44**(3), 752–4.

Walker, A. F. (1982). Physiological effects of legumes in the human diet: a review. *J. Plant Foods*, **4**, 5–14.

Weingartner, K. E., Erdman, J. W., Parker, H. M. & Forbes, R. M. (1979). Effect of soybean hull upon the bioavailability of zinc and calcium from soy flour-based diets. *Nutr. Rep. Int.*, **19**(2), 223–31.