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

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The Ca-binding capacity of butter beans, broad beans and lentils in the raw, cooked and fibre-rich fraction (FRF) forms, with Ca addition alone and with Fe, Zn, Mg and Cu (at amounts based on the recommended daily allowance), was investigated in conditions simulating the small intestine. Overall, more Ca was bound when Ca was added with Fe, Zn, Mg and Cu than when Ca was added alone. Cooking significantly increased the Ca-binding capacity of the raw legumes when Ca was added alone. The FRFs gave the highest Ca-binding capacity in comparison to the raw and cooked legumes. The results suggest that the mineral-binding capacity of dietary fibre may influence mineral absorption.

INTRODUCTION

Mature legume seeds are widely consumed in development countries and provide a major source of dietary minerals, particularly Ca and Mg. The significance of Ca is well known in relation to osteomalacia and rickets. Very little attention has been paid to the effect of legume foods on mineral bioavailability even though, over the last two decades, they have attracted interest as a source of dietary fibre (DF) in human nutrition.

Both dietary fibre (DF) (Eastwood & Mitchell, 1976; Jenkins, 1980) and phytate (Cheryan, 1980) have been investigated as possible factors in mineral bioavailability. James *et al.* (1978) demonstrated Ca-binding to DF (mainly uronic acids) from 29 plant food sources at pH 7·4. The consensus is that mineral interactions with DF and phytate play an important role in mineral bioavailability.

Minerals vary considerably with regard to the form in which they are present in foods. Ca and Mg were found in cereal grains mainly as phytin (Ca_5 Mg phytate) (Kent, 1986). Fe, Zn, Cu and other trace minerals are present in plant foods mainly as metalloproteins (e.g. enzymes) or in storage complexes (Hazell, 1985). For example, Fe can be in the monoferric phytate (Fe phytate) or the calcium iron phytate (Ca_3 Fe phytate) form (Ellis *et al.*, 1982). Elhardallou and Walker (1992) found in an *in-vitro* study that more Fe was bound to legumes, under simulated small intestinal conditions, in the presence of added Ca. Barton *et al.* (1983) found a Ca dose-related inhibition of Fe absorption in rat small intestine loops. This would suggest that the relative concentrations of minerals present in the gut lumen influence their bioavailability.

This study investigates the Ca-binding capacity of cooked butter beans, broad beans, lentils, their equivalent fibre-rich fractions (FRF), carboxymethylcellulose (CMC) and Solka floc, and the influence of the presence of other nutritionally important minerals using a 1 litre simulated small intestine digestion model. For Ca, Mg, Zn, Fe and Cu the recommended daily amounts (RDAs) were found to be 800, 350, 15, 10 and 2–3 mg/day (for an adult man), respectively (Food and Nutrition Board of the National Research Academy of Sciences, USA, 1980; FAO/WHO, see Pike & Brown, 1984). In the present study, the mineral concentration levels used were based on half the RDA for Fe and Cu (5 and 1 mg, respectively) and on less than that for Ca, Mg and Zn (100, 100 and 2 mg, respectively).

MATERIALS AND METHODS

Legume samples and model fibres

Mature and healthy seeds of butter beans (*Phaseolus lunatus* L.) were supplied by Whitworths, Wellingborough, Northants, UK; broad beans (*Vicia faba* L., minor) were provided by the Agricultural Research Corporation, Shambat, Sudan; and lentils (*Lens culinaris* L.) were purchased from the local market in Reading. Solka floc (Lillico, Betchworth, Surrey, UK) and CMC (Whatman, Mainstone, Kent, UK) were the chosen model fibres for cellulose and hemicellulose,

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respectively present in legumes. CMC contains a definite number of carboxymethyl groups and its mineral-binding saturation 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 contained only small amounts of endogenous minerals.

Raw and cooked sample preparation

Clean seeds of each material were fine-milled using the pin disk mill (Simon Handling Engineers: now Satake-Robinson, Stockport, UK). For cooking, seeds were 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 75 min. The cooked material was cooled to room temperature, blended, frozen (–25°C), freeze-dried (22°C), milled to pass a 60 mesh sieve (1·67 mm^2) and stored at room temperature in air-tight bottles.

Preparation of buffer solution, pH 7 (Gomori's buffer)

Twenty-five millilitres of 0·02 M tris-maleate (24·2 g Trizma base, Sigma, and 19·6 g maleic anhydride dissolved in 1 litre deionized water) and 24 ml 0·2 M NaOH were diluted to 100 ml. Gomori's buffer was used instead of phosphate-containing reagents, to avoid possible interference with mineral detection by the atomic absorption spectrophotometer (AAS).

Pancreatin preparation

Pancreatin (Grade 4 from porcine pancreas; Sigma, Poole, Dorset, UK) (3 g) was first suspended in phosphate buffer (27 ml), pH 7 (in the proportion of 61 ml 0·1 M Na_2HPO_4 to 39 ml 0·1 M NaH_2PO_4), stirred using a magnetic stirrer for 15 min and centrifuged at 10000 rpm for 20 min. The residue was re-extracted as described and the combined supernatants used as the pancreatin solution.

FRF preparation

Cooked legume flour (100 g, on dry basis) was treated with pancreatin solution (40 ml), made to 1 litre volume with Gomori's buffer, a few crystals of thymol added to control microbial growth and it was incubated overnight at 37°C with continuous stirring. The slurry was centrifuged to recover the solids which were extracted as described. The recovered solid (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 a 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) methods. Protein percentage ($N \times 6{\cdot}25$) was determined using the Leco FP228 nitrogen analyser. The endogenous Ca, Fe, Zn, Mg and Cu were determined using the AACC (1983) method.

Phytic acid determination

Phytate was extracted (Chang *et al.*, 1977) and measured (Latta & Eskin, 1980).

Mineral stock solutions

Mineral stock solutions (Ca, 10000 ppm; Fe, 500 ppm; Zn, 200 ppm; Mg 10000 ppm; and Cu, 100 ppm) were made by adding 3·6677 g $CaCl_2.2H_2O$, 0·2489 g $FeSo_4.7H_2O$, 0·0880 g $ZnSO_4.7H_2O$, 10·1411 g $MgSo_4.7H_2O$ and 0·0393 g $CuSo_4.5H_2O$ to 1 ml of concentrated HCl and diluting to 100 ml. Mineral concentrations in the stock solutions were checked by AAS against BDH Certified Standard solutions.

Atomic absorption standards

Working standards in the ranges 4–20, 1–5, 0·4–2, 0·6–3 and 0·2–1 ppm for Ca, Fe, Zn, Mg and Cu, respectively, were prepared by dilution of stock solutions.

Ca, Fe, Zn, Mg and Cu adsorption isotherms

To determine the extent of saturation of the binding sites, binding tests were investigated, for each mineral. From stock mineral concentrations, the volumes equivalent to 50, 100, 150 and 200 ppm for Ca; 2·5, 5·0, 7·5 and 10 ppm for Fe; 1, 2, 3, and 4 ppm for Zn; 50, 100, 150 and 200 ppm for Mg; and 0·4, 0·8, 1·2 and 1·6 ppm for Cu were added each to 1 g (on dry basis) of raw or cooked legume flour or the FRF amount equivalent to that in 1 g cooked legume flour (butter beans, 0·2445 g; broad beans, 0·3296 g; and lentils, 0·2770 g) in a 250 ml Erlenmeyer flask and made up to 100 ml using Gomori's buffer. Each flask was tightly covered using Nescafilm and/or clingfilm 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; filtration was slow, particularly for the viscous solutions of cooked legumes. Filtates were placed in glass sample bottles and kept at 2°C for analysis. For each test, thee replicates were run, and Gomori's buffer (100 ml) was treated as a control.

Buffer-soluble endogenous Ca with and without mineral mixture

The method used by Camire and Clydesdale (1981) was followed with some modifications. To 1 g raw or cooked legume flour, Solka floc or CMC (on dry basis) in a 250 ml Erlenmeyer flask, 99 ml of Gomori's buffer was added with stirring. The pH was adjusted to 7 (±0·05) with drops of 0·5 M NaOH where necessary. For the buffer-soluble endogenous Ca in the presence of the mineral mixture (Fe, Zn, Mg and Cu at 5, 2, 100 and

1 ppm, respectively), the volume of the added minerals (4 ml) and NaOH needed for the pH adjustment were considered when making to volume. Each was incubated, filtered and assayed as described.

Ca-binding with and without mineral mixture

For Ca alone, 1 ml of Ca stock solution (10000 ppm) was added to the sample (1 g or FRF equivalent) and the volume made up to 100 ml using Gomori's buffer. For Ca with Fe, Zn, Mg and Cu, 1 ml of each stock mineral solution (Fe, 500 ppm; Zn, 200 ppm; Mg, 10000 ppm; and Cu, 100 pm) was added and then 1·0–3·5 ml 0·2 M NaOH added within the 100 ml volume, to keep the pH at 7 ± 0·05. Each was incubated, filtered and assayed as described.

Apparatus

The AAS, Unicam SP9, was used to measure Ca, Fe, Zn, Mg and Cu at wavelengths of 422·7, 248·3, 213·9, 285·2 and 324·8 nm, respectively, with the corresponding lamp currents (mA) of 8–10, 12–15, 8–10, 4 and 4–5. The flame condition was stoichiometric for Ca, Fe, Zn and Mg and Lean-stoichiometric for Cu.

Statistical treatment

The analysis of variance was carried out for Ca-binding data. In the case of the observed trends that were slightly dissimilar, the differences between the three- or two-way interaction mean values were investigated in more detail. The standard error of the difference (SED) between these means was calculated using the formula:

$$\text{SED}\ (\bar{X}_1 - \bar{X}_2) = [\text{RMS}\ (1/n_1 + 1/n_2]^{1/2}$$

(where RMS is the root mean square; and n_1 and n_2 are the numbers of observations. Any differences found between mean values compared, which were greater than twice the calculated standard error of difference (2 SED), were considered significant.

RESULTS

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, for the protein contents 24·6, 33·9 and 28·5%, respectively, and for the ash contents, 4·6, 4·1 and 3·1%, respectively. These results were found to be in agreement with those in the literature. Meiners *et al.* (1976), studying the proximate composition of mature dry legume seeds, attributed the differences in moisture content to the variations in the relative humidity of the surrounding atmosphere at harvest and storage. The protein content of butter beans was recorded as 22·1% (Platt, 1962) and 25·3% (Duke, 1981). Salih and Elhardallou (1986), investigating 12 broad bean cultivars, found the protein content to be in the range of 28·5–37·7%. Protein in raw whole lentils was found to be 27·9% (Duke, 1981). The ash contents of raw butter beans and lentils were reported as 3·9 and 3·2%, respectively (Duke, 1981); Kay (1979) reported 4·0% for broad beans.

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 non-starch polysaccharides, protein, lignin and starch.

The endogenous Ca, Fe, Zn, Mg and Cu contents of the raw legume flour and equivalent FRF are presented in Table 1. The buffer-soluble endogenous Ca was noted to be relatively higher in the cooked form (Table 2). With Fe, Zn, Mg and Cu addition, the three raw or cooked legumes, FRFs and Solka floc, showed less (or no) Ca in the supernatant. This finding is supported by the enhanced Ca-binding found in the presence of Fe, Zn, Mg and Cu (Table 3), except for cooked and FRF forms of broad beans and cooked lentils.

For the Ca adsorption isotherm, Ca concentrations of 50, 100 and 150 ppm presented a linear relationship to the bound Ca, indicating the potential of the Ca (100 ppm)-treated legumes for extra Ca-binding sites. However, at a Ca concentration of 200 ppm, the percentage of bound Ca fell (showing the classic Langmuir

Table 1. The mean (± SD) endogenous Ca, Fe, Zn, Mg and Cu in raw butter beans, broad beans and lentils and their equivalent FRF[a] and in Solka floc and CMC (mg/100 g)[a]

Sample	Mean endogenous mineral (mg/100 g dry matter)				
	Ca	Fe	Zn	Mg	Cu
Butter beans					
Raw	106 ± 0·65	6·8 ± 0·16	4·4 ± 0·12	138 ± 0·95	0·32 ± 0·07
FRF	97·7 ± 0·62	18·1 ± 0·70	3·0 ± 0·26	28·6 ± 0·77	1·11 ± 0·02
Broad beans					
Raw	167 ± 1·06	11·0 ± 0·32	5·8 ± 0·27	155 ± 1·08	0·91 ± 0·07
FRF	173 ± 0·61	23·6 ± 0·52	2·9 ± 0·36	42·4 ± 0·77	1·40 ± 0·01
Lentils					
Raw	151 ± 1·03	12·2 ± 0·41	4·8 ± 0·1	108 ± 0·55	0·91 ± 0·07
FRF	163 ± 0·65	39·2 ± 0·66	6·2 ± 0·36	34·9 ± 0·71	3·00 ± 0·20
Solka floc	18·8 ± 0·20	11·3 ± 0·40	0·5 ± 0·07	12·0 ± 0·45	0·22 ± 0·00
CMC	4·3 ± 0·30	7·4 ± 0·07	0·4 ± 0·07	4·5 ± 0·07	0·20 ± 0·00

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

Table 2. The means (±SD, in ppm) and percentage of the buffer-soluble endogenous Ca of raw and cooked butter beans, broad beans and lentils and their equivalent FRFs and of Solka floc and CMC and the mean (±SD, in ppm) of buffer-soluble endogenous Ca after the addition of Fe, Zn, Mg and Cu[a]

Sample (on dry basis)	Mean buffer-soluble endogenous Ca	% Buffer-soluble endogenous Ca	Mean buffer-soluble endogenous Ca after addition of Fe, Zn, Mg and Cu[b]
Butter beans			
Raw (10 g)	8·12 ± 0·17	(77)	3·9 ± 0·13
Cooked (10 g)	9·54 ± 0·13	(90)	5·88 ± 0·15
FRF (2·445 g)	2·00 ± 0·14	(84)	1·66 ± 0·11
Broad beans			
Raw (10 g)	9·17 ± 0·08	(55)	3·48 ± 0·07
Cooked (10 g)	12·7 ± 0·16	(76)	8·98 ± 0·14
FRF (3·296 g)	4·04 ± 0·14	(71)	3·77 ± 0·12
Lentils			
Raw (10 g)	5·44 ± 0·06	(36)	3·44 ± 0·14
Cooked (10 g)	14·3 ± 0·57	(95)	7·07 ± 0·12
FRF 2·77 g)	3·65 ± 0·14	(81)	2·06 ± 0·10
Solka floc (10 g)	0·36 ± 0·04	(19)	0·00
CMC 910 g)	0·00		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. 1 ppm is equivalent to 1 mg/10 g raw or cooked legume or 1 mg/FRF equivalent amount of FRF from 10 g raw legume.

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

Fe, Zn, Mg and Cu were added in 5, 2, 100 and 1 ppm amounts, respectively, in a mineral-binding experiment under conditions simulating the small intestine.

curve), suggesting saturation of binding sites and perhaps some weakly bound Ca. 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 Mg adsorption isotherm, Mg concentrations of 50, 100 and 150 ppm presented a linear relationship to the bound Mg. At a Mg concentration of 200 ppm, the percentage of bound Mg fell (showing the classic Langmuir curve). Ca, Fe, Zn, Mg and Cu adsorption isotherms indicated that mineral additions of 100, 5, 2, 100 and 1 ppm, respectively, would be

Table 3. The means (±SD, in ppm) and percentage of Ca (added and buffer-soluble endogenous amounts) bound by the raw and cooked butter beans, broad beans and lentils and their equivalent FRFs, and by Solka floc and CMC with Ca and Ca + Fe + Zn + Mg + Cu addition under conditions simulating the human intestinal tract[a]

Sample (on dry basis)	The bound Ca (and its %[b]) by legumes, FRFs, Solka floc or CMC with the addition of Ca alone or in combination	
	Added Ca (100 ppm)	Added Ca, Fe, Zn, Mg and Cu (100, 5, 2, 100 and 1 ppm, respectively)
Butter beans		
Raw (10 g)	6·36 ± 0·16 (5·9)	27·6 ± 0·31 (26·6)
Cooked (10 g)	14·4 ± 0·19 (13·1)	28·5 ± 0·31 (27·0)
FRF (2·445 g)	9·79 ± 0·12 (9·6)	27·3 ± 0·22 (26·8)
Broad beans		
Raw (10 g)	21·0 ± 0·21 (19·2)	44·1 ± 0·34 (42·7)
Cooked (10 g)	38·1 ± 0·30 (33·8)	25·8 ± 0·30 (24·6)
FRF (3·296 g)	39·8 ± 0·19 (65·0)	35·5 ± 0·20 (34·3)
Lentils		
Raw (10 g)	4·21 ± 0·13 (3·9)	31·6 ± 0·33 (30·5)
Cooked (10 g)	18·7 ± 0·20 (16·4)	17·8 ± 0·24 (16·6)
FRF (2·77 g)	11·1 ± 0·19 (10·7)	39·4 ± 0·21 (38·4)
Solka floc (10 g)	4·26 ± 0·08 (4·8)	30·6 ± 0·12 (30·6)
CMC (10 g)	32·0 ± 0·10 (32·0)	96·8 ± 0·17 (96·7)

[a]The Ca bound (the average of 3 replicates) was calculated by subtracting the mean of Ca in the supernatant from the total of added (100 ppm) and buffer-soluble endogenous amounts. Values in ppm are equivalent to mg/10 g of the raw and cooked samples or mg/equivalent FRF, as the volume used in the analysis was 100 ml and the weights were 1 g and its equivalent FRF.

[b]% Ca bound = $\frac{\text{Amount of Ca bound}}{\text{Amount of added Ca + buffer-soluble endogenous Ca (Table 2)}} \times 100.$

Table 4. The mean phytic acid content (g/100 g dry matter ± SD) of raw and cooked butter beans, broad beans and lentils and their equivalent FRFs[a]

Sample (on dry basis)	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

[a]The figures are the means of 3 individual replicates. The FRFs (in g) equivalent to 100 g (dry wt) of butter beans, broad beans and lentils are 24·45, 32·96 and 27·70, respectively.

appropriate since they are within the linear portion of the adsorption isotherms.

The mean values of Ca bound are presented in Table 3. From the ANOVA of the Ca-binding data, the significance of the effects (particularly for the Ca addition and legume form factors) was noted and illustrated in Fig. 1. Overall, more Ca was bound to the FRF form: FRF > > cooked > raw, with a clearly significant difference between the three forms. The cooked forms, in total, showed no significant difference (< 2 SED) in Ca-binding at separate Ca addition or with other minerals.

With Fe, Zn, Mg and Cu, added Ca was found to result (in total) in significantly greater Ca-binding than when Ca was added alone. For butter beans no interactions were noted and the binding differences for the two Ca additions were equal for each form.

The phytic acid content of the investigated legumes in the raw, cooked and FRF forms was found to be in the range of 0·11–1·5% (Table 4), with a relatively high content in broad beans.

DISCUSSION

In total, significantly, more Ca (added alone or with other minerals) was bound to broad beans than butter beans and lentils (each in the raw, cooked or FRF forms); Ca-binding of lentils was significantly (>2 SED) greater than butter beans.

The Ca-binding of broad bean FRF with additional Ca alone was found to be significantly ($P<0·01$) greater than for other legume FRFs. There was no significant difference (< 2 SED) between the Ca-binding capacity of broad bean FRF with Ca addition above and that of lentil FRF with Ca addition in the presence of other minerals.

These observations can be attributed partly to the relatively higher FRF (Table 3) and phytic acid (Table 4) contents of broad beans. Similarly, Henderson and Ankrah (1985) recorded a higher phytate content (1·02%) for broad beans. The bound Ca to 29 food plants (fibre extracts) was found to be in proportion to the uronic acid content, strongly pH-dependent (optimum pH 7·4) and consistent with ionic binding by charged carboxylic-acid groups (James *et al.*, 1978). Lyon (1984), investigating Ca solubility in cereal products, showed phytate (ranging from 0·03% in cornflakes to 2·59% in ALL-bran) to have a paramount role in Ca precipitation at pH 7. Phytate was also considered to be responsible for the negative Ca balance found in a study with human subjects (Royal College of Physicians,

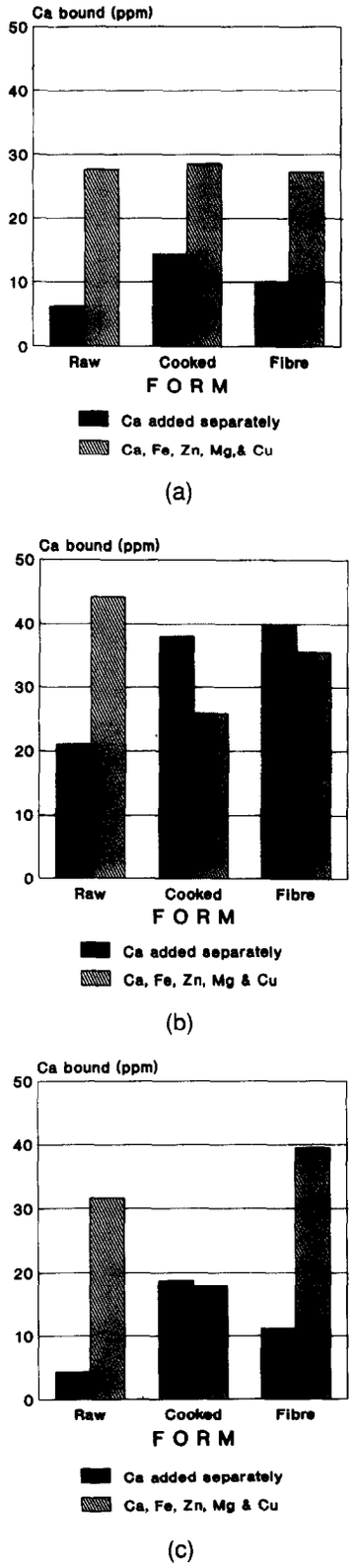

Fig. 1. The mean Ca-binding values of raw and cooked legumes and their equivalent fibre-rich fractions in the presence of Ca, alone or with Ca + Fe + Zn + Mg + Cu, under simulated human small intestinal conditions. (a) Butter beans, (b) broad beans, and (c) lentils.

1980). In the present study, the amount of 0·2 M NaOH in Gomori's buffer and that used to adjust the pH in the binding tests, may account for approximately 130 ppm Na^+. Most of the dietary sodium is derived from the salt (NaCl) added to cooked or processed foods such as bread (Pykel 1972). Sodium and potassium salts are entirely soluble, completely ionic and completely available for absorption (Hazell, 1985). However, monovalent Na^+ can displace other cations such as H^+, Ca^{2+} and Mg^{2+} from pectin to produce soluble pectates. Graf (1983) reported that Na^+ is several orders of magnitude weaker in interactions with phytic acid than divalent cations.

On addition of Ca alone, raw legumes showed a significant ($P < 0.01$) increase in Ca-binding after cooking. In studies of Ca-binding by wheat bran, Rendleman (1982) found cooking to increase the binding capacity. He suggested the availability of more Ca-binding sites (such as phytates) after cooking or acid treatment, previously occupied by other components such as proteins.

Solka floc treated with Ca alone and with Ca + other minerals, showed Ca-binding of 4·26 and 30·6%, respectively, and CMC showed 32·0 and 96·8% of bound Ca, respectively (Table 3), indicating more Ca-binding in the presence of other minerals as noted for legumes. However, the easily substituted carboxylate groups may account for the increased Ca-binding of CMC in comparison to cellulose. It was confirmed by Ogiwara and Kubota (1969), working on Ca-binding to cellulose (hardwood source), that the amount of Ca^{2+} (source: $CaCl_2.2H_2O$) adsorbed was about the same as the carboxyl group content. In legumes, carboxyl groups are available mostly in the uronic acid fraction of the hemicelluloses.

CONCLUSION

The present *in-vitro* study has demonstrated that some starchy legumes and their fibre-rich fractions have the capacity to bind Ca and that this effect is generally enhanced by the further addition of other minerals and by cooking. The ability of these foods to bind minerals may contribute to reduce bioavailability.

In populations where legume foods are a major food item, supplementation with Ca and other essential elements should be considered.

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