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Interactions of Fe(II), Ca(II) and Fe(III) with high dietary fibre materials: A physicochemical approach

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Interactions of Fe(II), Ca(II), and Fe(III) with five natural food materials, which should be used as fibre sources in the diet, such as pomaces from the production of white wine, cider and olive oil, and lemon peel and pulp, were investigated *in vitro*. The extent of mineral binding by these concentrates of fibre depended both on the nature and chemical composition of the sample used and on the type of mineral element studied; however, the majority of these samples exhibited a higher capacity to bind Fe(II) than Ca(II) and Fe(III). Two graphic methods have been used to provide basic information on the mineral binding mechanisms for the interactions of these cations with the high-fibre samples: the Scatchard plot, for examining binding by complex formation, and the equation of Langmuir, to predict cation retention by physical adsorption.

INTRODUCTION

Interactions between micronutrients, such as minerals and vitamins, and dietary fibre and closely associated substances (phytate, tannins, etc.) have been the focus of attention in recent years (Southgate, 1987; Torre *et al.*, 1991). In fact, much research has been done *in vitro* and *in vivo* on the contribution of purified components of dietary fibre (Fernandez & Phillips, 1982; Kohn, 1987; Platt & Clydesdale, 1987; Wieber *et al.*, 1988; Ha *et al.*, 1989; Schlemmer, 1989; Torre *et al.*, 1992), fractions of fibre (McBurney *et al.*, 1986; Platt & Clydesdale, 1987; Wieber *et al.*, 1988; Ha *et al.*, 1989; Schlemmer, 1989; Torre *et al.*, 1992) and whole natural foods rich in fibre (Thompson & Weber, 1979; Rendleman, 1982; Laszlo, 1987) to metal ion balances. The aim of most of these investigations is to make evident that fibre may be an important determinant of the utilisation of minerals in the diet. However, little research has been done to elucidate the factors and mechanisms governing the interactions of metallic ions with the plant cell wall material (Irwin *et al.*, 1984; Laszlo, 1987; Platt & Clyesdale, 1987) and also the relationship between the extent and selectivity of these interactions and the distribution of the main single constituents of fibre and related compounds (Rasper, 1979).

From a physicochemical point of view, the plant cell wall material is a macromolecular assembly made of a great diversity of polymer chains packed together (Laszlo, 1987; Eastwood & Morris, 1992). The constituents of these polymers contain numerous fixed polyvalent functional groups, mainly hydroxyl, and methoxy groups. Thus, the fibre matrix may be viewed as a naturally occurring substrate, which exhibits a characteristic capacity and specificity to associate metal ions in solution.

The present study has been undertaken to measure *in vitro* the iron (ferrous and ferric ions) and calcium-binding capacity of five fibre-rich materials and also to elucidate some of the physicochemical mechanisms which may be involved in this process. These cations have been chosen as models of common microelements and macroelements, respectively, of importance in nutrition. On the other hand, the tested samples are by-products from food and agricultural factories which are known to be rich in dietary fibres and thus may be used as natural sources of fibre enrichment in the diet. In spite of the complexity of these plant food materials rich in fibre in contrast to the single polymers or fractions of fibre, we have chosen the former as a more realistic approximation to the high-fibre whole foods in the diet of consumers.

For the assessment of ion-interaction phenomena and the interpretation of binding data we have adopted two of the most developed and simplified models

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reported in the literature which have been successfully applied for the prediction of mineral binding in natural and synthetic substrates: the complex formation and adsorption.

MATERIALS AND METHODS

Materials

The concentrates of fibre used were white wine pomace (WWP), from Cooperativa de Manzanares, Ciudad Real; apple pomace (AP), from Sidra El Gaitero, S. A., Villaviciosa, Asturias; olive pomace (OP), from Carbonell, S. A.; lemon peel (LP) and lemon pulp (LPP), obtained from Citricos de Murcia, S. A., Grupo Kas, Murcia, and Vital S. A., Gandía, Valencia.

The five crude fibre sources were first dried at 60°C or lyophilised (lemon pulp and lemon peel) and stored frozen. All dried material was ground to pass a 0·5 mm screen prior to further treatment. The samples were then treated with a chloroform–methanol 2 : 1 (v/v) mixture and, subsequently, with four volumes (25 ml) of hot 95% ethanol, in order to remove pigments, lipids and ethanol-soluble sugars. The residues left by these samples after the washing steps were mixed with 40 ml of acetone for 5 min, centrifuged for 10 min at 1500 × *g*, and air-dried at room temperature. Dry matter (DM) content for these residues was determined by drying at 110°C to a constant weight.

Analytical data of the fibre composition (Goñi *et al.*, 1989; Saura-Calixto *et al.*, 1989; Mañas *et al.*, 1990; Saura-Calixto *et al.*, 1991) of these samples are shown in Table 1.

Individual components of fibre were also used: lignin, obtained from Therapharm Ltd (Downham Market, Norfolk, UK) and cellulose (powder, 20 μm), purchased from Sigma Chemical Co. (St Louis, MO, USA).

Reagents

All reagents were of analytical grade. Anhydrous calcium chloride ($CaCl_2$), fused granular about 0·5–2·0 mm, was obtained from Merck (D-6100 Darmstadt, Germany). Iron sources were as follows: ferrous sulphate heptahydrate (Sigma Chemical Co. St Louis, MO, USA) and iron(III) stock certified atomic absorption reference solution in 20% HCl (Fisher Sci. Co., Medford, MA, USA).

Solutions of metallic cations were prepared with distilled deionised water.

All glassware was acid-washed with HNO_3 1 : 1 (v/v) and rinsed with distilled deionised water.

Atomic absorption standards

Certified atomic absorption reference solutions of 1000 ppm Ca (Carlo Erba, Milan, Italy) and 1000 ppm Fe (Fisher Sci. Co., Medford, MA, USA), were used.

Apparatus

A Perkin-Elmer Model 2380 atomic absorption spectrophotometer (AAS) with an air–acetylene flame, impact bead, and corrosion-resistant nebuliser was used to measure Fe and Ca at 248·3 nm and 422·7 nm, respectively. Monoelemental hollow cathode lamps were used for each element.

The apparatus for pH measurements was a MicropH 2000 Crison pH meter, with a glass/reference electrode.

Calcium and iron analysis

For calcium and iron determinations, the concentration of each metal was calculated from the direct comparison of the signal of absorbance in the spectrophotometer of solutions of these mineral ions with the calibration curve prepared using appropriate standards (in the range 0–5 ppm of metallic cation in solution). The analysis of calcium required the addition of lanthanum chloride (final concentration of 0·4% w/v) to each sample and standard in order to prevent the formation of calcium phosphate compounds.

Analyses of samples and calibration curve were carried out in duplicate.

Analysis for total endogenous calcium and iron

Approximately 1·00 g of each fibre sample was digested (in triplicate) with concentrated HNO_3, according to the procedure described by Platt and Clydesdale (1987).

Table 1. Content and composition of dietary fibre of some by-products from food and agricultural factories (Goñi *et al.*, 1989; Saura-Calixto *et al.*, 1989; Mañas *et al.*, 1990; Saura-Calixto *et al.*, 1991)[a]

	Fibre			Klason	Uronic acids	
Sample	Total	Insoluble	Soluble	lignin	Insoluble	Soluble
White wine pomace	58·6	56·3	2·3	41·2	2·60	1·30
Olive pomace	69·4	65·7	3·7	37·2	2·30	1·80
Apple pomace	62·5	48·3	14·2	18·2	5·75	9·94
Lemon pulp	45·8	26·0	19·8	2·9	4·78	7·24
Lemon peel	50·9	28·2	22·7	5·5	5·33	19·9

[a]Dietary fibre analysed by the AOAC method (Prosky *et al.*, 1988); Klason lignin was determined gravimetrically after acid treatment of the IDF residue. Results expressed as a percentage of original dry matter. Values represent the mean of at least three determinations.

Procedure

Binding of calcium and iron by fibre was measured by the procedure of mixing the sample with a solution which contained a known free concentration of metallic ion and following centrifugation after equilibration for quantitative measurement of soluble mineral ion in the supernatant.

Solutions (approximately 0·4% of sample, w/v) which contained one of the three minerals in the range of concentration 0–4·5 μeq/ml Fe(II), 0–6 μeq/ml Ca(II) or 0–3 μeq/ml Fe(III), at a constant pH, were prepared. The desired pH of these solutions was adjusted with diluted HCl or NaOH, in order to keep the final ionic strength as low as possible.

The pH is one of the essential factors which affect iron solubility, stability and oxidation state (Nojeim *et al.*, 1981; Fernandez & Phillips, 1982). For this reason, the pH was varied from 4·5 to 5·0 in the experiments with ferrous iron (Nojeim *et al.*, 1981; Fernandez & Phillips, 1982; Johnson, 1989; Smith, 1983) while the pH range selected for ferric iron was 1·5–3·0, due to the strong tendency of this trivalent cation to hydrolyse and form polynuclear species which could interfere with the estimates of iron binding by the samples at the less acidic conditions of the medium (Platt & Clydesdale, 1987; Miller & Berner, 1989). In any case, iron solutions were prepared shortly before they were to be used.

Approximately 100 (±0·1) mg, in duplicate, of sample were suspended in 25 ml of metallic solution. The suspension was shaken (in screw-capped centrifuge tubes of 50 ml capacity) at room temperature for 3 h, which time was sufficient to achieve equilibrium between samples and mineral ions in aqueous solution. The reaction mixture was then centrifuged for 15–20 min at 1500 × *g* and the resulting clear supernatant was removed and analysed for equilibrium mineral ion concentration. The equilibrium pH of the supernatants was also recorded. In order to determine the contribution of the endogenous minerals to the total mineral content of the system, parallel control or blank experiments were done in which the metallic cation was omitted.

The amount of citrus samples used in the experiments with calcium was approximately 50 mg, since the tendency of these fibre concentrates to aggregate in solutions of this cation was a problem of importance in the study of calcium binding.

Calculations

The amount of bound mineral (BC) in an insoluble form by the samples was calculated from the mineral equilibrium concentration in the supernatant (EC), the initial metal ion concentration which was added to the sample (IC), and the mass of the ethanol-insoluble residues (*m*). The expression was:

$$BC = (IC-EC^*)/m \text{ (expressed in } \mu eq/g \text{ DM)} \quad (1)$$

where

$$EC^* = EC-EC_b \ (\mu eq/ml) \quad (2)$$

was the residual equilibrium concentration in the supernatant, corrected by the soluble endogenous mineral concentration of blank or control experiments (EC_b).

Assays were performed at least twice. Results are expressed as the mean value of binding determinations. The repeatability, given as the coefficient of variation, was in all cases < 4%.

RESULTS AND DISCUSSION

Theory

Considering the plant cell wall material as a macromolecular structure which consists of a three-dimensional array of polymer chains containing a high enough proportion of fixed anionic and cationic functional groups and with capacity of hydration and swelling, we may propose at least three mechanisms of interactions between this plant cell wall material and metallic ions: complex formation, by either electrostatic forces or chelation, physical adsorption, and ion-exchange.

When considering the mechanism of complex formation, interactions of metallic ions with the plant cell wall material proceed in accordance with the multiple equilibria law, with formation of a stoichiometric bond. On the other hand, all interactions in adsorption occurring in the plant cell wall material start on the surface of its macromolecular constituents and are frequently caused by inter-ionic attraction. Thus, of great importance are the more accessible sites of the surface of these polymers. The mechanism of ion-exchange has not been examined since the interpretation of experimental data according to the ion-binding phenomenon in natural systems can be extremely complicated. In fact, the process of ion-exchange is based on a theoretical treatment of great complexity, which requires the knowledge of parameters experimentally inaccessible for most biological systems.

For evaluation of the binding parameters of interactions by either complex formation or adsorption we have applied two theoretical models which have been described in the literature: the graphic model of Scatchard, for examining metal ion interactions with fibre by complex formation (Rosenthal, 1967), and the Langmuir equation, to predict ion binding by adsorption in the simplest situations where the first ions adsorbed cover up the most active sites so that additional adsorption is decreased (Schwartz & Elving, 1983). Both models are based on simplified equations requiring the measurement of a limited number of parameters in the experimental conditions we have employed in our laboratory and which may be achieved in physiological circumstances.

The equation of Scatchard plot is given by

$$BC/EC = K_{eff} \times BC_{max} - K_{eff} \times BC \quad (3)$$

and the Langmuir adsorption isotherm follows the equation:

$$IC/BC = (1/K_{ads} \times BC_{max}) + (1/BC_{max}) \times IC \quad (4)$$

Table 2. Total and soluble endogenous calcium and iron content of five high dietary fibre materials[a]

Sample	Total (mg/g)		Soluble (%) as a function of pH			
			Iron		Calcium	
	Iron	Calcium	1·5–3·0	3·0–4·5	5·0–6·0	6·0–8·0
White wine pomace	1·30 ± 0·03	22·34 ± 1·46	100	2·0	53	48
Olive pomace	0·63 ± 0·05	6·36 ± 0·38	58	0·0	49	55
Apple pomace	0·12 ± 0·00	0·31 ± 0·02	0·0	0·0	18	0·0
Lemon pulp	<0·02[b]	6·46 ± 0·29	0·0	0·0	0·0	0·0
Lemon peel	<0·02[b]	12·13 ± 0·60	0·0	0·0	0·0	0·0

[a]Results expressed on dry weight basis of the ethanol-insoluble residues. Mean of three determinations.
[b]Concentration of metallic element less than or equal to the detection limit of the analytical method.

These two graphic methods used to plot the experimental results yield a straight line in a graph for the simplest case of one mobile ion and one type of mutually noninteracting molecular binding sites. In the Scatchard plot, if the macromolecular structure has more than one type of metal binding site, a curved line is obtained rather than a straight line and the estimation of the binding parameters requires a more complex graphic analysis (Pennock, 1973).

Estimation of the two parameters for the binding can be obtained by these graphic models. In the Scatchard graph the slope is a measure of the stability constant (K_{eff}) which gives us an idea of the affinity of a given sample for a specific cation, and the intercept on the abscissa shows the amount of mineral bound by the respective binding sites (BC_{max}) (Platt & Clydesdale, 1987). Moreover, when plotting the Langmuir equation, the slope and the ordinate are a measure of the limiting amount of mineral adsorbed in the surface and the constant of associations (K_{ads}), respectively.

These equations are useful to predict the extent of cation association with the plant cell wall material in its integrity (plant cell wall polysaccharides, lignins, proteins, inorganic constituents, and other related components).

Mineral binding

The endogenous total iron and calcium content of the five concentrates of fibre and the percentage of solubilization of these cations as a function of pH is shown in Table 2. These results demonstrate that the residual calcium content of these samples ranged from 0·03 to 2·2% (w/w DM), while the endogenous iron content was of much less importance (< 0·1% in all cases).

The pH treatment in control experiments had significant effects on the binding of endogenous minerals. In fact, over the pH range from 5·0 to 8·0 and pH <3·0 a significant percentage of the residual calcium and iron content, respectively, was released from pomaces. In most cases, the endogenous minerals became re-bound when the pH was raised. Nevertheless, the amount of minerals solubilised from citrus was negligible at any pH.

The results of these studies evidenced that the two minerals were bound differently to the various concen-

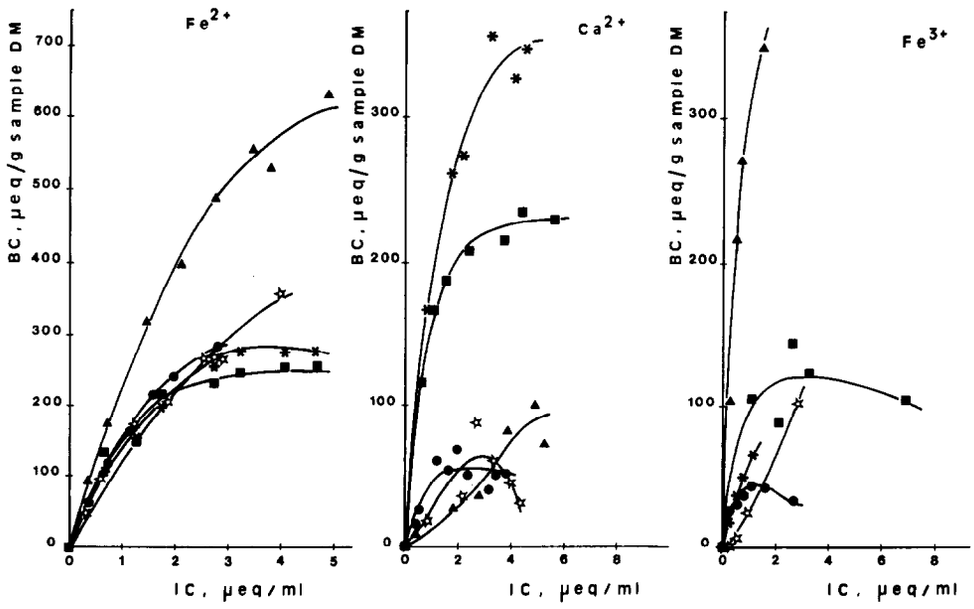

Fig. 1. Effect of initial metal ion concentration on mineral binding by five fibre concentrates at a given pH. WWP (▲); OP (●); AP (■); LPP (*); LP (☆). Results are referred to dry matter of the 95% ethanol-insoluble residues. The values of initial pH and mass of sample (mg) were, respectively: Fe(II): 4·81 ± 0·18 and 96·6 ± 4·7; Ca(II): 5·72 ± 0·16 and 97·1 ± 2·7 (49·4 ± 2·0 for citrus samples); Fe(III): 1·90 ± 0·10 (3·23 ± 0·02 for WWP) and 109·8 ± 13·0.

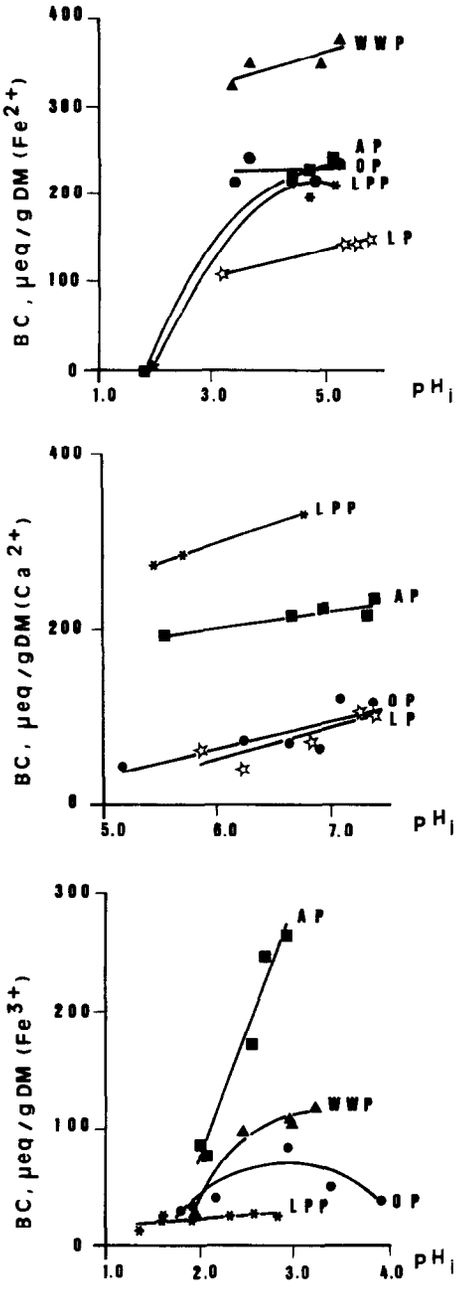

Fig. 2. Dependence of the binding of Fe(II), Ca(II) and Fe(III) by five fibre concentrates upon pH, at a given initial mineral concentration. Results are referred to dry matter of the 95% ethanol-insoluble residues. The values of initial mineral concentration (μeq/ml) and mass of sample (mg) were, respectively: Fe(II), WWP: 1·63 ± 0·04, 92·7 ± 0·9; OP: 0·85 ± 0·03, 97·7 ± 3·9; AP: 1·92 ± 0·03, 96·9 ± 1·7; LPP: 1·78 ± 0·04, 95·7 ± 0·9; LP: 2·20 ± 0·05, 94·9 ± 1·2. Ca(II), OP: 2·57 ± 0·18, 105·9 ± 2·6; AP: 2·18 ± 0·03, 101·2 ± 3·9; LPP: 1·72 ± 0·05, 42·4 ± 3·6; LP: 2·35 ± 0·04, 48·4 ± 0·6. Fe(III), WWP: 0·51 ± 0·03, 106·3 ± 7·7; OP: 0·56 ± 0·03, 101·3 ± 5·5; AP: 2·64 ± 0·02, 97·6 ± 1·3 LPP: 0·43 ± 0·05, 140·8 ± 14·3·

trates of fibre, according to the studies of Thompson and Weber (1979), and, also, the importance of control experiments for correcting from the release of naturally occurring minerals in binding studies (Laszlo, 1987).

Effect of initial mineral concentration on binding

The amount of Fe(II), Ca(II) and Fe(III) bound to the concentrates of fibre when fixed amounts of these samples were equilibrated with variable concentrations of these minerals, at a constant pH, is illustrated in Fig. 1.

As shown in these graphs, the quantity of polyvalent cations bound by high dietary fibre materials increased at higher levels of mineral addition. The relationship was linear at low concentrations of cation in solution and reached a plateau at higher concentrations, when the concentrates of fibre became saturated with minerals. The differences observed in the maximum retention depended on the nature and chemical composition of the sample used and, also, on the type of mineral element; however, we can state that the majority of the concentrates of fibre bound far more of the Fe(II) than the two other cations.

The greater capacity of these samples to bind Fe(II) than Fe(III) may be due to the higher stability of Fe(II) in food systems (Lee & Clydesdale, 1979; Nojeim & Clydesdale, 1981). On the other hand, the amount of calcium retained by white wine and olive pomaces was minimal over the complete range of initial calcium concentration in solution. This may be explained by the significant release of endogenous calcium from these samples over the pH range 5·0–8·0, which contributed to the total calcium content of the system, modifiying the equilibrium between free calcium in solution and the sample (Laszlo, 1987).

Effect of pH on binding

The effect of pH on mineral binding was also studied for all samples so as to give complementary information (Fig. 2). These results indicate that increasing the pH of metallic solutions increased the amount of bound mineral. The marked increase in Fe(II) binding at pH values higher than 3·0 indicated the contribution of proton-dissociating chemical groups, such as β-carboxyl, phenolic and amino groups of the polymeric constituents of these samples, to the uptake of this ion. The greater ability of the samples in the binding of calcium at higher pH was not so marked, indicating that the dissociation of hydrogen ions from the functional groups was not preferentially related to the binding of this cation. The pronounced effect of pH on Fe(III) retention as the pH rose in the range 1·5 to, approximately, 3·5 was not so easily explained since the mass action law did not favour a change of ionisation of the functional groups in the fibre matrix at the less acidic conditions of the solution.

Application of theoretical models for the interpretation of experimental results

In order to provide a physicochemical insight in mineral binding by the fibre concentrates, under the conditions in which this study has been carried out, we have applied the two given models to our experimental results. Due to the fact that it is very difficult to know the amount of metallic ion which is retained by one or another mechanism, we have started from the hypothesis that the element is bound by just one mechanism; if our experimental results fit well to the proposed model

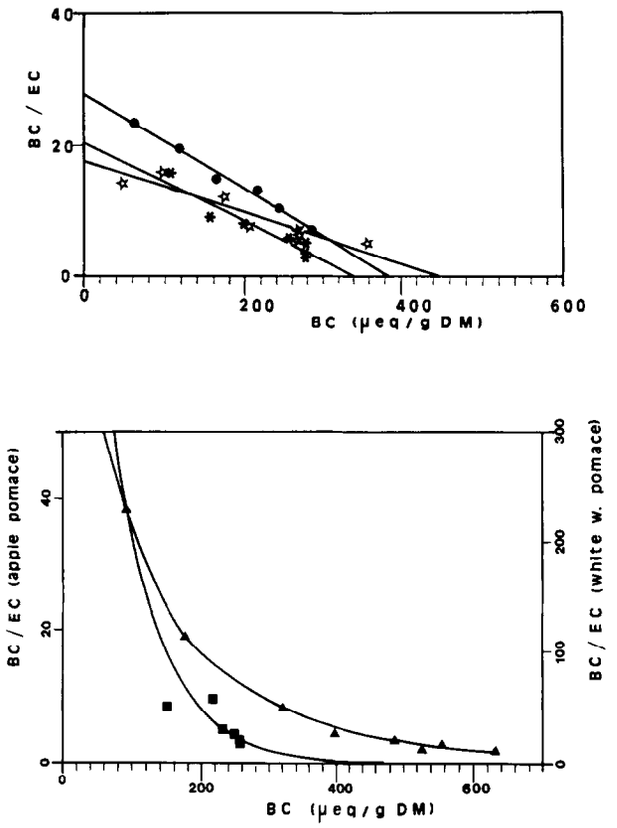

Fig. 3. Scatchard plots of the binding of Fe(II) by five fibre concentrates. Along the ordinate is plotted the quotient bound mineral concentration to equilibrium concentration and along the abscissa is plotted the bound mineral concentration. WWP (▲); OP (●); AP (■); LPP (*); LP (☆). Estimates of binding are based on the means of measurements whose results are illustrated in Fig. 1.

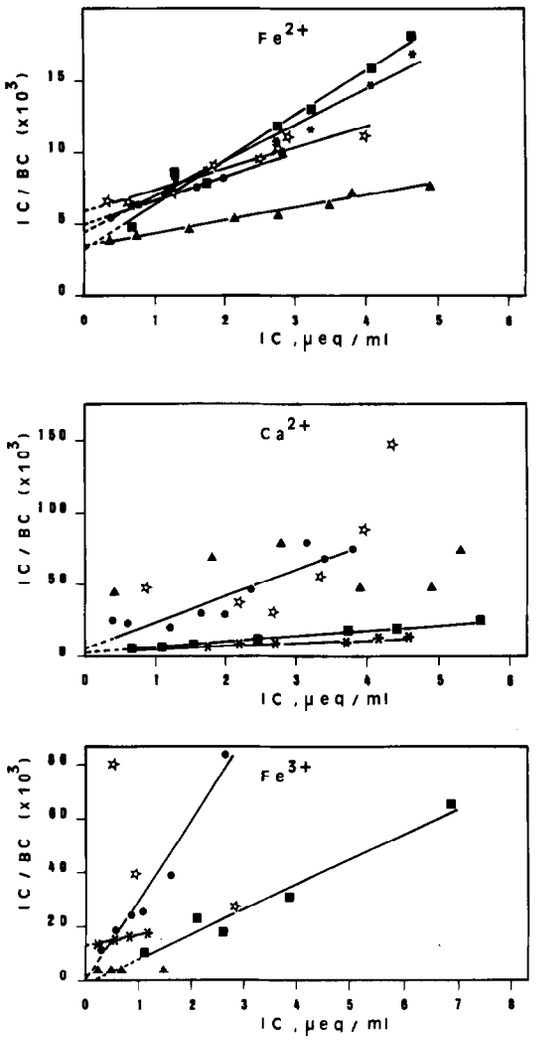

Fig. 4. Application of the equation of Langmuir to the binding of Fe(II), Ca(II) and Fe(III) by five fibre concentrates. Along the ordinate is plotted the quotient initial mineral concentration to bound concentration and along the abscissa is plotted the initial mineral concentration. WWP (▲); OP (●); AP (■); LPP (*); LP (☆). Estimates of binding are based on the means of measurements whose results are illustrated in Fig. 1.

it must be admitted that the cation is preferentially retained by this mechanism, although this is not necessarily the only mechanism that could describe the mineral binding by the fibre concentrates.

The results of Fe(II) binding replotted according to Scatchard (Fig. 3) demonstrate that Fe(II) retention by all the samples proceeded in accordance with the theory of multiple equilibria, with formation of stoichiometric bonds. Experimental points were fitted by a straight line, with a very good correlation coefficient, for olive pomace and lemon peel and pulp, suggesting the presence in these samples of one binding site with a high complexation ability for Fe(II). Curved lines obtained for apple and white wine pomaces indicated two or more sites for the binding of Fe(II) (Pennock, 1973; Platt & Clydesdale, 1987). For these samples, each binding curve could be resolved into two straight lines (Rosenthal, 1967), which showed the presence of two binding sites of different nature in each one of these fibre concentrates.

For calcium, with the exception of apple pomace, there were no binding lines which satisfied the equation of Scatchard. Hence, the majority of these fibre concentrates did not bind calcium by complex formation. It is very likely that the high endogenous calcium content of these samples might be blocking those negatively charged units of the cell wall matrix which acted as ligands of the calcium ions added to the system. Moreover, Fe(III) was not bound in accordance with the multiple equilibria law to any sample.

By assuming the mechanism of adsorption (Fig. 4), it seemed that the isotherms of mineral retention on the fibre concentrates followed, in most cases, the Langmuir pattern and good fits with the Langmuir equation are generally obtained for divalent cations. Adsorption of Fe(II) occurred on all samples. Moreover, with the exception of white wine pomace and lemon peel, the model of adsorption could be suggested to adequately explain the binding of calcium ions. However, we should propose, again, a different mode of cation-binding for ferric ions.

The mineral binding characteristics corresponding to the mechanisms of complex formation and adsorption are presented in Table 3. Comparisons were made with mineral binding data corresponding to insoluble constituents of fibre (lignin and cellulose). Examination of the interactions of lignin and cellulose with Fe(II),

Ca(II) and Fe(III) showed that binding of Fe(II) and Ca(II) by lignin could be explained by both complex formation or adsorption, although these mechanisms could not be proposed to describe the retention of Fe(III). On the other hand, minerals were associated with cellulose preferentially by adsorption.

With respect to the high-fibre materials, for Fe(II) binding the stability constant obtained from Scatchard analysis decreased in the following order: white wine pomace > apple pomace > olive pomace > lemon pulp > lemon peel. Thus, the ferrous iron binding affinity by complex formation could be related to the lignin content in the fibre concentrates (higher in pomaces than in citrus). Dealing with the adsorption mechanism, whenever the association constants were to be compared, it could be stated that mineral binding forces by adsorption were greater for those ions of greater ionic radius: Ca^{2+} (0·99 Å) > Fe^{2+} (0·74 Å) > Fe^{3+} (0·64 Å).

From Table 3 it is also obvious that values of the association constants corresponding to complex formation are higher (by a factor of 10^2 or 10^3) than those of adsorption.

On the other hand, the maximum bound concentration predicted by each one of the proposed models differed greatly and were, in general, higher than maximum retention data found experimentally. As an example, it is interesting to note the marked differences found among the limiting Fe(II) and Fe(III) concentration values corresponding to olive pomace and lemon pulp, respectively. This might suggest the competition of the different mechanisms of interaction in the binding of mineral ions by the concentrates of fibre. In fact, such competition of mechanisms may be attributed to the different contribution of each of the components in the sample.

The extent to which the mineral binding properties of the five tested fibre materials correlated with the distribution of the main individual components of fibre in these samples could be determined according to the linear correlation coefficients summarised in Table 4. According to these data, Fe(II) retention was highly correlated with all of those components of the cell wall material which had easily ionisable functional groups in their structures. The binding of this cation increased with an increase in the insoluble fraction in the samples; this means that lignin and the insoluble uronic acids exhibited a high Fe(II) binding capacity in contrast with the soluble fibre constituents.

On the other hand, Ca(II) was found in highly significant positive correlation with the soluble fibre fraction

Table 3. Comparison of the binding characteristics for the interactions of Fe(II), Ca(II) and Fe(III) with two insoluble components of fibre (lignin and cellulose) and with high-fibre materials, according to the proposed physicochemical mechanisms of interaction

	Maximum bound concentration (BC_{max}) by a given graphical model[a]			Interaction constant (M^{-1})		Predominant mechanism[b]	
System	Experimental value[c]	Complex formation	Physical adsorption	Complex formation (K_{eff})	Physical absorption (K_{ads})	Complex formation	Physical absorption
Fe(II)							
Lignin	240·0	247·5	282·0	$8{\cdot}71 \times 10^3$	$2{\cdot}44 \times 10^3$	++	+
Cellulose	40·0	—	45·7	—	$2{\cdot}31 \times 10^3$	–	+
White wine pomace	625·0	137·5 557·5	1134·0	$1{\cdot}67 \times 10^5$ $3{\cdot}51 \times 10^3$	$5{\cdot}20 \times 10^2$	+++	+
Olive pomace	285·0	386·4	622·0	$3{\cdot}70 \times 10^3$	$6{\cdot}33 \times 10^2$	++	+
Apple pomace	255·0	122·0 244·0	320·0	$1{\cdot}43 \times 10^5$ $9{\cdot}34 \times 10^2$	$1{\cdot}90 \times 10^3$	+++	+
Lemon pulp	275·0	341·3	402·3	$3{\cdot}00 \times 10^3$	$1{\cdot}10 \times 10^3$	+	+
Lemon peel	350·0	448·7	664·3	$1{\cdot}95 \times 10^3$	$5{\cdot}18 \times 10^2$	++	+
Ca(II)							
Lignin	220·0	229·5	237·8	$2{\cdot}40 \times 10^4$	$6{\cdot}80 \times 10^3$	++	+
Cellulose	20·0	—	—	—	—	—	+
White wine pomace	90·0	—	—	—	—	—	—
Olive pomace	50·0	—	55·7	—	$2{\cdot}59 \times 10^3$	—	+
Apple pomace	230·0	236·0	260·0	$1{\cdot}14 \times 10^4$	$3{\cdot}10 \times 10^3$	++	+
Lemon pulp	345·0	—	427·0	—	$2{\cdot}17 \times 10^3$	—	+
Lemon peel	75·0	—	—	—	—	—	
Fe(III)							
Lignin	440·0	—	—	—	—	—	—
Cellulose	50·0	—	131·6	—	$4{\cdot}53 \times 10^2$		+
White wine pomace	400·0	—	—	—	—	—	—
Olive pomace	45·0	43·7	—	$1{\cdot}26 \times 10^4$	—	+	—
Apple pomace	125·0	—	—	—	—	—	—
Lemon pulp	70·0	168·6	230·3	$2{\cdot}22 \times 10^3$	$1{\cdot}03 \times 10^3$	+	+
Lemon peel	100·0	—	—	—	—	—	—

[a]Values expressed as μeq M^{n+}/g sample (DM).
[b]Qualitative appreciation starting from the values of the interaction constants.
[c]Bound concentration calculated from the plateau (value of saturation) of binding curves in Fig. 1.

Table 4. Linear correlation coefficients of mineral binding (at a given initial mineral concentration and pH) with the composition of five fibre concentrates

Component (%)	Fe(II) IC (μeq/ml) = 1·37 ± 0·14 pH_i = 4·81 ± 0·18	Ca(II)[a] IC (μeq/ml) = 1·61 ± 0;18 pH_i = 5·73 ± 0·23	Fe(III) IC (μeq/ml) = 1·13 ± 0·19 pH_i = 2·16 ± 0·60
Klason lignin	0·811	0·999(–)	0·625
Insoluble uronic acids	0·793(–)[b]	0·961	0·451(–)
Soluble uronic acids	0·601(–)	0·990	0·541(–)
Insoluble dietary fibre	0·571	0·717(–)[c]	0·385[c]
Soluble dietary fibre	0·784(–)	0·996	0·522(–)

[a]The line of regression was calculated without the binding data of citrus samples.
[b]Negative correlation was found for a given component of the sample.
[c]Regression equations are not significant at $P < 0·05$.

and its constituents (mainly soluble uronic acids), which corroborated the different mechanism of retention observed for this cation with respect to Fe(II). It should be noted that the values of retention for citrus samples were not included in these regression analyses for calcium; the correlation values would change if citrus samples were included since the binding experiments with these fibre concentrates were conducted with half the amount of sample, as stated in the experimental part of this paper. Lastly, with the exception of the relation with lignin, the binding of Fe(III) by all of the tested samples was not significantly correlated with the composition of these. The differences observed between the behaviour of Fe(III) and that of the two divalent cations may involve the high valence and small size of this ion; however, more work is needed in order to draw such a conclusion.

CONCLUSION

The *in-vitro* study described herein shows that the experimental mineral binding by high dietary fibre materials can be successfully explained by the theoretical mechanisms of complex formation and adsorption. Fe(II) retention is preferentially governed by the mechanism of complex formation, while Ca(II) binding proceeds very likely in accordance with the model of physical adsorption. From our results, the mode of Fe(III) binding to fibre concentrates remains an open question and further research is required.

By comparing the mineral binding capacity predicted by each one of the proposed models for the five tested fibre concentrates used in this study and that experimentally observed, it is evident that the association of minerals may be the result of the competition or cooperation of at least two of the given mechanisms. Moreover, the mode of binding is very much dependent on the relative content of the different fibre constituents in each sample.

Although in-vitro studies cannot really simulate the complex nature of the absorption processes within the intestine (physiological conditions of nutritional status, gastrointestinal secretions, physical form of food, mineral solubility) and the interactions that occur with other food components affecting mineral availability, the research presented in this article may be used to acquire increased understanding of the interactions between mineral elements and plant cell wall materials observed *in vivo*.

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