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In vitro binding of calcium, iron and zinc by non-starch polysaccharides

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Abstract

The in vitro binding capacity of eight non-starch polysaccharides (agar, κ-carrageenan, gum xanthan, gum arabic, gum karaya, gum tragacanth, pectin and gum guar) was measured by equilibrium dialysis in neutral and acidic (0.1M HCl) solutions in the presence of divalent cations (Ca^{2+}, Zn^{2+}). No significant binding was observed in acidic conditions while, in neutral solutions, the extent of binding was correlated ($P < 0.1$) to the cation-exchange capacity of the polysaccharides. It is apparent that the interactions are essentially electrostatic in nature, due to the presence of ionised carboxyl (uronic/pyruvic acids) and sulphated groups, in polyanionic polysaccharides. By contrast, significant binding occurs with Fe^{3+} in acidic conditions, presumably due to complexation (chelation). These data provide a clear insight into how non-starch polysaccharides interact with minerals and the potential nutritional consequence in terms of bioavailability.

Keywords: In vitro binding; Bioavailability; Non-starch polysaccharides; Calcium; Iron; Zinc

1. Introduction

Epidemiological data have suggested that the intake of dietary fibre is a protective factor against the incidence of a wide range of diseases prevalent in industrialised countries (Burkitt & Trowell, 1975). However, some of the fibre components have the ability to bind cations and therefore reduce the bioavailability of nutritionally important minerals and trace elements. A well-known example of such interaction in the plant cell walls is the complexation of Ca^{2+} with non-esterified polygalacturonan chains of pectin (Brett & Waldron, 1996), via a mechanism called the "egg-box" model (Grant, Morris, Rees, Smith & Thom 1973). However, in addition to acidic polysaccharides (i.e. pectin, alginate), dietary fibres are associated with other compounds which also have the potential to bind minerals such as phytate, polyphenols (e.g. tannins) and organic acids (e.g. oxalic acid) (British Nutrition Foundation, 1990). Therefore, it is important to clearly identify chemically the functional groups responsible for cation binding.

The issue of the effects of dietary fibre on mineral bioavailability has been the subject of a number of reviews (Davies, 1978; James, 1980; Kelsay, 1986; Munoz & Harland, 1993; Southgate, 1987; Torre, Rodriguez & Saura-Calixto 1991). These reviews indicate that there is a paucity of knowledge with respect to binding mechanisms and the nutritional significance of in vitro data. For example, it is often difficult to discriminate between the binding capacity due to uronic acids and phytic acid in cell wall preparations (Southgate, 1987).

Gums are used extensively in food manufacturing but their micronutrient binding capacity is poorly understood. This has major implications with respect to food production, fortification, health products and overall bioavailability. Hence there is a need to understand these interactions. Therefore, a range of purified neutral and polyanionic polysaccharides (classified as soluble dietary fibres) were selected and investigated to understand their cation binding characteristics and potential significance in vivo.

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2. Materials and methods

2.1. Materials

Agar, carrageenan (predominantly kappa), gum xanthan (from *Xanthomonas campestris*), gum arabic (from Acacia tree), gum karaya (from Sterculia tree), gum tragacanth, low-methoxyl pectin (from citrus fruits) and gum guar, were purchased from Sigma Chemical Co. All general chemicals were of analytical grade (certified AR, Fisher Scientific).

2.2. Analytical methods

The moisture content of the polysaccharides was determined by heating circa 1 g in an oven, provided with a fan, for 16 h at 105±2°C and 2 h at 130±2°C. The ash content was determined in a muffle furnace by slowly heating 0.5–2.0 g polysaccharide (in porcelain crucibles) up to 500°C for 16 h. The protein content of the polysaccharides was estimated (N×6.25) from the quantitative analysis of nitrogen by the Kjeldahl method (Egan, Kirk, & Sawyer 1981).

For polysaccharide analysis, the samples (5–10 g) were prepared free from interfering oligosaccharides by refluxing three times in a Soxhlet with 150ml boiling 85% (v/v) ethanol, followed by washing with acetone (2×50ml) and air-drying. Neutral sugars in polysaccharides were determined by the method of Englyst and Cummings (1988) without the preliminary step of starch hydrolysis with α-amylase and pullulanase. Dry polysaccharides (free from oligosaccharides) were directly solubilised in 12M H_2SO_4 then hydrolysed in 1M H_2SO_4 (2 h, 100°C). Monosaccharides were reduced and derivatised as alditol acetate then separated and quantified with a Perkin Elmer Autosystem gas-liquid chromatograph fitted with a fused silica capillary column (Supelco) and a flame ionisation detector. The operating conditions were: carrier gas (helium), injection (270°C), split flow mode (65ml. min^{-1}), oven (260°C), detector (265°C), FID gases (air 450ml. min^{-1}, hydrogen 45ml. min^{-1}). The alditol acetates were quantified by integration (PE Nelson model 1020) from peak areas relative to β-D-allose used as internal standard, and corrected for FID response factors and sugar losses during hydrolysis determined with standards. The uronic acids in polysaccharides were determined by using the colorimetric (3,5-dimethylphenol) method of Scott (1979). The degree of methylation was estimated by difference between saponified (1 h in 0.6M NaOH at room temperature) and unsaponified samples. The pyruvate content in gum xanthan was quantified by ^{13}C CP/MAS NMR spectroscopy (Rinaudo, Milas, Lambert & Vincendon, 1983). The spectra were obtained at room temperature at low field (25 MHz) using a Bruker MSL 100 NMR. Over 2000 scans (with MAS speeds of ~4 MHz, 1 ms contact time and 10 MHz line-broadening) were accumulated and Fourier-transformed while the pyruvate content was estimated by integration of the chemical shift of $-CH_3$ and $-C{=}O$ at 22.2 and 174.6ppm, respectively. The amount of sulphate esters in sulphated algal polysaccharides was estimated from the quantitative analysis of barium sulphate by the turbidimetric method of Dodgson (1961), modified by Dodgson and Pryce (1962).

Atomic absorption spectrometry determination of minerals (Ca, K, Fe, Na, Zn) were done using a Perkin Elmer model 3100 AAS. The sample preparation involved dry ashing according to the method of Miller-Ihli (1996). The operating conditions for all the elements are listed in Table 1. The samples were quantified with reference to standards diluted from 1000 ppm stock solutions from commercial sources (Primar, Fischer Scientific) and laboratory-made primary standards (Smith & Parsons, 1973). All glassware and containers were thoroughly acid cleaned prior to use. Matrix effects were checked by the method of standard addition (Bader, 1980).

The viscosity of 0.1% (w/v) polysaccharide solutions was determined at 25°C using a Rheology International RI 2L cone and plate (0.8°) viscometer previously calibrated with a 100 mPa.s silicone standard (Brookfield). The pH of 0.1% (w/v) polysaccharide solutions was determined at 20°C with a calibrated Piccolo ATC pH-meter (Hanna Instrument).

2.3. Calcium and zinc binding by equilibrium dialysis

Dialysis tubings (molecular weight cut-off 12–14 kDa, Medicell International, UK) were previously cleaned by heating twice at 80°C for 30 min in a 2% sodium bicarbonate, 1 mM EDTA solution. The tubing was then thoroughly rinsed with deionised water and stored at 5°C in a 0.1% sodium azide solution.

Polysaccharide solutions (0.1% w/v) were prepared by constant stirring overnight or boiling (agar, carrageenan) followed by slow cooling and constant stirring. Fifty millilitres of 0.1% (w/v) polysaccharide solution was then poured into standard lengths of dialysis tubing (15 cm). The tubes were equilibrated 30 min at room temperature in 450 mL solvent (water or 0.1 M HCl) with constant stirring (~80 turns/min with a magnetic stirrer) in a 600 mL beaker covered with a protective film to prevent evaporation. One millilitre of 1 mg ml^{-1} (Zn^{2+}) or 4 mg ml^{-1} (Ca^{2+}) mineral solution was then added and allowed to equilibrate for 60 min (with constant stirring). After equilibrium, a 50 ml aliquot from the outside solution was transferred into a class A volumetric flask, mixed with 10 mL 6% HNO_3 or 6% HNO_3-3% $LaCl_3.7H_2O$ then stored in acid-cleaned LDPE Nalgene bottles. The amount of mineral was measured by atomic absorption spectrometry (Section

Table 1
Perkin–Elmer model 3110 atomic absorption spectrophotometer operating conditions[a]

Element[b]	Wavelength (nm)	Slit width (nm)	Hollow cathode lamp current intensity (mA)	Standard concentration range (ppm)		Detection limit[c] (ppm)
				S1	S2	
Ca	422.7	0.7	15	4.00	12.0	0.013
Fe	248.3	0.2	30	6.00	–	0.026
K	766.5	0.7	12	2.00	4.00	0.038
Na	589.0	0.2	12	0.50	1.00	0.013
Zn	213.9	0.7	5	1.00	3.00	0.004

[a] Air-acetylene flame, standard nebuliser and flow spoiler, no background correction.
[b] Standards, samples and blanks are made up in 1% (v/v) HNO_3 (iron, zinc) or 1% (v/v) HNO_3 and 0.5% (w/v) $LaCl_3$.$7H_2O$ (calcium, potassium, sodium) matrices.
[c] Average of $n = 5$ measurements at dilutions $\times 5$ and $\times 10$ the expected detection limit.

2.3) and the extent of binding was normalised with a reference where the polysaccharide solution was replaced by water.

2.4. Direct iron binding

Into 60 ml acid-cleaned LDPE Nalgene bottles, 10 ml of 200 μg ml^{-1} Fe(III) [from $Fe(NO_3)_3.9H_2O$] in 0.01M HCl was added with a positive displacement pipette. To these bottles, 10 ml of a 0.1% (w/v) polysaccharide solution was added and the bottles were rapidly mixed. The bottles were placed in a shaking incubator at 35°C for 16 h. After incubation, the solutions were filtered (Whatman GF/A) and a 0.5 ml aliquot of the supernatant was analysed for ferric iron by the thiocyanate method (Jeffery, Bassett, Mendham & Denny, 1989). A standard solution of 100 μg ml^{-1} Fe(III) from $Fe(NO_3)_3.9H_2O$ (1 l deionised water containing 10 ml conc. HCl) was prepared, together with 20% (w/v) KCNS and 4M nitric acid. Triplicate 0.5 ml aliquots of standard and samples were transferred to 10 ml screw-capped test tubes. Then, 5 ml water, 0.5 ml KCNS and 0.5 ml nitric acid were added and the tubes were thoroughly mixed and allowed to stand (in the dark) 20 min at room temperature. The absorbance was then measured against a reagent blank at 480 nm.

3. Results and discussion

3.1. Proximate composition and physico-chemical properties of polysaccharides

The proximate compositions (moisture, ash, protein and carbohydrate) of the polysaccharides are shown in Table 2. The moisture content was measured as weight

Table 2
Promimate composition of polysaccharides

	Moisture (g/100g)	Ash (g/100g)	Protein (g/100g)	Carbohydrate (g/100g)	
				Calculated[a]	Experimental[b]
Algal polysaccharides					
Agar	11.4±0.1	2.5±0.0	0.4±0.0	85.7±0.1	37.4±0.7
Carrageenan	11.4±0.1	33.4±0.1	0.4±0.0	54.8±0.1	25.6±1.3
Microbial polysaccharides					
Gum xanthan	10.9±0.0	14.6±0.0	4.6±0.0	69.8±0.1	58.4±1.7
Gum exudates and pectin					
Gum arabic	14.2±0.1	3.4±0.4	2.0±0.0	80.4±0.4	78.5±1.7
Gum karaya	16.3±0.1	8.0±0.0	0.3±0.0	75.4±0.1	49.9±1.8
Gum tragacanth	11.5±0.1	3.0±0.0	3.3±0.0	82.2±0.1	78.2±0.6
Pectin	8.5±0.2	2.8±0.0	6.4±0.0	82.3±0.2	80.3±1.4
Seed gums					
Gum guar	11.5±0.1	0.7±0.0	4.5±0.1	83.4±0.1	80.5±0.9

[a] Calculated = 100−{moisture + ash + protein}.
[b] Experimental = neutral sugars (gas-liquid chromatography) + uronic acids (colorimetry)

loss at 105°C overnight and 130°C for 2 h. Although both results are highly correlated ($r = 0.9940$, $P < 0.001$), the later gives a moisture content 8.5% higher than the former. Therefore, the weight loss, after drying at 130°C for 2 h was used as the best estimate. Although all polysaccharides were stored under identical conditions, the broad range of 8.5–16.3% moisture suggest large differences in terms of water-polysaccharide interactions. The level of purity of the polysaccharides, as protein content, was measured by Kjeldahl nitrogen (N×6.25). Some polysaccharides showed a good index of purity (agar, carrageenan and gum karaya), with 0.3–0.4% protein, while gum guar, gum xanthan, and pectin had much higher levels of protein (4.5–6.4%). These proteins can be either contaminants or associated with some polysaccharidic chains, via covalent links (proteoglycans), although the existence of these cross-links in the plant cell walls is still under investigation (Brett & Waldron, 1996).

The monosaccharide content of the carbohydrate fractions was measured (after hydrolysis) by gas-liquid chromatography. The neutral sugar composition is shown in Table 3 and agrees well with literature values. When compared to the theoretical amount of carbohydrate (100−{moisture + ash + protein}), reasonable agreement was found for gum arabic, gum guar, gum tragacanth and pectin. The discrepancy for the other polysaccharides is due to two major experimental causes.

1. For agar and carrageenan, the 3,6-anhydrogalactosyl monomer of the disaccharide unit is rapidly degraded by the hydrolysis in 1M sulphuric acid. As a consequence, the yield of monosaccharides is nearly half that of the theoretical amount and other analytical methods that protect and yield quantitative recovery of the 3,6-anhydrogalactosyl residue should be used (Stevenson & Furneaux, 1991)
2. By contrast, for gum karaya and gum xanthan, the large amount of uronic acids which are extremely resistant to acid hydrolysis would decrease the release of monosaccharides directly linked to the uronic acid moieties (Stephen et al., 1990). Therefore, for each polysaccharide, a more systematic study would be required in order to optimise hydrolysis conditions (complete hydrolysis of all glycosidic linkages) and minimise the losses of released monosaccharides

Of particular importance for this study, is the polyelectrolyte nature, due to the charged groups of the polysaccharides. As shown in Table 4, with the quantification of carboxyl (uronic/pyruvic acids) and sulphate groups, the polysaccharides should be classified as neutral or polyanionic:

1. gum guar and agar (due to the extremely low content of sulphate groups) are essentially neutral;
2. carrageenan is a sulphated galactan with a content of sulphate groups (13.3±0.3 g SO_3^-/100 g) close to the idealised structure of κ-carrageenan (i.e. 11.4 g SO_3^-/100 g with a theoretical carbohydrate content of 54.8±0.1 g/100 g);
3. gum exudates, pectin and gum xanthan have various levels of carboxyl groups, from 13.3% uronic acids in gum arabic to 75.5% in pectin.

The quantification of the charged groups leads to an estimation of the cation exchange capacity of the polysaccharides (Table 4) which is a key parameter of their interactions with di- and tri-valent cations (Section 3.2).

Table 3
Structural analysis of polysaccharides: monosaccharide composition by gas-liquid chromatography

	Tota neutral sugars (g/100 g)l	Pentoses (%, by mole)		Hexoses (%, by mole)			Deoxy sugars (%, by mole)	
		D-Arabinose	D-Xylose	D-Glucose	D-Mannose	D-Galactose	L-Rhamnose	L-Fucose
Algal polysaccharides[a]								
Agar	37.4±0.7	–	–	1.9	–	92.7	–	–
Carrageenan	25.6±1.3	–	–	6.0	–	87.2	–	–
Microbial polysaccharides								
Gum xanthan	44.6±1.7	–	–	62.4	37.6	–	–	–
Gum exudates and pectin								
Gum arabic	65.2±1.7	29.4	–	0.6	–	56.6	13.4	–
Gum karaya	22.4±1.8	0.5	–	2.3	–	64.5	32.6	–
Gum tragacanth	43.0±0.5	38.2	20.5	15.1	–	14.3	1.7	10.3
Pectin	5.3±0.2	19.2	–	11.3	–	61.9	7.6	–
Seed gums								
Guar gum	79.7±0.9	1.8	0.9	3.1	58.5	35.2	0.5	–

[a] Agar and carrageenan contain 5.4 and 6.8% unknown, respectively.

Table 4
Structural analysis of polysaccharides, charged groups and cation exchange capacity

	Carboxyl groups (COO^-)					Sulphate groups (as SO_3^-)	Cation exchange capacity[d] (mequivalent/g)
	Tota uronic acid (g/100g)	D-Gal*p*A[a] (as% uronic acid)	D-Glu*p*A (as% uronic acid)	Degree of methylation[b] (as% uronic acid)	Pyruvate[c] (g/100g)		
Algal polysaccharides							
Agar	–	–	–	–	–	1.2±0.2	0.23
Carrageenan	–	–	–	–	–	13.3±0.3	2.08
Microbial polysaccharides							
Gum xanthan	13.8±0.4	nd[e]	nd	–	14.6±0.4	–	1.66
Gum exudates and pectin							
Gum arabic	13.3±0.0	14	86	ns[f]	–	–	0.86
Gum karaya	27.5±0.1	72	28	ns	–	–	1.64
Gum tragacanth	35.2±0.4	97	3	6	–	–	2.10
Pectin	75.0±1.4	100	ns	14	–	–	3.69
Seed gums							
Guar gum	0.8±0.0	nd	nd	–	–	–	0.05

[a] D-Gal*p*A and 4-*O*-methyl-D-Glu*p*A
[b] Degree of methylation (DM) is determined between saponified and unsaponified samples
[c] Pyruvate (as 4,6-*O*-carboxyethylidene-βD-Man*p*) is estimated by ^{13}C CP/MAS NMR.
[d] Cation-exchange capacity = total charged monomers (mg/g) / < MW > (g/mole) where < MW > is the average molecular weight of the monomers in the polyaccharide. For uronic acids, total charged monomers (mg/g) = total uronic acid (mg/g)× {1−(DM/100)}.
[e] nd, Not determined
[f] ns, Not significant

The mineral content of the polysaccharides, as determined by dry-ashing and atomic absorption spectroscopy, is shown in Table 5. The large ash contents in carrageenan, gum xanthan and gum karaya are essentially due to the presence of bound monovalent cations potassium and sodium and the divalent cation calcium. The trace metals, iron and zinc, are in the range of 17.2–89.9 μg/g (db) and 2.2–14.3 μg/g (db) respectively, for all the polysaccharides investigated. Comparison with literature is difficult since systematic data on the mineral composition of non-starch polysaccharides are scarce.

The pH of 0.1% (w/v) polysaccharide solutions is shown in Table 6. All the polysaccharides are slightly acidic with the exception of carrageenan. The viscosity of 0.1% (w/v) polysaccharide solutions is also shown in Table 6. The higher viscosity of gum xanthan and gum guar is essentially due to the 1→4-β glycosidic linkage of the polymer backbone. Both polymers belong to the ribbon conformational family, according to the classification of Rees (1977), which favours a pulled out ribbon-like shape. By contrast, gum exudates are highly branched polysaccharides and the viscosity, in salt-free

Table 5
Mineral content of polysaccharides

	Ca (mg/g, db[a])	K (mg/g, db[a])	Na (mg/g, db[a])	Fe (mg/g, db[a])	Zn (mg/g, db[a])
Algal polysaccharides					
Agar	1.80±0.15	0.44±0.10	6.13±0.21	49.2±1.2	14.3±0.3
Carrageenan	30.3±0.26	102.39±0.82	8.71±0.17	74.2±2.9	5.6±0.3
Microbial polysaccharides					
Gum xanthan	0.87±0.05	35.62±0.09	28.52±1.05	17.2±1.2	2.6±0.4
Gum exudates and pectin					
Gum arabic	7.82±0.08	8.14±0.13	0.19±0.04	21.0±1.1	2.7±0.8
Gum karaya	22.12±0.21	15.92±0.10	0.65±0.10	52.3±1.3	7.2±0.4
Gum tragacanth	9.26±0.24	3.50±0.01	0.21±0.01	89.9±1.5	2.2±0.5
Pectin	1.53±0.06	1.61±0.12	7.58±0.22	51.9±1.2	4.0±0.3
Seed gums					
Gum guar	0.79±0.02	1.73±0.04	0.22±0.01	39.3±2.3	14.2±0.8

[a] db, Dry weight basis.

Table 6
pH and rheological properties of polysacchrides

	pH[a]	Viscosity[b] (mPa.s)
Algal polysaccharides		
Agar	5.8±0.4	Gel
Carrageenan	7.3±0.6	31.6
Microbial polysaccharides		
Gum xanthan	5.8±0.4	43.4
Gum exudates and pectin		
Gum arabic	5.0±0.3	13.2
Gum karaya	4.8±0.1	27.9
Gum tragacanth	5.2±0.3	25.9
Pectin	3.6±0.1	18.6
Seed gums		
Gum guar	5.1±0.4	27.6

[a] pH of 0.1% (w/v) solutions at 20°C.
[b] Viscosity of 0.1% (w/v) solutions at 20°C, at a shear rate of 1200 s^{-1} with a Rheology International RI 2L cone plate (0.8°) viscometer.

solution, reflects structural differences (polymer backbone, degree of branching, molecular weight) and electrostatic interactions (repulsion between charged groups).

3.2. Cation binding by polysaccharides

Interactions between the divalent cations (calcium and zinc) and the polysaccharides were measured by equilibrium dialysis in low ionic strength solutions. As shown in Fig. 1, a quantitative recovery of Zn^{2+} at pH 1 indicates the absence of significant binding in conditions approaching the acidity of the stomach. At this pH, all the charged groups are fully protonated ($-COOH$, $-SO_3H$) hence no electrostatic interactions with divalent cations are expected. For calcium, the same behaviour was observed, in addition to a large release of endogenous calcium from the polysaccharides (this effect was not observed for zinc due to a release of endogenous zinc well below the detection limit). For example, in the case of carrageenan, 55.3±0.5% of its endogenous calcium is released after 1 h of dialysis.

By contrast, in salt-free neutral solutions, significant binding was observed as shown in Fig. 1. It is clear that neutral polysaccharides, agar and gum guar, have little or no affinity for divalent cations while gum xanthan, carrageenan and pectin show a large extent of binding. The basis of the electrostatic interaction is the deprotonation of the functional charged groups (at least for carboxyl groups) since the intrinsic pK has been reported to be 3.2 for polygalacturonic acid (Malavíková, Rinaudo & Milas, 1994) and in the range 2.6 (at high ionic strength) to 2.8 (at low ionic strength) for gum xanthan (Holzwarth, 1976).

Fig. 2 indicates a good correlation ($r = 0.9871$; $P < 0.001$) of the extent of Ca^{2+} and Zn^{2+} binding in neutral salt-free solution by the polysaccharides. The negative correlation between calcium or zinc recovery and the cation exchange capacity ($r = -0.6615$; $P < 0.1$ and $r = -0.5945$; $P < 0.001$ respectively) suggests an essentially electrostatic interaction which is in agreement, at least for pectin and carrageenan, with the literature (Kohn, 1987; Rochas & Rinaudo, 1980). Nair, Asp, Nyman and Persson (1987) have also shown that

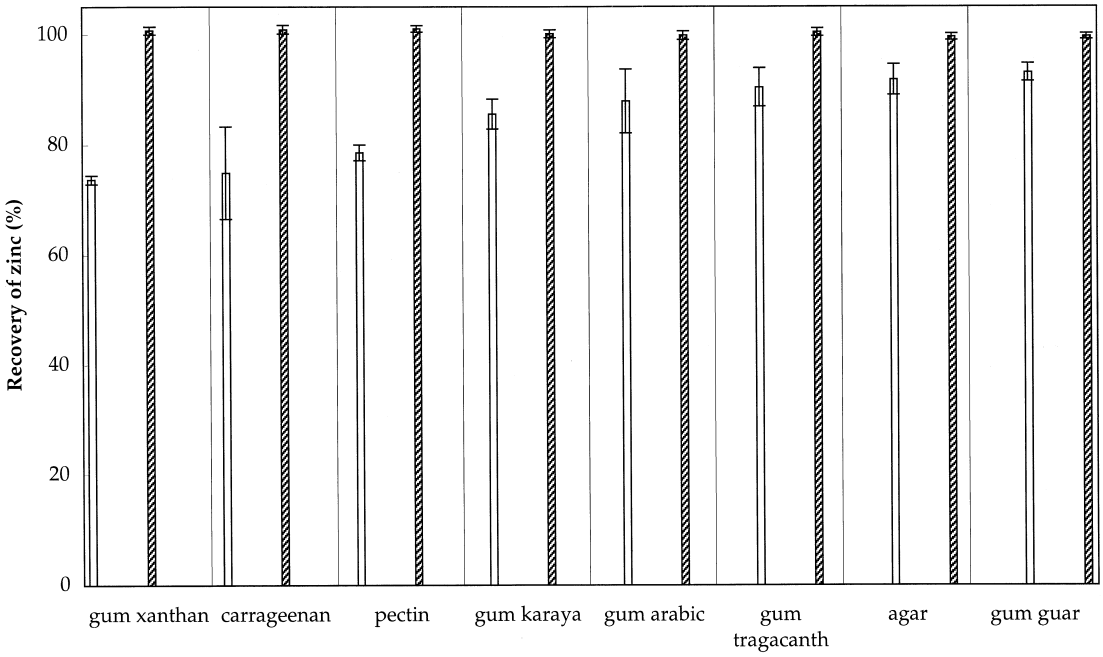

Fig. 1. Recovery of zinc after equilibrium dialysis in the presence of 0.1% (w/v) polysaccharide solution in water (white bars) and 0.1N HCI (shaded bars).

the extent of binding of cadmium, copper and zinc with low- and high-methoxyl pectin, gum tragacanth (Sterculia gum) and gum guar, is directly related to the content of free carboxyl groups. This is in agreement with the suggestion of Branch, Southgate and James (1975) that the ionisation of the carboxyl groups of free (i.e. not methyl esterified) uronic acids is significantly correlated to calcium binding by dietary fibre from plant foods.

In the case of ferric iron (Fe^{3+}), due to the precipitation of iron hydroxydes as pH is increased, direct binding was quantified in acidic conditions at pH2 in 0.01N HCl. Again, as shown in Fig. 3, pectin, gum xanthan

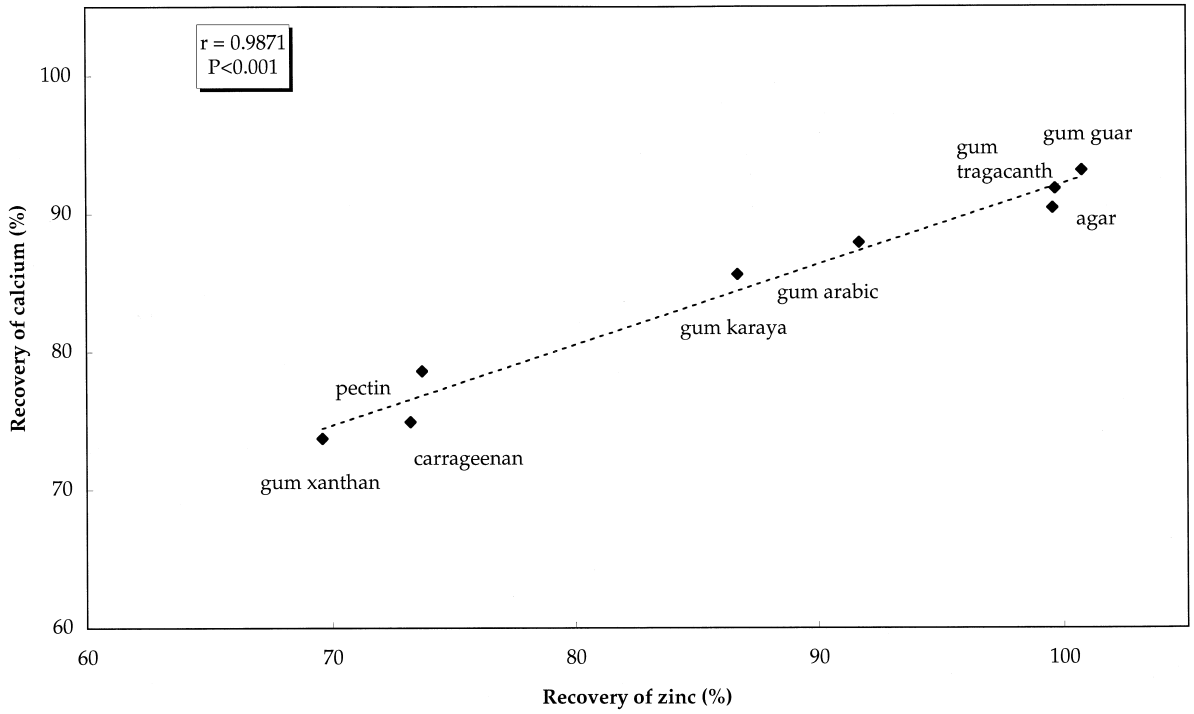

Fig. 2. Comparsion of calcium and zinc recovery after equilibrium dialysis in the presence of 0.1% (w/v) polysacchride solution in water.

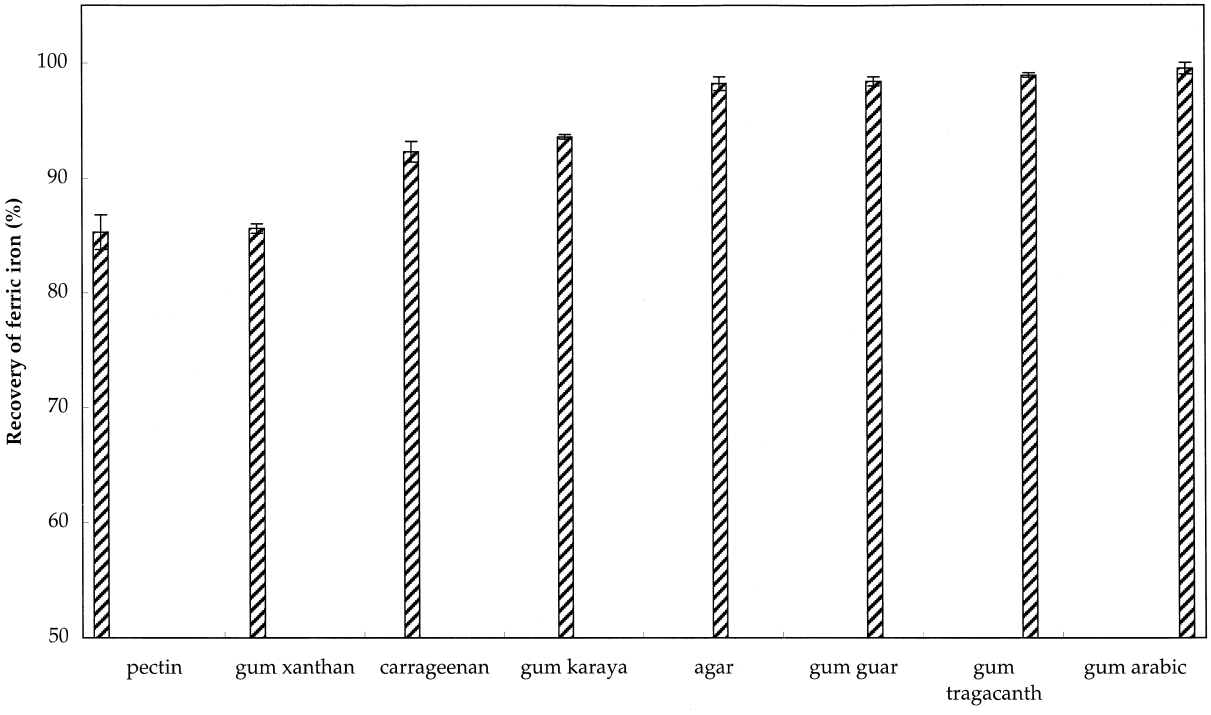

Fig. 3. Recovery of ferric iron in the prsence of polysaccharide solution in 0.01N HCI.

and carrageenan show the largest extent of binding while neutral polysaccharides agar and gum guar have no affinity for Fe^{3+}. A significant precipitate was observed for pectin, gum xanthan, carrageenan and gum karaya. This may be due to the formation of insoluble complexes with iron as observed in the case of pectin with Cu^{2+} (Debongnie, Mestdagh & Rinaudo, 1987; Kohn, 1987). Similarly, in acidic pH, Fe^{3+} coordinates with the carboxylic groups of D-galacturonic acid to form Fe^{3+}-D-GalA_3 complexes (Deiana, Gessa, Solinas, Piu & Seeber, 1989). Finally, the complex formation of iron (by chelation) with alginate in acidic pH has also been postulated by Berner and Hood (1983).

3.3. Extrapolation of in vitro cation binding by polysaccharides with their in vivo bioavailability

The binding of nutritionally important minerals as described above suggests a similar fate in vivo due to the conditions encountered in the small intestine when, after neutralisation of the gastric juice in the duodenum, the pH increases up to 6.5–7.0 (in the jejunum and the ileum). However, a large number of mineral balance studies, essentially with pectin and gum guar (for comprehensive reviews, see Munoz & Harland, 1993; Torre et al., 1991), with both animal and human models, conclude that there is no significant effect on mineral bioavailability in the presence of soluble dietary fibre. Therefore, it is important to review and discuss, critically, these conflicting results between in vitro and in vivo studies.

A first — apparently trivial — point is that, as cation-exchangers, some of the soluble dietary fibres occur as salts (Table 5) and a large amount of these in the diet would significantly increase mineral intake (Southgate, 1987) and this should be taken into account in mineral balances.

In addition to the pH dependence of cation-binding by polyanionic polysaccharides, the ionic strength is also a critical parameter. The in vitro binding studies of Schlemmer (1989) have shown a maximum binding of Ca^{2+} and Zn^{2+} by pectin, alginate and κ-carrageenan at pH 5.0–7.5 and low ionic strength ($I < 0.009$) in diluted HCl/NaOH. By contrast, limited or no binding occurs at higher ionic strength ($I \sim 0.10$), in the physiological sodium bicarbonate/CO_2 buffer within the same pH range, presumably due to partial shielding of the polyanions by an electrical double layer formed by the ions of the buffer.

The main caveat of in vitro studies with isolated dietary fibre is that the interactions with other food components are completely ignored. Hence, in vitro availability measurements with various foods in simulated gastrointestinal conditions are more realistic models since they contain all other potential ligands for mineral binding present in the diet (Miller, Schricker, Rasmussen & van Campen, 1981; Wolters, Diepenmaat, Hermus & Varagen, 1993; Wolters, Schreuder et al., 1993). Measuring the dialysability of calcium, copper, iron, magnesium and zinc in 57 food products (cereals, nuts, vegetables and fruits), Wolters, Diepenmaat et al. (1993) found no significant effect between the amount of uronic acids in foods and in vitro availability of minerals. Although the degree of esterification of uronic acids was not measured, the effect of pectins as they occur in plant cell walls was clearly differentiated from phytic acid which showed a negative effect for all minerals investigated except copper. In vitro dialysability of iron is also reduced by the presence of fibre in foods (presumably due to phytic acid) but significantly increased by the presence of chelators such as ascorbic acid (Miller et al., 1981). Similarly, Berner and Hood (1983) have suggested that alginate-iron complexes are disrupted during simulated in vitro digestion and therefore, iron dialysability is unaffected by alginate but strongly dependent on the presence of chelators.

The fate of soluble dietary fibres in the colon is also extremely important with regard to mineral bioavailability. Since the fibres escape digestion in the small intestine, they represent a potentially important source of carbon and energy for the numerous anaerobic saccharolytic bacteria in the colon (Salyers, 1979). There is supporting evidence from in vivo human studies that pectin is almost completely fermented since little or no recovery has been observed for faecal samples of subjects which were given dietary pectin supplements (Cummings et al., 1979; Gramstorff Fetzer, Kies & Fox, 1979). In addition, the high level of pectin degradation is substantiated by in vitro fermentation studies (Barry et al., 1995) and a predictive hierarchy of fermentatibility of soluble dietary measured as short-chain fatty acids production has been established (Adiotomre, Eastwood, Edwards & Gordon Brydon, 1990): pectin > gum arabic > gum guar > gum tragacanth > gum xanthan > gum karaya. As a consequence, the absence of significant effects of some soluble dietary fibres on the mineral balance in human studies, is likely to be due to fermentation and subsequent absorption in the colon as suggested for calcium and pectin (James, 1980).

4. Conclusions

This study has confirmed how non-starch polysaccharides have the ability to bind cations in vitro as a consequence of their polyanionic nature. However, many components in the diet and the physiological conditions (pH, ionic strength, colonic bacteria) in the gastro-intestinal tract will potentially interfere with these binding characteristics.

It has been estimated that an average Western diet, containing 17.2 g fibre and 12.3 mmol uronic acids per

day, has the ability to bind 3.8 mmol (152 mg) calcium (James, 1980; James, Branch & Southgate, 1978). This estimation makes allowance for any variation in the degree of methylation of pectin, with low degree of substitution associated with higher calcium binding capacity. With a daily recommendation of 18 g/d non-starch polysaccharide in the adult diets of the UK (Committee on Medical Aspects of Food Policy, 1991), large amounts of cation binding would be expected from in vitro studies. However, most of mineral balance studies in man conclude that there is no significant effect on mineral bioavailability in the presence of dietary pectin. This is substantiated by the chelating ability of other components in the diet, the pH and ionic strength conditions in the gastro-intestinal tract and the extent of degradation in vivo by colonic bacteria. In conclusion, in vitro data should not be used for extrapolation to in vivo systems. Instead, mineral bioavailability require to be validated by careful in vivo mineral balance studies.

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