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Effect of pH and hemicellulase digestion on calcium binding by selected gums

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The following study was conducted to investigate calcium binding by locust bean gum (Loc), gum arabic (Ara) and xanthan gum (Xan) under various pH conditions and during hemicellulase digestion. Binding behavior of the gums was pH-dependent. Ara and Xan bound most Ca at neutral pH, whereas Loc bound most Ca at pH 9.5. For all three gums, binding decreased below pH 5. Xan bound an average of 70% of added Ca in the range studied (0–3000 μM). Ara bound about 26% of added Ca, whereas Loc bound 5–25% of added Ca. Based on Scatchard analysis, there were two types of binding sites for Ca for Xan and Ara, and one type for Loc. Hemicellulase digestion decreased binding ability for all three gums. Ara and Loc released endogenous Ca, while Xan lost 35–45% of its Ca binding capacity after a 24 h in-vitro digestion.

INTRODUCTION

In the food industry, gums are used for their wide range of properties, including adhesive, binding, emulsifying, gelling, stabilizing, thickening and water-binding functions (Pomeranz, 1985).

A number of researchers have investigated the behavior of insoluble fibers toward different cations (Thompson & Weber, 1979; Camire & Clydesdale, 1981; Fernandez & Philips, 1982; Rendleman, 1982; Mod *et al.*, 1982; Laszlo, 1987; Platt and Clydesdale, 1987). Relatively few studies have been conducted on the interactions between gums and cations (Zemel & Zemel, 1985; Nair *et al.*, 1987; Ha *et al.*, 1989; Kelly & Potter, 1990).

In order to address the means by which locust bean gum (a seed galactomannan), gum arabic (a plant exudate), and xanthan gum (a microbial polysaccharide) bind Ca under different conditions, the following study was conducted. It encompassed the following objectives: to determine free and endogenous Ca levels of the gums; to determine changes in binding of Ca during changes of pH; to determine changes in binding occurring during hemicellulase digestion; and to determine number and types of sites for Ca binding using Scatchard analysis.

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MATERIALS AND METHODS

Gums and enzymes

Gum Arabic (Ara) from Acacia tree, locust bean gum (Loc) from seeds of *Ceratonia siliqua* L., and gum xanthan (Xan), prepared by fermentation of dextrose with *Xanthomonas campestris* were purchased from Sigma Chemical Co. (St. Louis, MO, USA). A crude hemicellulase preparation containing cellulase activity (level not specified) from *Aspergillus niger* was obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Endogenous Ca content

To determine the endogenous Ca content of the gums, samples were wet-ashed (Niedermeier *et al.*, 1971), followed by analysis on a Hitachi 170–50 atomic absorption spectrophotometer at 422·7 nm wavelength with an air–acetylene flame. Samples were diluted in 0·5% lanthanum chloride solution and readings compared to a standard curve.

Potentiometric titrations

Potentiometric titrations for the determination of free Ca were carried out using a PHM 84 research pH meter with an ABU 80 autoburette and TTA 80 titration assembly all from Radiometer Copenhagen (Westlake, OH, USA). The ion-sensitive electrode was a F

2112 Ca selectrode and a K 401 Calomel electrode serving as reference electrode (Radiometer Copenhagen, Westlake, OH, USA). Before titrations a semi-logarithmic standard curve was prepared using 0·1 M $CaCl_2$ standards from Ricca (Arlington, TX, USA) diluted accordingly. The same standard was added to samples during titration. To prevent the possible formation of $CaCO_3$, nitrogen gas (Sunox, Charlotte, NC, USA) was bubbled through the samples throughout the titration. For pH determinations a Ross Sure Flow combination pH meter (Orion Research Inc., Boston, MA, USA) connected to an Accumet pH Meter, model 825MP (Fisher, Norcross, GA, USA) was employed.

Gums were added to distilled, deionized water at concentrations of 0.25% (w/v) for Loc and Ara, and 0·10% (w/v) for Xan and stirred for a minimum of 2 h and a maximum of 7 h. Aliquots of the stirred gums were taken, Ca standard was added and solutions stirred for approximately 10 min before mV readings were obtained. The pH titrations were performed by adding 0.01 M NaOH or 4 mM HCl. NaOH was added first to raise the pH to 9·5, followed by addition of HCl to drop the pH stepwise to 4·1. Readings below pH 4 were avoided due to the sensitivity limit of the selectrode. After the addition of NaOH or HCl, at least 3 min were allowed for the sample to equilibrate before a mV reading was taken.

The readings obtained were pH-dependent from pH 5 downward and pH 9 upward. Therefore, water was titrated with the corresponding standards over the pH range (9·5–4·1) to adjust for the dependency. Free Ca was determined directly from the potentiometric titrations, whereas bound Ca was calculated as the difference between the water reading and free Ca levels in the gum systems.

Enzyme digestion

For Loc, enzyme preparation was added to a final concentration of 0·1% (w/v). For Xan and Ara, enzyme preparation was added to a final concentration of 2% (w/v). Addition of the enzyme preparation at the 2% level resulted in the addition of 30 μM Ca, which was subtracted from the data obtained during digestion.

Upon enzyme addition the samples were kept in a 38 ± 0·5°C water bath for 48–96 h. At selected time periods during the digestions, samples were stirred to obtain a homogeneous sample and aliquots were removed to which graded amounts of $CaCl_2$ standard were added to determine the binding ability of the digested gums. Where the pH fell below 4 during enzyme digestion, 0·01 M NaOH was added to raise the pH to 4. The amount of NaOH added was considered a dilution factor and was multiplied by the result obtained for free Ca during potentiometric titrations. Standards were measured at the pH of the gum being investigated.

Loss of polysaccharide structure was determined by measuring the reduction in viscosity over time with Cannon–Fenske routine viscometers (Industrial Research Glassware, Kenilworth, NJ, USA) size 300 for Loc and Xan, and size 150 for Ara. Viscosity was expressed in centistokes (1 cSt = 1 mm^2/s). Samples for viscosity determinations were taken at the same time periods as for the addition of Ca.

Scatchard analysis

To obtain data for the equation (Scatchard, 1949)

$$r/c = k(n = r)$$

bound Ca (r) was calculated as endogenous Ca + added Ca − free Ca. Unbound Ca (c) equaled free Ca obtained by potentiometry. Corrections for nonspecific binding in curved line models were made by determining the limiting bound/unbound ratio, multiplying it by the free ligand concentration at each point, and then subtracting it from the bound Ca to give specific binding (Chamness & McGuire, 1975). The limiting bound/unbound ratio for Ara was 0·2, and 0·6 for Xan. Binding curves were obtained by least squares method for Loc and by the radial line method to fit the relation $OC = OA + OB$ (Pennock, 1973) for Ara and Xan.

Statistical analysis

Mean comparisons were carried out to determine significant differences between means. The significance of terms (pH, level of Ca addition, hours of digestion) to the model of bound Ca in a full quadratic model was tested by Analysis of Variance (ANOVA) using type I sums of squares. All statistical analyses were performed with the Statistical Analysis System (SAS, Cary, NC, USA).

RESULTS AND DISCUSSION

Endogenous Ca content

The endogenous Ca content of the gums, as determined by atomic absorption spectrophotometry, was 5·80 ± 0·06 μg/mg, 0·81 ± 0·01 μg/mg, and 0·83 ± 0·01 μg/mg for Ara, Loc, and Xan, respectively (mean ± SEM) and were within the range of published data (Artaud *et al.*, 1976; Reymond *et al.*, 1983; Anderson & Morrison, 1989; Ha *et al.*, 1989).

Table 1. Binding of calcium at initial pH of gums[a]

Level of addition (μM)	Gum arabic		Locust bean gum		Xanthan gum	
	μM	%	μM	%	μM	%
100	35·5	33·5	5·4	5·3	100	100
200	55·0	29·1	4·0	2·1	180	95·2
500	89·0	20·0	6·0	1·3	361	81·1
1 000	258	27·1	90·0	9·4	593	62·2
2 000	456	24·2	237	12·6	865	45·8
3 000	638	23·3	443	16·2	964	35·3

[a] For method of calculation refer to text

Table 2. Effect of pH on bound calcium levels in locust bean gum[a]

Level of addition (μM)	pH[b]						
	4·1	4·5	5·0	6·0	7·0	8·0	9·5
100	−11·5 ± 4·9	−9·2 ± 4·2	9·0 ± 3·3	11·0 ± 6·9	13·9 ± 6·9	16·7 ± 7·3	36·6 ± 3·2
200	−9·4 ± 9·9	6·93 ± 12·3	19·6 ± 16·9	38·4 ± 11·2	37·7 ± 9·6	28·1 ± 9·4	42·1 ± 10·3
300	−20·2 ± 5·6	−5·8 ± 9·7	21·2 ± 5·9ab	22·1 ± 7·3a	22·8 ± 13·4a	31·0 ± 13·6	55·4 ± 14·8
400	0·5 ± 15·0	30·1 ± 18·8	53·6 ± 16·0	55·1 ± 17·1a	56·4 ± 19·9a	59·8 ± 21·8a	68·7 ± 9·5
500	49·4 ± 13·2	90·5 ± 18·2	60·8 ± 18·4	48·7 ± 16·1	75·0 ± 13·0	69·4 ± 14·1	62·9 ± 11·2
1 000	−2·3 ± 5·5	34·3 ± 6·1	121 ± 34·2	122 ± 22·2	110 ± 25·6	138 ± 13·8	109 ± 16·5
2 000	−146 ± 8·0	−153·2 ± 17·7	−158 ± 33·4	−48·7 ± 41·4	−14·0 ± 51·7	−37·3 ± 66·9	71·5 ± 90·1
3 000	−171 ± 110	−49·1 ± 105	−78·2 ± 150	−102 ± 63·7	−19·8 ± 68·7	−26·7 ± 57·8	47·5 ± 68·8

[a] Data are given as μM bound calcium ± standard error of mean. Gum concentration 0·25% w/v.
[b] Means followed by the same letter in one row are equal at LS means comparison $P < 0·05$.

Effect of pH on bound Ca

Averages of bound Ca levels for the three gums at initial pH are given in Table 1. The initial pH values were 5·4–5·5 for Ara, 6·1–6·3 for Loc, and 6·1–6·2 for Xan. Xan bound about 100% at the 100 μM Ca addition, but about 35% at the 3000 μM addition. Ara bound between 20 and 33·5% of added Ca in the range studied. Loc bound the least of the three gums with 5% at lower levels of addition and about 16% at the 3000 μM level of addition.

Neutral polysaccharides, such as the galactomannan Loc, have a low affinity for cations at neutral pH (Rendleman, 1978). However, in alkaline conditions, hydroxylic protons are released and mineral–polysaccharide complex formation is possible (Rendleman, 1978). This behavior of Loc was evident at pH 9·5 where the highest amount of Ca was bound (Table 2). Statistically important terms for the model of bound Ca for Loc were linear and quadratic terms of level of addition; the linear term of pH, and the linear term of interaction.

Ara consists of a galactose backbone to which side-chains consisting of galactose, arabinose, rhamnose, and glucuronic acid are attached (Ha *et al.*, 1989). The polysaccharide is highly branched and carries a negative charge. Maximum binding of Ca to Ara occurred at pH 7 and 8 (Table 3). Important terms for the statistical model of bound Ca for Ara included linear and quadratic terms of level of addition, pH and interactions.

Xan consists of a cellulosic backbone to which trisaccharide chains consisting of two mannose and one glucuronic acid residue are attached (Anderson & Andon, 1988). The mannose residues can carry acetyl and pyruvate groups (Rees *et al.*, 1982). Maximum binding of Ca occurred at pH 7 and 8 (Table 4). Statistically important terms for the model of bound Ca for Xan included linear and quadratic terms of level of addition, pH, and interactions.

All gums bound less Ca at lower pH levels. For Ara and Loc a drop was apparent from pH 5 downward, and for Xan from pH 6 downward (Tables 2–4). A pH-dependency for mineral binding has also been observed by other authors (Camire & Clydesdale, 1981; Ha *et al.*, 1989; Schlemmer, 1989).

For higher levels of Ca addition (1500–2000 μM), a drop in Ca binding is obvious for all three gums. This behavior was only found during the titration study, where pH was raised to 9·5 and then dropped stepwise to 4·1. It is possible that the combination of high pH and high amounts of Ca affected structural components of all three gums which resulted in diminished binding when compared to Ca additions at initial pHs. This effect was most pronounced in Loc, where additions of 2000 and 3000 μM Ca resulted in release of previously bound endogenous Ca, as seen in the negative numbers for bound Ca. Ca binding in Ara and Xan was also diminished, but not as much as in Loc. Lambert *et al.* (1984) demonstrated conformational changes in Xan induced by NaOH, which could explain the different

Table 3. Effect of pH on bound calcium levels in gum arabic[a]

Level of addition (μM)	pH[b]						
	4·1	4·5	5·0	6·0	7·0	8·0	9·5
100	−39·7 ± 3·8	−29·2 ± 5·5	9·3 ± 4·6ab	10·4 ± 13·5a	7·7 ± 11·8a	12·9 ± 14·3	22·4 ± 16·7
300	−1·70 ± 2·7	51·1 ± 4·4	68·6 ± 6·0	78·4 ± 5·1	88·3 ± 2·0	80·3 ± 10·8	102 ± 1·0
500	62·2 ± 10·4	128 ± 5·8	126 ± 8·2	114 ± 6·9	140 ± 15·4	156 ± 5·1	148 ± 0·2
1 000	108 ± 17·1	208 ± 4·1	360 ± 11·3	317 ± 10·9	308 ± 12·7	333 ± 20·3	257 ± 45·9
1 500	124 ± 20·3	250 ± 7·5	441 ± 40·1	445 ± 0·3	467 ± 15·8	455 ± 41·9	457 ± 13·7
2 000	−162 ± 55·2	−39·0 ± 83·9	51·1 ± 71·4	122 ± 29·2	136 ± 32·8	129 ± 35·8	139 ± 16·9
3 000	−266 ± 85·2	57·0 ± 57·4	114 ± 55·1	118 ± 53·2	240 ± 43·9	215 ± 27·7	129 ± 32·3

[a] Data are given as μM bound calcium ± standard error of mean. Gum concentration 0·25% w/v.
[b] Means followed by the same letter in one row are equal at LS means comparison $P < 0·05$.

Table 4. Effect of pH on bound calcium levels in xanthan gum[a]

Level of addition (μM)	pH						
	4·1	4·5	5·0	6·0	7·0	8·0	9·5
200	38·3 ± 2·7	87·7 ± 3·6	134 ± 1·7	169 ± 0·7	170 ± 1·0	165 ± 1·0	155 ± 0·9
300	68·8 ± 3·4	139 ± 3·3	209 ± 2·2	228 ± 2·0	239 ± 1·0	240 ± 1·3	242 ± 1·2
400	85·0 ± 0·9	166 ± 4·5	256 ± 6·1	290 ± 4·3	296 ± 4·0	301 ± 4·2	298 ± 4·4
500	129 ± 7·8	230 ± 6·9	307 ± 4·1	341 ± 7·0	369 ± 2·2	376 ± 3·3	349 ± 5·1
1 000	168 ± 21·2	301 ± 13·0	543 ± 15·4	567 ± 15·5	560 ± 25·4	580 ± 36·6	530 ± 64·4
1 500	148 ± 31·2	297 ± 57·3	485 ± 48·4	521 ± 40·0	545 ± 66·0	609 ± 39·1	568 ± 69·0
2 000	189 ± 18·4	255 ± 37·4	457 ± 9·8	643 ± 17·0	637 ± 56·8	628 ± 47·5	622 ± 32·3
2 500	262 ± 77·6	469 ± 42·3	488 ± 49·9	683 ± 62·0	726 ± 57·1	819 ± 75·5	678 ± 113
3 000	211 ± 88·8	509 ± 131	579 ± 71·3	773 ± 64·1	835 ± 77·1	856 ± 57·5	775 ± 65·0

[a] Data are given as μM bound calcium ± standard error of mean. Gum concentration 0·10% w/v.

binding behavior at initial pH (Table 1) as compared to the titration study.

Another aspect to consider is that the gums were not purified and there may have been some minor interferences from impurities. HPLC analysis of all three gums showed the presence of monosaccharides, which could have resulted in soluble chelates with Ca (Rendleman, 1966). The samples were not analyzed for other organic impurities.

Scatchard analysis (Scatchard, 1949) of the gums resulted in curved line models for Ara and Xan (Figs 1 and 2) and a straight line for Loc (Fig. 3). Curved lines indicate two or more binding sites and can be resolved into their single components (Pennock, 1973) and intrinsic association constant k, and the number of binding sites, n, can be calculated. Xan had the highest affinity for Ca for both specific and unspecific binding site, with $k_1 = 5{\cdot}28$ and $k_2 = 6{\cdot}30 \times 10^{-2}$ and corresponding n of 54 and 413, respectively. The intrinsic association constants for Ara were one order of magnitude lower, with $k_1 = 8{\cdot}72 \times 10^{-1}$, and $k_2 = 1{\cdot}46 \times 10^{-3}$. However, the number of binding sites was higher than in Xan with $n_1 = 133$ and $n_2 = 560$. The intrinsic association constant $k = 8{\cdot}43 \times 10^{-2}$ for Loc was similar to the non-specific binding site in Xan. Ha *et al.*. (1989) found two types of binding sites for Ara and one type of binding site for Loc. Possible explanations for the variation in numbers between Ha *et al.*. (1989) and the present study are the difference in gum concentration and the limited data points in Ha *et al.*'s study, which introduce a higher error rate (Klotz, 1982).

Effect of hemicellulase digestion

Upon hemicellulase digestion, all gums lost all or some of their Ca binding ability (Tables 5–7). Digestion of Ara and Loc resulted in free Ca levels that exceeded the levels which were added at lower levels of Ca addition, indicating a release of previously bound Ca possibly due to breakdown of the polysaccharide structure. Loc released all endogenous Ca at levels of addition up to 1000 μM (Table 5). At higher levels of Ca addition, measured free Ca was less than the amount added. Ara did not release all endogenous Ca and reached the maximum amount of free Ca at 24 h (Table 6). The increase in free Ca at 72 h in Ara is probably due to dilution rather than further break-

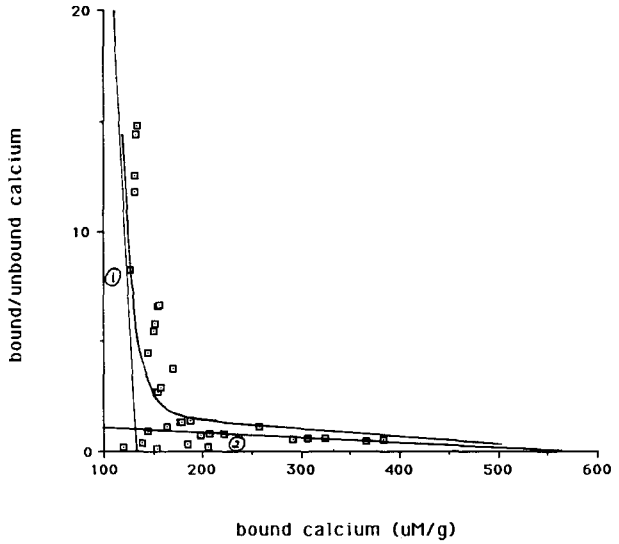

Fig. 1. Scatchard plot for Ca binding by 0·25% (w/v) solutions of gum arabic at initial pH. Line 1 depicts the specific binding site; line 2 the non-specific binding site for Ca.

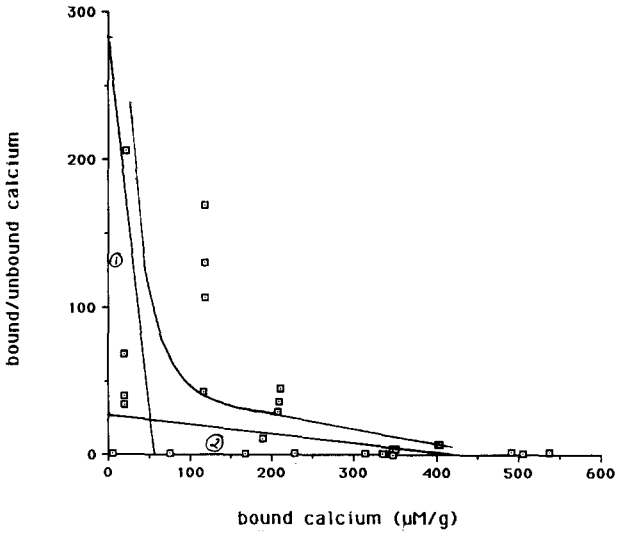

Fig. 2. Scatchard plot for Ca binding by 0·10% (w/v) solutions of xanthan gum at initial pH.

Table 5. Effect of hemicellulase digestion on calcium binding by locust bean gum[a]

Level of addition (μM)	Time (h)			
	0	1	24	48
0	22·3 ± 8·7ab	28·7 ± 7·6a	55·2 ± 2·7	41·3 ± 3·9
100	94·6 ± 16·8	128 ± 26·4a	149 ± 6·3	126 ± 11·1a
200	185 ± 21·8	226 ± 41·8a	251 ± 9·5	224 ± 16·4a
500	439 ± 23·3	573 ± 70·6	561 ± 14·7	591 ± 57·9
1 000	860 ± 40·7	978 ± 71·8	1053 ± 40·6	1016 ± 73·3
2 000	1651 ± 40·3	1768 ± 109	1932 ± 66·4	1885 ± 125
3 000	2291 ± 38·7	2391 ± 108	2706 ± 104	2673 ± 205

[a] Data are given as μM free calcium ± standard error of mean. Gum concentration 0·25% w/v.
[b] Means followed by the same letter in one row are equal at LS means comparison $P < 0·05$.

down. The pH dropped to about 3 during digestion and large amounts of 0·01 M NaOH had to be added to bring the pH to 4, which led to a dilution of the initial gum concentration of about 30%. Xan never completely lost its ability to bind Ca (Table 2), but binding decreased by 35–45%. A Scatchard plot of Xan digested for 48 h (Fig. 4) indicated that mostly specific binding sites were lost.

Statistically important terms for the models of free Ca of digested Loc and Xan include linear and quadratic terms for level of Ca addition, time of digestion, and interaction terms for Loc and Xan. For Ara, the linear terms of level of addition, time, and interaction, as well as the quadratic term of time, were significant.

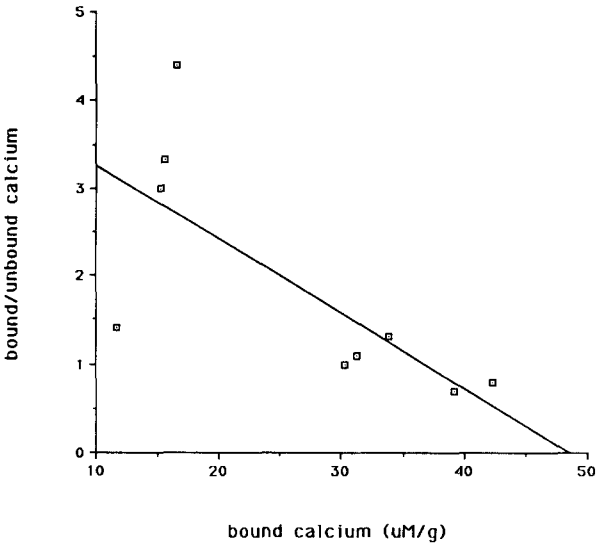

Fig. 3. Scatchard plot for Ca binding by 0·25% (w/v) solutions of locust bean gum.

The scope of this study was to characterize the Ca-binding ability of these gums during hemicellulase digestion. No attempt was made to determine specific activity of the enzyme mixture used. Depolymerization of the gums was measured by observing changes in viscosity over time with Cannon–Fenske routine viscometers (Stauffer, 1989). This did not yield specific data on depolymerization or the extent thereof, nor whether selective hydrolysis resulted in the loss of Ca binding, but was a means to assess hemicellulase action. The changes of viscosity during hemicellulase

Table 6. Effect of hemicellulase digestion on calcium binding by gum arabic

Level of addition (μM)	Time (h)				
	0	3	24	48	72
0	27·6 ± 2·8	39·2 ± 2·9	171 ± 10·9	189 ± 4·1	203 ± 11·6
100	66·5 ± 4·0	103 ± 7·7	245 ± 7·8ab	249 ± 23·0a	289 ± 7·5
200	134 ± 5·8	181 ± 14·2	317 ± 14·4	348 ± 12·2	382 ± 11·7
500	356 ± 15·1	437 ± 29·3	595 ± 20·7	616 ± 21·1	682 ± 25·3
1 000	695 ± 40·1	904 ± 43·4	1 082 ± 16·5	1 043 ± 27·7	1 187 ± 39·2
2 000	1 432 ± 83·1	1 691 ± 103	1 955 ± 36·1	1 915 ± 69·5	2 200 ± 93·6
3 000	2 096 ± 129	2 384 ± 98·4	2 722 ± 89·7a	2 726 ± 83·5a	3 170 ± 130

[a] Data are given as μM free calcium ± standard error of mean. Gum concentration 0·25% w/v.
[b] Means followed by the same letter in one row are equal at LS means comparison $P < 0·05$.

Table 7. Effect of hemicellulase digestion on calcium binding by xanthan gum[a]

Level of addition (μM)	Time (h)					
	0	3	24	48	72	92
0	0·4 ± 0·1abc	0 ± 0ade	9·4 ± 2·6f	5·0 ± 2·8bdfgh	2·7 ± 1·0ceg	10·2 ± 1·9h
100	1·4 ± 0·5a	0 ± 0a	45·9 ± 3·8bc	45·0 ± 7·3bd	41·0 ± 5·2cd	59·4 ± 7·2
200	9·1 ± 3·2	0 ± 0	113 ± 7·0a	124 ± 13·8b	108 ± 8·2a	127 ± 14·1b
500	84·4 ± 9·9a	88·3 ± 12·3a	333 ± 14·6	397 ± 31·5	351 ± 8·6	364 ± 36·0
1 000	360 ± 31·1	422 ± 45·9	790 ± 34·7	908 ± 27·7	818 ± 8·9a	813 ± 47·7a
2 000	1 023 ± 35·8	1 363 ± 137	1 537 ± 47·0	1 852 ± 76·0	1 712 ± 9·8	1 677 ± 102
3 000	1 770 ± 40·9	1 942 ± 163	2 331 ± 70·0	2 769 ± 98·1	2 606 ± 50·7	2 517 ± 148

[a] Data are given as μM free calcium ± standard error of mean. Gum concentration 0·10% w/v.
[b] Means followed by the same letter in one row are equal at LS means comparison $P < 0·05$.

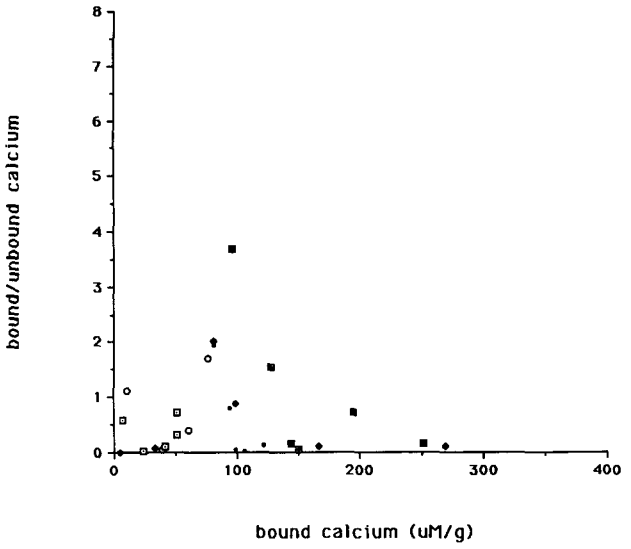

Fig. 4. Scatchard plot of 48 h hemicellulase-digested xanthan gum. Different symbols depict different samples.

digestion are given in Table 8. Ara, the least viscous of the gums, dropped from 1·22 to 1·10 cSt. The viscosity of Loc dropped from 1·93 to 1·01 cSt, and Xan dropped from 5·09 to 1·76 cSt after incubation at 38°C.

CONCLUSIONS

Locust bean gum (Loc), gum arabic (Ara) and xanthan gum (Xan) were investigated to determine their affinity for Ca under varying pH conditions and after hemicellulase digestion. All gums bound increasing amounts of Ca at levels of addition from 100 to 3000 μM. Loc bound least and Xan the most added Ca. Scatchard analysis revealed two types of binding sites for Ca for Ara and Xan, and one type of binding site for Loc.

Raising the pH to 9·5 concomitant with a Ca addition of above 1500–2000 μM led to reduced binding capacity in all three gums as compared to additions made at the initial pH. The drop in binding capacity was most pronounced in Loc and least pronounced in Xan. This loss of binding capacity may reflect structural changes in the gums induced by a high pH.

Binding of Ca in all three gums resulted in decreased binding at pH 5 and below for Loc and Ara, and a drop in binding from below pH 6 for Xan. Maximum binding occurred around neutral pH for Ara and Xan and at pH 9.5 for Loc.

Hemicellulase digestion resulted in loss of binding capacity for all three gums. Incubation of Loc and Ara with hemicellulase for 24 h resulted in release of previously bound endogenous Ca. In Xan, hemicellulase digestion decreased binding capacity between 35 and 45%.

In the present study, isolated fibers to which one mineral had been added were investigated. It is important to gain insight into polysaccharide–mineral interactions by studying simple systems. However, additional work that studies binding behavior between gums and several minerals are needed to learn more about interactions.

Table 8. Viscosity changes during hemicellulase digestion[a]

Time (h)	Viscosity (cSt)[b]		
	Ara	Loc	Xan
0	1·22	1·93	5·09
3	1·18	1·03	4·32
24	1·11	1·02	1·89
48	1·10	1·01	1·82
72	1·10	—	1·78
96	—	—	1·76

[a] Gum concentration 0·25% (w/v) for Ara and Loc, 0·10% (w/v) for Xan; 38°C.
[b] 1 St = 10^{-4} m^2/s.

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