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Chemical and physical properties of yellow mustard (*Sinapis alba* L.) mucilage

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The yield of crude mucilage (CM) obtained from yellow mustard (*Sinapis alba*, L.) was 5% of the total seed weight. CM was separated into a water-soluble fraction (WS, 55·6%) and a water-insoluble fraction (WI, 38·8%). Proximate analysis of CM and its fractions revealed carbohydrates as the major component (80–94%) with ash (1·7–15·0%) and protein (2·2–4·4%) as minor constituents. Glucose (22–35%) was the major monosaccharide present followed by galactose (11–15%), mannose (6·0–6·4%), rhamnose (1·6–4·0%), arabinose (2·8–3·2%) and xylose (1·8–2·0%). All mucilage fractions exhibited interfacial activity and shear thinning behaviour typical of xanthan gum dispersions. Of the mucilage fractions studied, the WS fraction was least affected by pH, temperature and solutes (NaCl, sucrose).

INTRODUCTION

White or yellow mustard seeds, *Sinapis alba* L., are grown primarily for their use as condiments. The outer seed coat of mustard seeds was first reported, almost 60 years ago, to be rich in mucilaginous material (Bailey & Norris, 1932). The consistency of prepared mustard products, such as salad dressings and food pastes, was later attributed by Weber *et al.* (1974) to the presence of mucilage. The mucilage in four yellow mustard cultivars grown at four different locations in Western Canada was reported by Woods & Downey (1980) to range from 0·34% to 2·05% with an overall average of 1·28%. A number of researchers have since shown mustard seed mucilage to be composed primarily of polysaccharides containing glucose, arabinose, xylose, rhamnose, galactose, mannose and galacturonic acid (Vose, 1974; Theander *et al.*, 1977; Siddiqui *et al.*, 1986). A recent report by Anguilar & Ziegler (1990) showed both temperature and electrolytes had a negative effect on the viscosity of aqueous dispersions of mustard seed mucilage. These studies were all based on mucilage extracted using the Weber method which only extracted 2% mucilage (Weber *et al.*, 1974; Anguilar & Ziegler, 1990). Studies conducted in our laboratory yielded higher amounts of mucilage (~5·0%) from yellow mustard seeds using a modified extraction procedure (Sharafabadi, 1987). The material obtained was white and cotton-like in appearance, exhibiting shear-thinning behaviour similar to xanthan gum dispersions.

The initial objective of the present study was to maximize the extraction yield of mucilage from yellow mustard seeds. Since mustard mucilage was shown to contain two fractions, a water soluble (WS) fraction and a water insoluble (WI) fraction, further objectives included separation of these fractions to determine their chemical composition and physical properties. The potential food application of mustard mucilage was assessed by comparison with commercial gums.

MATERIALS AND METHODS

Materials

Whole mustard seeds (*Sinapis alba* L. cv Tilney) were obtained from United Grain Growers (UGG), Winnipeg, Manitoba, Canada. Xanthan, guar and arabic gums were purchased from Sigma Chemical Co., St. Louis, USA. All chemicals used were of reagent grade.

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Extraction, fractionation and analyses of yellow mustard seed mucilage

Crude mucilage (CM) and its respective water soluble (WS) and water insoluble (WI) fractions were obtained following the procedure of Sharafabadi (1987) which was modified by successive aqueous extraction with seed:water ratio of 1:6 (Fig. 1).

Moisture and ash contents were determined using the AOAC oven method (AOAC, 1980). Protein was determined by a microkjeldahl method using a Kjeltec Auto 1030 Analyzer (Tecator, Sweden). Phosphorus and sulphur were determined using the method described by McKeague (1978), while other minerals were determined by atomic absorption (Perkin Elmer 560 Atomic Absorption Spectrometer, wet-ashed). Uronic acids were measured colorimetrically according to Blumenkrantz & Asboe-Hansen (1973). Monosaccharide determination was carried out by GLC using a SP-2330 glass capillary column, 30 m × 0·75 mm i.d., according to Englyst *et al.* (1982). ^{13}C-NMR spectra (500 Hz) were recorded on a Bruker AMX500 FT spectrometer at 85°C; polymer concentration 2·0% (w/v) in D_2O, 40000 pulses, pulse repetition time 1·3 s and r.f. pulse angle 80·0°.

Gel permeation chromatography was conducted on a Sephacryl S-300 (HR, 1·6 × 70 cm) column, eluted with 0·1 M NaCl solution. Samples dissolved in the same buffer (1 mg/ml) were applied onto the column, and fractions of 2 ml were collected. Blue dextran 2000 and D-glucose were used to determine the void and total volumes respectively, while linear dextran T-70 (Pharmacia Ltd, Montreal, PQ) was used as a relative molecular weight marker. Carbohydrates in the fractions were determined by the anthrone method (Loewus, 1952), and uronic acids were according to the method of Blumenkrantz & Asboe-Hansen (1973).

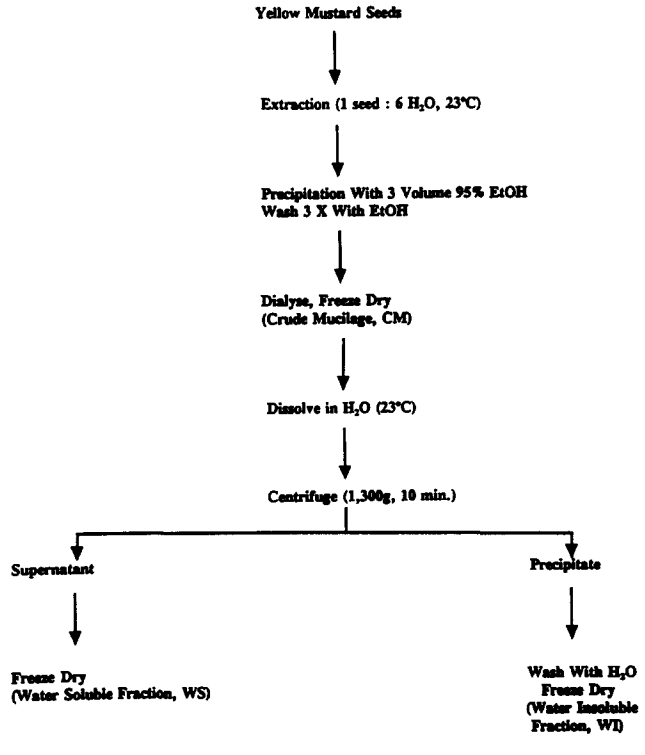

Fig. 1. Procedure for extraction and fractionation of yellow mustard mucilage.

Interfacial properties

The interfacial activity of yellow mustard mucilage was examined following the procedure of Izydorczyk *et al.* (1991). Emulsion capacity and stability tests were performed as described by Yasumatsu *et al.* (1972) with the following modification: 0·50 g of gum or mucilage was suspended in 40 ml of distilled water followed by the addition of 40 ml of corn oil. All mixtures were then emulsified using a polytron at 10000 rpm for 1 min and centrifuged at 1300 *g* for 5 min. Emulsion capacity was calculated as:

$$\frac{\text{height of emulsion layer}}{\text{total height of fluid}} \times 100\%$$

Emulsion stability was determined by heating the emulsion at 80°C for 30 min, cooling with tap water for 15 min and then centrifuging at 1300 *g* for 5 min. Emulsion stability was calculated as:

$$\frac{\text{height of remaining emulsion layer}}{\text{total height of fluid}} \times 100\%$$

Foaming capacity and stability of mustard mucilage and commercial gums were determined by the method of Yasumatsu and co-workers (1972) with a slight modification: 0·3% of mucilage (gum) solutions were made in 0·1% Ovalbumin (Sigma Chemical Co.). The reported data represent means of triplicate measurements.

Rheological properties

All rheological properties were determined on a Bohlin VOR Rheometer (Bohlin Reologi, Sweden). A concentric cylinder geometry, with height of 63·0 mm and radii of the inner and outer containers of 12·5 mm and 13·75 mm, respectively, was used throughout the rheological study (Mazza & Biliaderis, 1989). The samples were subjected to shear sweeps between 3·682 sec^{-1} to 734·3 sec^{-1}. Viscosity measurements were conducted using aqueous solutions of 0·3%, 0·5% and 1·0% (w/w). The influence of pH, salt and sugar on the viscosity of mucilage solutions was examined at 0·5% (w/w), while temperature effects were examined at 1·0% (w/w). Dynamic rheological measurements on 1·0% (w/w) solutions and dispersions of mucilage as well as of commercial gums were carried out as a function of oscillatory frequency (*f*: 0·5–20·0 Hz) with a maximum input strain of 4% at 22°C. The rheological parameters used to evaluate the viscoelastic properties of these materials were the storage modulus (G'), loss modulus (G"), dynamic viscosity $\eta' = G''/2\pi f$) and phase angle, δ ($\tan \delta = G''/G'$). Data presented are means of triplicate measurements.

Table 1. Yield of mucilage from yellow mustard seeds upon sequential aqueous extraction (100 g seeds/600 g H_2O)

Extraction	1	2	3	4	5	Total
Weight (g)	3·45	0·93	0·54	0·25	0·12	5·29
Percent (%)	65·2	17·6	10·2	4·7	2·3	100

RESULTS AND DISCUSSION

Chemical compositions

The yield of mucilage obtained upon successive extractions of yellow mustard seeds is shown in Table 1. The initial extraction removed 65·2% of the mucilage, with the second and third successive extractions accounting for 17·6% and 10·2% of the total mucilage, respectively. The first three fractions accounted for 93·0% of the total mucilage extracted which represented 4·9% of the total seed. CM was fractionated into a WS fraction and a WI fraction according to the procedure outlined in Fig. 1. Following extraction, the mucilage was precipitated with 3 volumes of 95% ethanol, dialysed and then freeze-dried to provide the crude mucilage (CM). CM was further fractionated into a water soluble (WS) fraction and a water insoluble (WI) fraction by centrifugation and accounted for 55·6% and 38·8% of the crude mucilage, respectively, with an overall recovery of 94·4%.

The results of proximate analysis of crude mucilage and its fractions are shown in Table 2. Crude mucilage contained 80·4% carbohydrates, 4·4% protein and 15·0% ash. Following dialysis, the ash content was reduced substantially from 15·0% to 4·8%, while the amount of carbohydrates increased from 80·2% to 91·1%. The carbohydrate content was similar for both WS and WI fractions, while the ash content was higher in the WS fraction and protein content higher in the WI fraction. The monosaccharide composition of yellow mustard mucilage and its fractions is summarized in Table 3. Glucose appeared to be the predominant sugar, followed by galactose, mannose, rhamnose, arabinose and xylose. The only exception was for the WI fraction where arabinose was higher than rhamnose. Uronic acid content was highest in the WS fraction accounting for 18·6% and lowest in the WI fraction at 10·2% with CM in between at 14·7%.

^{13}C NMR (Fig. 2) confirmed the presence of uronic acid by the characteristic resonance at δ = 174·93 ppm and rhamnose by the typical C-6 resonance at δ = 17·99 ppm. Because of the complexity of the spectra, due to the heterogeneous nature and complex structure of the polysaccharide species present in the WS fraction, a complete assignment of resonances to characteristic carbons of monosaccharide residues was not feasible. Nevertheless, in the anomeric carbon region (95–108 ppm) at least six resonances were present which correspond with the main sugars identified by monosaccharide analysis (Table 3).

Table 2. Chemical compositions of yellow mustard seed mucilage and its fractions

Component (on dry base)		CM[c]	CM[d]	WS	WI
Water	(%)	6·9	8·7	10·2	8·1
Ash	(%)	15·0	4·8	4·3	1·7
Protein	(%)[a]	4·4	4·1	2·2	4·2
Fat	(%)	0·2	—[e]	—	—
Carbohydrate	(%)[b]	80·4	91·1	93·5	94·1
Potassium	(%)	2·1	0·04	0·01	0·02
Calcium	(%)	2·2	1·2	1·6	0·48
Magnesium	(%)	1·3	0·6	0·4	0·16
Phosphorus	(%)	2·1	0·6	0·4	0·09
Sulphur	(%)	1·4	0·29	0·16	0·02
Iron	(ppm)	212·5	260·0	223·0	358·0
Zinc	(ppm)	53·5	70·0	123·0	208·0
Manganese	(ppm)	73·0	70·0	72·0	27·0
Copper	(ppm)	13·5	12·0	34·0	18·0

NB: CM = mucilage; WS = water soluble fraction; WI = water insoluble fraction.
[a] N × 6·25.
[b] By difference.
[c] Crude mucilage before dialysis.
[d] Cude mucilage after dialysis.
[e] Not determined.

Gel filtration chromatography of CM and WS fractions on the Sephacryl S-300 column (Fig. 3) indicated the presence of both high and low molecular weight polysaccharide species, the most prominent being the peak at the void volume. Uronic acids were detected in both high and low molecular weight regions of the eluted carbohydrates.

Interfacial properties

The effect of yellow mustard mucilage fractions on the surface tension of water is shown in Fig. 4. Increasing the mucilage concentration up to 0·05% substantially reduced surface tension. Further additions of mucilage only decreased the surface tension slightly. WS exhibited the greatest reduction in surface tension compared

Table 3. Yield, monosaccharide composition and uronic acid content of yellow mustard mucilage and its fractions[a]

Mucilage and fraction	Sample yield (%)	Uronic acid (%)	Glucose (%)	Galactose (%)	Mannose (%)	Rhamnose (%)	Arabinose (%)	Xylose (%)
CR	—	14·64 ± 0·62	23·54 ± 0·77	13·83 ± 0·30	6·07 ± 0·20	3·15 ± 0·16	3·02 ± 0·17	1·80 ± 0·09
WS	55·6 ± 2·2	18·68 ± 1·36	22·26 ± 1·57	15·21 ± 0·70	6·31 ± 0·30	3·93 ± 0·0	3·22 ± 0·08	1·77 ± 0·10
WI	38·8 ± 1·8	10·30 ± 1·07	34·95 ± 1·35	11·70 ± 0·76	6·35 ± 0·01	1·65 ± 0·35	2·84 ± 0·03	2·00 ± 0·11

NB: CM = crude mucilage; WS = water soluble fraction; WI = water insoluble fraction.
[a] n = 3, mean ± SD.

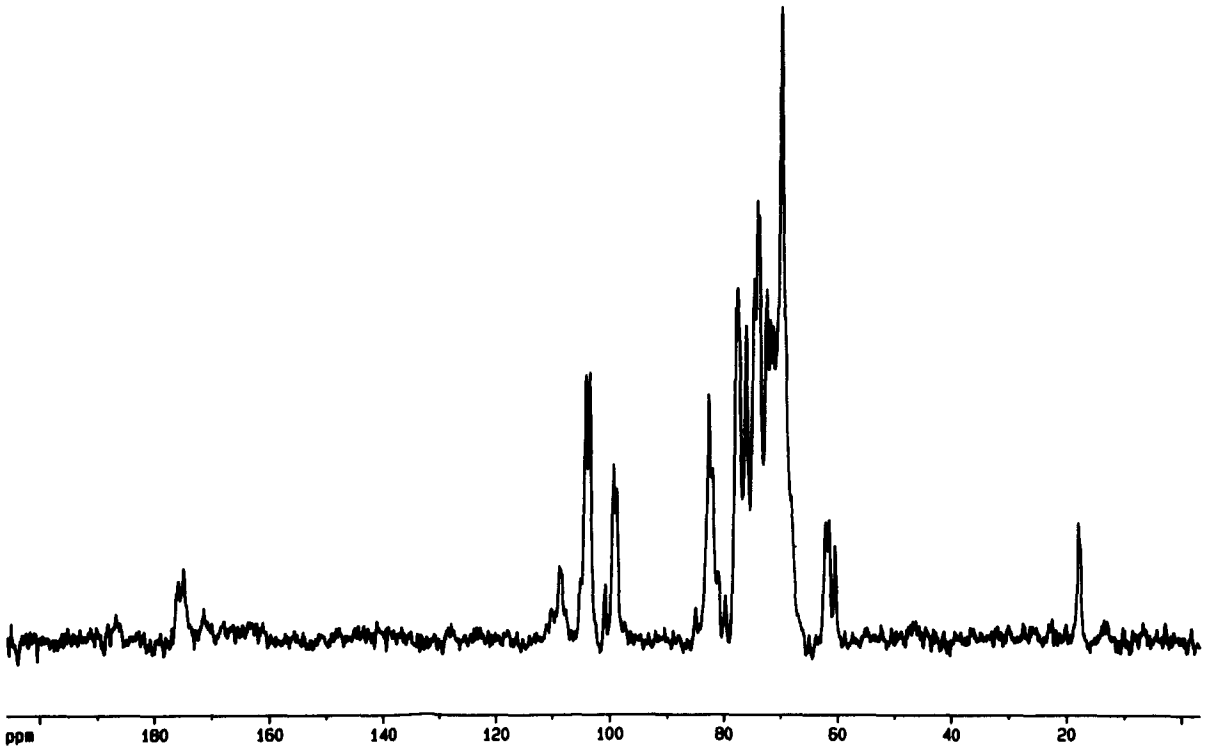

Fig. 2. ^{13}C-NMR spectrum of the water soluble fraction of yellow mustard mucilage (in D_2O). The chemical shifts were assigned relative to 1,4-dioxan.

to CM or WI fraction. The surface and interfacial activities of some plant hydrocolloids (guar and locust bean gums etc.) were recently ascribed to be due to the presence of residual surface active constituents/impurities in them (Gaonkar, 1991). The protein present in CM and its fractions (Table 2) could thus contribute to the surface activity of the polysaccharides; interestingly, the WS fraction, although having the lowest protein content, was the most surface active of all mucilage fractions.

The emulsion capacity and stability of yellow mustard mucilage and its fractions were compared to commercial gums as shown in Fig. 5. Before dialysis, the CM fraction exhibited the highest emulsion capacity and stability as compared to the other mucilage fractions or commercial gums. While dialysis reduced the emulsion capacity and stability of CM substantially, it still exhibited higher emulsion capacity and stability compared to the commercial gums.

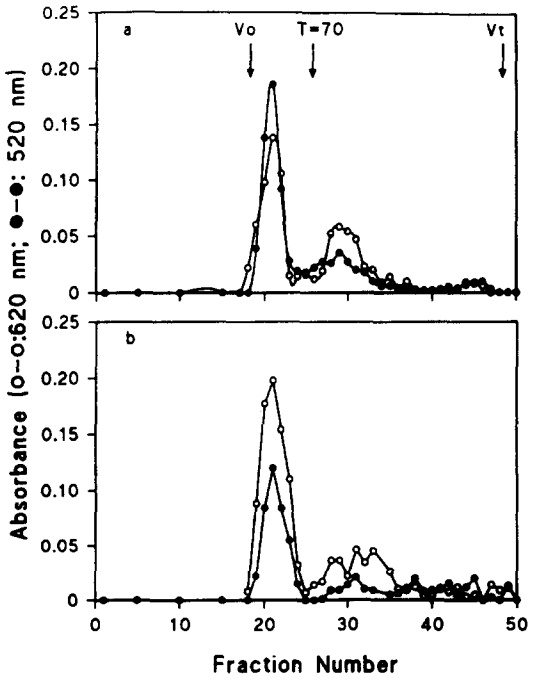

Fig. 3. Chromatographic profiles of (a) CM, and (b) WS fractions on a Sephacryl S-300 HR column (1·6 × 70 cm) eluted with 0·1N NaCl solution, flow rate 1 ml/min, temperature 23°C; arrows indicate peak elution volumes of dextran standards (Blue T-2000, Vo; T-70; Glucose, Vt) used as molecular weight markers (○—○ total carbohydrate; ●—● uronic acids). (CM = crude mucilage; WS = water soluble fraction.)

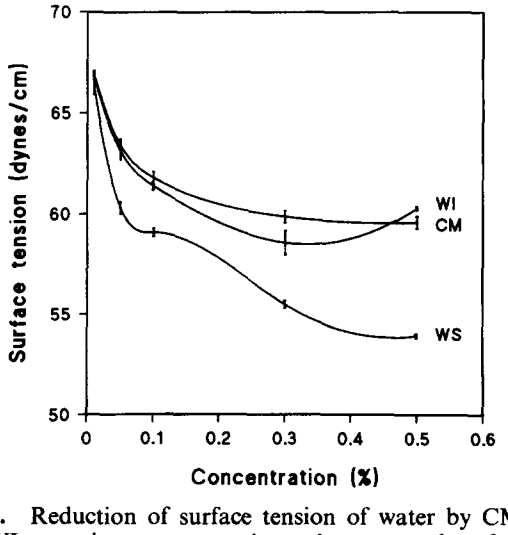

Fig. 4. Reduction of surface tension of water by CM, WS and WI at various concentrations; the corrected surface tension of distilled water was 69·0 ± 0·7 dyne/cm at 23·0 ± 0·5°C. (CM = crude mucilage; WS = water soluble fraction; WI = water insoluble fraction.)

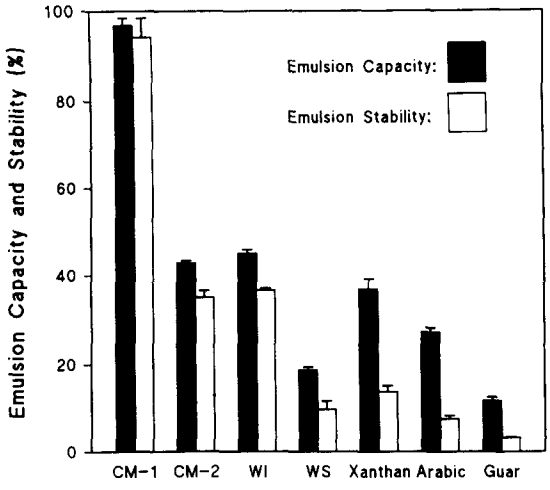

Fig. 5. Emulsion capacity and stability of yellow mustard mucilage fractions and other commercial gums; CM-1 and CM-2 are crude mucilage samples before and after dialysis.

The foaming capacity and stability of yellow mustard mucilage fractions and commercial gums are summarized in Table 4. Prior to dialysis, the CM fraction exhibited the highest foaming stability among the yellow mustard mucilage fractions. Following dialysis, the foaming capacity of CM increased, but its foam was less stable. Similar trends were observed for both WS and WI fractions which exhibited higher foaming capacity but poorer foaming stability.

Rheological properties

The flow profiles of yellow mustard mucilage and its two fractions vs shear rate are shown in Fig. 6. The shear thinning behaviour exhibited by yellow mustard mucilage and its fractions resembled those of xanthan gum dispersions at all concentrations. In contrast, guar gum solutions showed shear thinning behaviour only at higher concentration (e.g. 1·0% w/w) and Newtonian behaviour at a lower concentration (0·3%).

The most widely used mathematical expression for pseudoplastic rheological behaviour of hydrocolloid solutions/dispersions is the power law model described by Ostwald (Whitcomb *et al.*, 1980):

$$\eta = K\dot{\gamma}^{n-1} \quad (1)$$

where η is the apparent viscosity (pas), $\dot{\gamma}$ is the shear rate, K is the consistency index (Pa s) and n is the flow index which measures the pseudoplasticity of the system. A comparison of n and K values of yellow mustard mucilages against xanthan and guar gums is given in Table 5. The K values increased with increasing polysaccharide concentration, but the n values decreased. This is in agreement with the findings of Whitcomb *et al.* (1980) on guar gum solutions; the higher the concentration, the more pronounced becomes the pseudoplastic behavior of a system.

The small strain oscillatory rheological testing of yellow mustard mucilage provided evidence which confirmed earlier observations (Fig. 6) that yellow mustard mucilage in solution behaves more like xanthan gum than guar gum (Fig. 7). Both xanthan gum and yellow mustard mucilage solutions/dispersions exhibited a gel-like structure with G' > G" (storage and loss moduli) over the entire frequency range examined. In contrast, guar gum solutions of identical concentration (1·0% w/w) behaved like typical visco-elastic fluids, where G" > G' at low frequencies, and the reverse occurs at high frequencies. This type of rheological response was also evident in the phase angle changes vs the frequency of the hydrocolloid solutions. The tangent of phase angle, δ, defined as the ratio of G"/G', expresses the relative contributions of the viscous and elastic components to the viscoelastic properties of a material. The constant increase of both G' and G" vs frequency resulted in relatively constant phase angle values for both xanthan gum and yellow mustard mucilage solutions over the entire frequency range examined (Fig. 8). In contrast, the phase angle values of guar gum solutions were highly dependent on frequency.

The effects of pH on the rheological properties of mucilage solutions (0·5% w/w) are shown in Fig. 9. Viscosity at a shear rate 93·32 s^{-1}, approximating mouth-feel conditions (Sherman, 1975), increased by either

Table 4. Effect of yellow mustard mucilage on foaming capacity and stability of 0·1% bovine serum albumin solutions

Time (h)	Foam volume (ml)						
	CM[a]	CM[b]	WS	WI	Xanthan	Arabic	Guar
0·0	23·5 ± 0·5[c]	38·3 ± 0·6	52·3 ± 1·5	39·0 ± 1·0	36·5 ± 0·9	41·8 ± 1·0	12·8 ± 0·3
0·5	19·2 ± 1·0	16·5 ± 1·3	29·2 ± 0·7	16·7 ± 0·8	36·5 ± 1·0	36·3 ± 1·2	9·3 ± 0·3
1·0	18·3 ± 0·8	13·7 ± 0·3	20·3 ± 0·4	15·0 ± 1·3	36·2 ± 0·5	32·8 ± 1·3	8·2 ± 0·0
3·0	15·9 ± 0·1	6·3 ± 1·0	0·0 ± 0·0[d]	0·0 ± 0·0	18·7 ± 1·5	20·6 ± 0·6	7·3 ± 0·6
5·0	14·8 ± 0·3	1·0 ± 0·0	0·0 ± 0·0	0·0 ± 0·0	17·5 ± 1·3	8·0 ± 0·9	6·6 ± 0·5
6·0	14·7 ± 0·3	0·0 ± 0·0	0·0 ± 0·0	0·0 ± 0·0	17·2 ± 1·0	6·6 ± 1·3	5·9 ± 0·1
23·0	13·3 ± 0·3	0·0 ± 0·0	0·0 ± 0·0	0·0 ± 0·0	13·2 ± 1·3	0·5 ± 0·5	4·0 ± 0·0

NB: CM = crude mucilage; WS = water soluble fraction; WI = water insoluble fraction.
[a] CM before dialysis.
[b] CM after dialysis.
[c] n = 3, mean ± SD.
[d] Diminished.

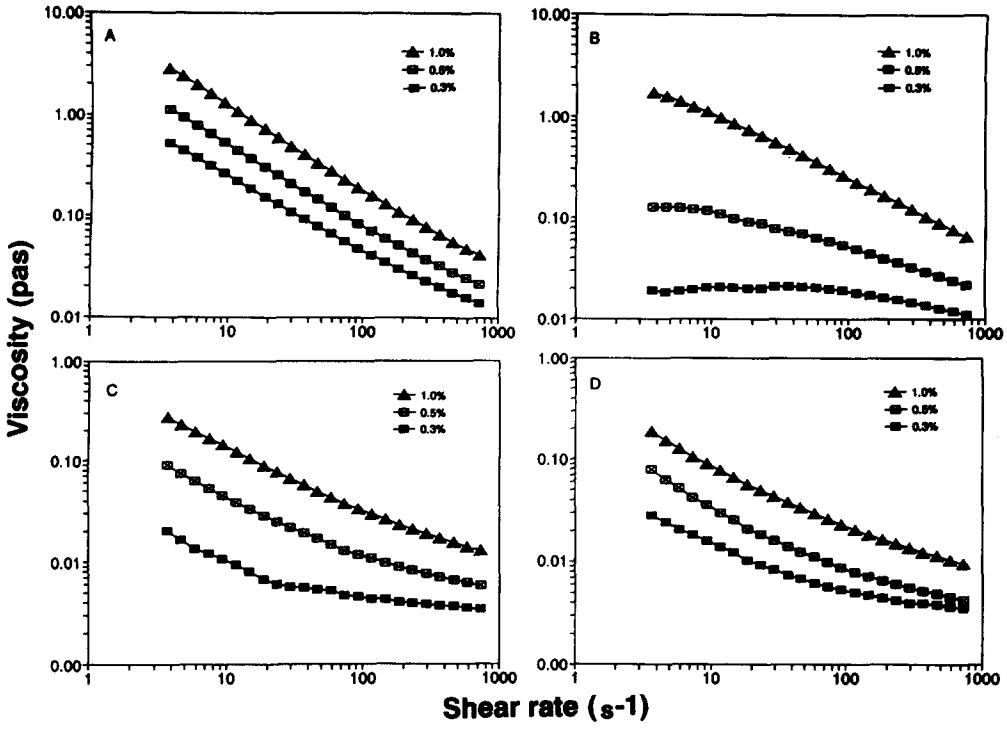

Fig. 6. Effect of shear rate on the apparent viscosity of (A) xanthan gum, (B) guar gum, (C) CM, and (D) WS at concentration between 0·3–1·0%, 22·0 ± 0·1°C. (CM = crude mucilage; WS = water soluble fraction.)

Table 5. Comparison of n and K values of mustard mucilage fractions against xanthan and guar gums solutions/dispersions (at 22·0°C, shear rate range: 3·682–734·3 s^{-1})

Concentration	CM		WS		WI		Xanthan		Guar	
	n	K (Pa s)	n	K (Pa s)	n	K (Pa s)	n	K (Pa s)	n	K (Pa s)
1·0%	0·427	0·480	0·466	0·280	0·436	0·957	0·188	7·813	0·350	4·830
0·5%	0·507	0·123	0·492	0·099	0·557	0·163	0·243	2·767	0·629	0·271
0·3%	0·740	0·016	0·638	0·031	0·639	0·064	0·309	1·167	0·885	0·028

NB: CM = crude mucilage; WS = water soluble fraction; WI = water insoluble fraction.

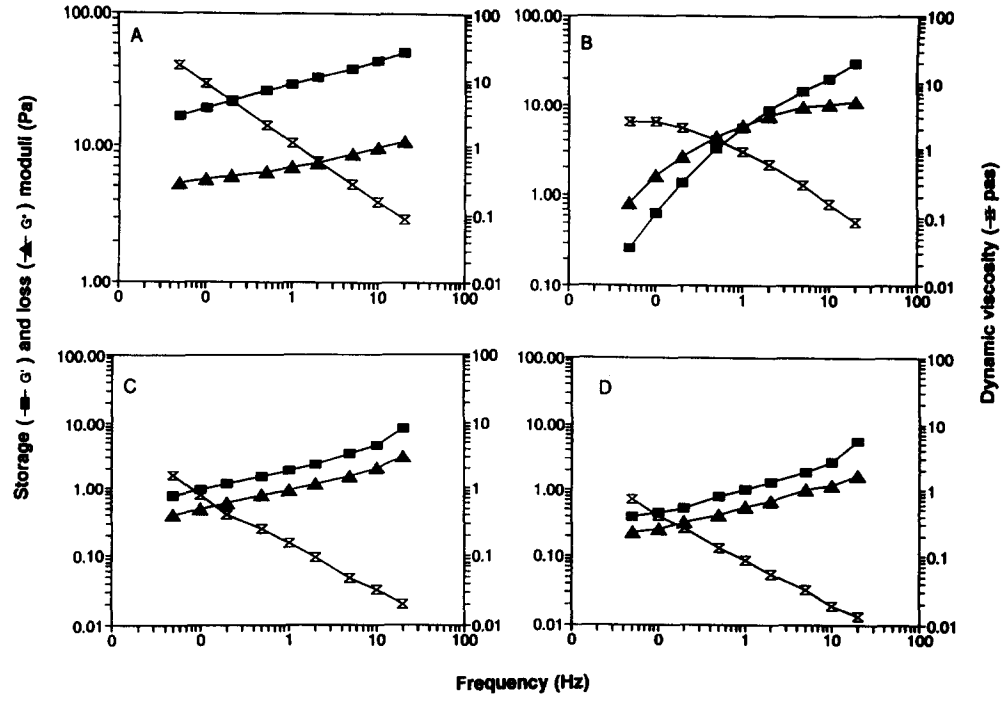

Fig. 7. Frequency dependence of storage (G') and loss (G") moduli, and dynamic viscosity (η') of (A) xanthan gum, (B) guar gum, (C) CM, and (D) WS for 1·0% (w/w) solutions/dispersions. (CM = crude mucilage; WS = water soluble fraction.)

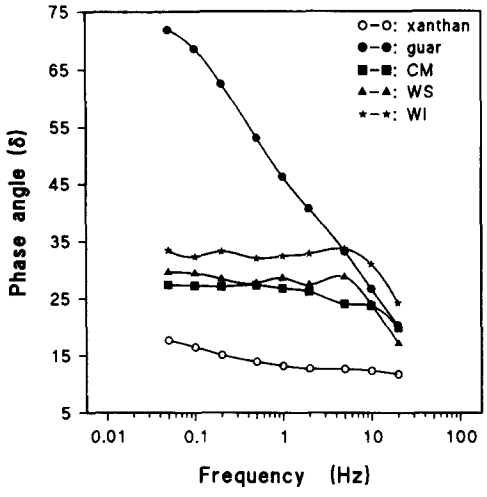

Fig. 8. Phase angle value profiles as a function of frequency of yellow mustard mucilage and commercial gum for 1% (w/w) solutions/dispersions. (CM = crude mucilage; WS = water soluble fraction; WI = water insoluble fraction.)

addition of dilute HCl or NaOH solutions in agreement with earlier work of Weber and co-workers (1974). The increase in viscosity at both low and high pH regions suggested that acid or alkali environments alter the conformation of the polysaccharides and most likely affect intermolecular interaction due to modification of electrostatic effects. Furthermore, the viscosity increase of WI in the alkaline region could be due to a more effective dispersion/solubilization of the insoluble mucilage fraction under these conditions. The extent of the influence of pH on the apparent viscosity of mucilage solutions (suspensions) was in the order of WI > CM > WS.

Similar trends in viscosity for CM and its fractions (0·5% w/w) were observed with the addition of either NaCl (0·4–3·5 M) or sucrose (0·15–1·2 M). Solutions of CM and WS generally exhibited higher viscosity values

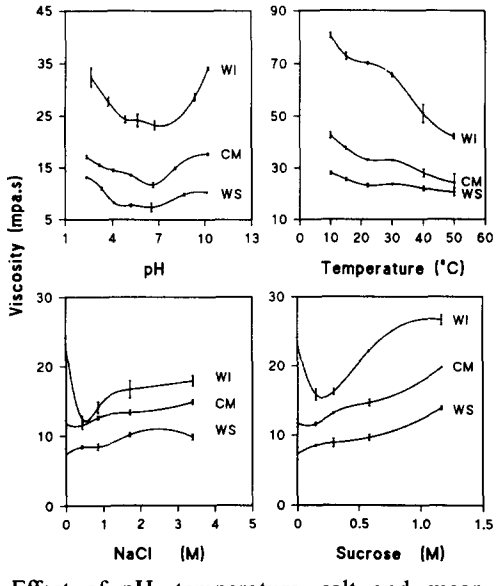

Fig. 9. Effect of pH, temperature, salt and sugar on the apparent viscosity of yellow mustard mucilage fractions at shear rate 92·32 s^{-1}. (Concentration for temperature effect: 1·0% w/w; concentrations for pH, salt and sugar effect: 0·5% w/w; pH for temperature, salt and sugar effects: 6·3).

with increasing additive concentration. In contrast, WI dispersions exhibited an initial reduction in viscosity at low solute concentrations (<0·5 M NaCl, <0·25 M sucrose). At much higher solute concentration, the rheological responses of WI were similar to those of CM and WS fractions.

The effect of temperature on the apparent viscosity of yellow mustard mucilage solutions and dispersions is also presented in Fig. 9. An increase in temperature resulted in a continuous reduction in viscosity for samples. In the case of WI, the reduction in viscosity was more pronounced compared to the other two fractions. This may suggest the presence of extended interparticle associations for this material at low temperatures.

The results of this study demonstrated the similarity between the interfacial activities and rheological properties of mucilage obtained from yellow mustard seeds and xanthan gum solutions/dispersions which suggest yellow mustard mucilage could be used as a substitute for xanthan gum in food dispersions.

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