Isolation and Preliminary Characterization of *Lesquerella fendleri* Gums from Seed, Presscake, and Defatted Meal

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Polysaccharide gums were separated from *Lesquerella fendleri* seed. Fracturing the seed and air classification yielded, 34% by weight, fractions enriched in gums. Water-swollen epidermal seed cells, after fracturing in a Waring Blendor and filtering, yielded a fraction that is 21% of the seed. Crude gums from hexane-extracted meal ranged from 35 to 47% depending on the amount of centrifugal force used to remove seed residue. Viscosity enhancement was greatest for the gums isolated from the fractured epidermal cells and for a gel fraction separated by a two-step process. A typical gum has a Rha:Ara:Xyl:Man:Gal:Glc molar ratio of 0.5:1.0:0.1:0.1:1.4:0.5 and contains 15% galacturonosyl residues, 14% protein, and 7% ash that is mainly Ca and Mg, which may serve as cross-linking cations for the uronic acid residues. Protein is associated with the polysaccharide at a level of 10–33% in various isolates.

In a survey of new sources of polysaccharide gums at this laboratory (Tookey et al., 1962; Tookey and Jones, 1965), *Lesquerella fendleri* was found to contain 19.5% gums based on hot water extraction of whole ground seeds and precipitation with ethanol. Values in the survey were corrected for protein content and components not hydrolyzable with dilute acid. A search for new oilseed crops revealed that lesquerella seed oil contains hydroxy fatty acids that have potential industrial uses (Mikolajczak et al., 1962). Recently, there have been significant plantings of lesquerella by the U.S. Department of Agriculture (USDA) Water Conservation Laboratory and by private firms elsewhere. The National Center for Agricultural Utilization Research (NCAUR) has an ongoing program to develop new industrial products from lesquerella oil. In addition, a cooperative venture has been initiated and partially funded by the Alternative Agriculture Research Center (AARC) for the commercialization of lesquerella as a new crop. In light of this recent interest and in order to fully utilize this new crop's potential, we investigated the chemical and physical properties of its gum component and practical means for its isolation. Gums have a number of commercial applications but are used primarily to increase the viscosities of aqueous solutions or to form gels (Bolker, 1974). Lesquerella gums are unique in that other gums used commercially are not extracted from oilseeds or oilseed presscake.

L. fendleri gum has been examined for its entrapment of mosquito larvae (Barber et al., 1974). In that work the composition of the mucilage from the surface of the seeds was classified into hydrolyzable component sugars and "suspected" cellulose (insolubles after hydrolysis in 2% H_2SO_4 at 80 °C, 20 h). Component sugars were listed as arabinose, galactose, rhamnose, and xylose. Galacturonic or glucuronic acids were found in some fractions. Sugar ratios and quantitation were not reported. The suspected cellulose fraction was 27.3% of the mucilage and revealed a large glucose component after hydrolysis with 80% H_2SO_4 for up to 45 min at 120 °C. Under these hydrolysis conditions, many pentose and uronic acid residues would be degraded and not detected in subsequent analyses (Biermann, 1988).

Since *L. fendleri* is a crucifer, it is reasonable to compare its gum composition to those of crucifer seed gums that have been thoroughly analyzed. *Sinapis alba* L. (yellow mustard) seed has 5% mucilage, 56% of which is water soluble (Cui et al., 1993a). The crude mucilage was composed of 15% glucuronic and galacturonic acids, 24% glucose, 14% galactose, 6% mannose, 3% rhamnose, 3% arabinose, and 1.8% xylose. Ash (15%) and protein (4.4%) were the other major components in addition to the polysaccharides. The water-soluble component was further characterized as a mixture of a pectic polysaccharide composed of galacturonic acid, galactose, and rhamnose; a β-1,4-glucan; and polysaccharides composed mostly of neutral sugars with nonreducing end residues of glucuronic acid (Cui et al., 1993b). *Lepidium sativum* (cress) seed has a capsular gel and a water-soluble gum similar to those of lesquerella seeds, but the gums make up only 2.6% of the seed weight (Bailey, 1935; Tyler, 1965). *Brassica sinapis alba* (white mustard) also has a cold-water-soluble polysaccharide that has been reported to contain as much as 50% microcrystalline cellulose in micelles (Grant et al., 1969). *Brassica campestris* (rapeseed) has been shown to contain 0.25% of a complex polysaccharide mixture (Siddiqui and Wood, 1977, and references cited therein).

Polysaccharides have been isolated from complex pectic mixtures by fractional solubilization with chemicals or solvents of increasing strength [e.g., Ericson and Elbein (1980) and Odonmazig et al. (1985)] or by fractional precipitation with solvents or metal complexes. Analyses have been based on hydrolysis with acid or enzymes and subsequent chromatographic separations of oligomers. Methods for analyses of pectin compositions have been reviewed by Doner (1986). Although the name "pectin" has been applied to poly-

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Table 1. Mechanical Separation of *L. fendleri* Hexane-Defatted Meal[a]

	size, μm	% by wt	% N	viscosity,[b] mPa·s
air-classified	<15	10.5	4.93	4
	15–18	7.4	4.74	15
	18–24	30.4	4.37	55
	24–30	13.4	3.93	110
	>30	38.4	3.04	280
sieved[c]	30–44	0.9	2.36	
	44–53	7.6	2.83	480
	53–63	24.5	3.05	460
	63–74	37.3	2.95	350
	74–88	17.1	3.09	270
	88–105	6.2	3.34	160
	>105	6.5	3.10	10

[a] Pin-milled. [b] 1% meal in 2% aqueous NaCl saturated with CaCO$_3$. [c] Sieved starting with the air-classified fraction >30 μm; see text for details.

Table 2. Aspirated, Fractured Whole Seed of *L. fendleri*

air velocity, % AI[a]	% by wt	viscosity[b] (2 h), mPa·s	viscosity (remixed 20 s), mPa·s	% oil	% N	% fiber
0		20	100	22.9	4.60	45.8
7.5	4.3	40	250	14.5	3.23	67.5
10	9.6	20	260	9.89	3.08	82.8
12.5	19.8	30	200	17.5	3.87	98.3
15	30.4	40	90	29.7	5.22	16.7
17.5	21.7	40	110	35.8	6.02	12.6
residue	12.1	30	80	24.6	4.85	27.3

[a] The air intake (AI) baffle was opened to increasing percentages of fully open (100%) to increase air flow and thus elute fractions sequentially by increasing density. 0% AI is the unclassified starting material. [b] Meal (1%) in a 2% NaCl solution saturated with CaCO$_3$ as in Table 1, except that the viscosity was measured after initial blender mixing and after 2 h at room temperature and a second mixing for 20 s.

(galacturonic acid)s with various degrees of methoxylation and acetylation, the Doner paper deals with acidic polysaccharides in general.

Properties of acidic polysaccharide gums depend greatly on structure and are as diverse as their compositions. Properties also change drastically in synergistic combinations with other gums (Dea, 1987). The wide variations of composition and structure within the class render each gum unique, ranging from gum arabic, an exudate, which has a low viscosity in solution, to water-insoluble, capsular gels from seeds, which thicken solutions by entirely different physical mechanisms than do soluble polysaccharides.

EXPERIMENTAL PROCEDURES

Materials. Hexane-extracted meal was supplied by Agrigenetics Corp. after processing at Texas A&M University to remove oil. It contained 2–3% residual oil. Presscake meal from single pressed seed was supplied by International Flora Technologies (Apache Junction, AZ). It contained 11.1% oil, 5.04% ash, and 24.4% crude protein. *L. fendleri* seed NU64621 was from the seed collection at NCAUR. Standard sugars, uronic acids and lactones, and xylan were purchased from Aldrich (Milwaukee, WI). Gum arabic, xanthan gum, and guar gum were purchased from Sigma (St. Louis, MO). Cellulose used in the hydrolysis studies was sterile cotton, U.S.P., from Johnson and Johnson (New Brunswick, NJ). Microcrystalline cellulose for FTIR spectra was from Supelco, Inc. (Bellefonte, PA). Reagent grade chemicals were used in all tests unless otherwise noted. Deionized, organics free water with an 18-MΩ resistance was used in laboratory experiments; water purified by reverse osmosis was used in pilot-plant experiments.

Viscosities. Unless otherwise stated, viscosities were measured with a Labline Model 4537 viscometer (no. 3 spindle, 30 rpm, 25 °C). The measurement was taken 30 s after immersion of the spindle in a 400-mL beaker filled with gum dispersions. Multiple measurements were always within 10% of each other.

Mechanical Separations. Hexane-extracted meal was ground in an Alpine pin mill, Model 160Z, and then air classified by particle size in a Pillsbury laboratory-scale air classifier. Particles larger than 30 μm were further sieved through silk screens of the size range described in Table 1.
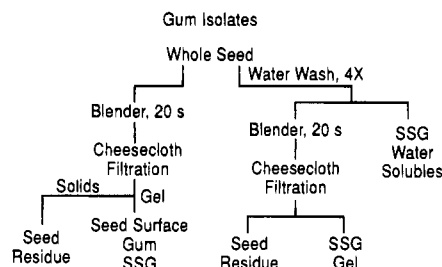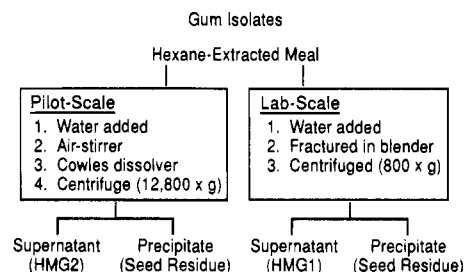
Whole seed was fractured in an Entoleter 5HP centrifugal machine, type ESA, with a Model 111226 scourer aspirator from Entoleter Inc. (New Haven, CT). The fractured seed was classified on a General seed blower from General Sheet Metal (New Brunswick, NJ). Fractions were eluted at various air intake openings as indicated in Table 2.

Laboratory-Scale Aqueous Separations. *From Whole Seed.* Lesquerella seed swells in water to 13 times its original volume, and this occurs mainly in the seed surface gum (SSG)

Figure 1. Isolation of gums from whole seed.

Figure 2. Isolation of gums from hexane-extracted meal.

or capsular gel layer. Washing with water was done in two different ways in separate experiments (Figure 1). A 1-min rinse was used to remove superficial dust in both experiments, but in one case, four additional water washes of the fully swollen seed were made at 30-min intervals to remove 92% of SSG water-soluble components. After the seeds were washed to extract water-soluble components, the swollen surface cells were fractured by stirring in a blender at high speed for 20 s. Gel from washed and fractured seed was easily squeezed through a double layer of cheesecloth to separate the SSG gel and largely intact seed. A 1:1 dilution of the SSG gel reduced viscosity sufficiently so that centrifuging at 12 000 rpm (19800g) removed the final traces of non-gel seed residue. The total seed surface gum, i.e., capsular gel layer (SSG), was recovered by shear mixing the swollen seed and centrifuging as above but without first washing out soluble components.

From Hexane-Extracted Meal. Hexane-extracted meal (HM) (63 g), mixed with 1.5 L of water and stirred in a blender for 60 s, gave a viscous, gelatinous mixture that was not separated by centrifuging at 2000 rpm (800g). After dilution to 3 L, however, brown seed residue was sedimented under the same conditions. The top layers were clear to light tan and gelatinous. The combined gelatinous layer was freeze-dried (HMG1 in Figure 2).

Pilot-Plant-Scale Separations. Meal (2 kg) that had been commercially deoiled by extraction with hexane was agitated with an air stirrer in 100 L water for 2 h. It was then stirred with a Cowles Model 7VT dissolver, which is a high-shear mixer. Samples were taken at 2.5 and 5 min to test for release of the gel fraction. The resulting suspension was centrifuged in a Sharples 3-in. bowl centrifuge (12780g). One pass was
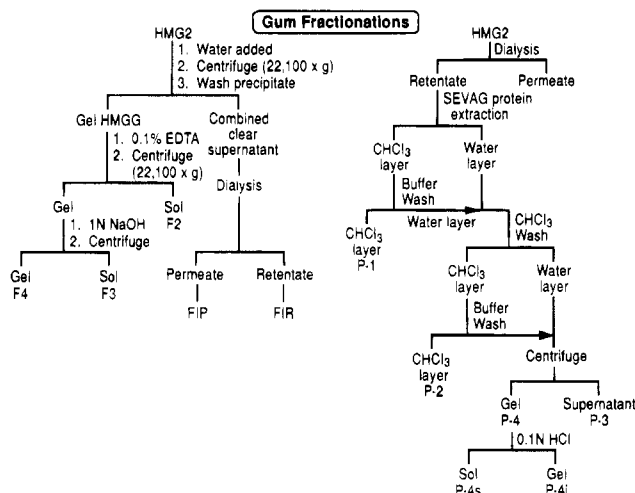
Figure 3. Fractionation of HMG2 gum isolate.

sufficient to retain the seed residue but not the gel layer, although the bowl was nearly completely filled. The gel layer was partially drum-dried and partially freeze-dried (HMG2 in Figure 2).

The same pilot-plant procedure was used for separation of gums from presscake meal (PCG).

Fractionation of HMG2 Gums. HMG2 was fractionated according to a number of procedures before analyses of component sugars, uronic acids, component amino acids, and metal ions (Figure 3). In the first procedure, water-insoluble seed residue was centrifuged from a 1% dispersion of HMG2 at 22100g. The supernatant was diluted by 50%, centrifuged, washed, and centrifuged again. A clear layer and a gel layer formed after each centrifugation. The clear layers were decanted and combined as water-soluble components (F1). The combined gel layers were made 0.1% in Na_2EDTA, stirred for 15 min, and recentrifuged. A clear layer above the gel was decanted, and the gel was washed again with 0.1% EDTA. On the basis of supernatant and gel layer volumes, the combined washes removed 75% of EDTA-extractable components. The remaining gel was diluted 1:1 with 2 N NaOH, stirred, and centrifuged. The supernatant (84% by volume) was decanted (F3) and the centrifugate gel (F4) not washed. F1 through F4 were dialyzed against water, and the permeate of F1 (F1P) and all retentates were freeze-dried.

The second separation was made on HMGP, the dialysis retentate of HMG2 (Figure 3). To remove protein, a 0.5% solution was treated with 0.5% Na_2CO_3 at 50 °C for 2 h, neutralized with acetic acid, mixed with $CHCl_3$/pentanol, and centrifuged according to the Sevag procedure (Staub, 1965). The $CHCl_3$ layer was washed with buffer and the washed $CHCl_3$ extract (protein) saved as P-1. The combined aqueous supernatant and wash were reextracted in the same way, and the second $CHCl_3$ extract (P-2) was saved. Excess $CHCl_3$ was removed by nitrogen purge, but some pentanol remained in the water and gel phases. The initial polysaccharide concentration was below 0.35% at this point, and a clear supernatant layer (61% by volume) formed and was decanted (P-3). The protein-depleted gel layer (P-4) was dialyzed, freeze-dried, and further separated into 0.1 N HCl extractable (P-4s) and residual gel (P-4i) components by stirring for 15 min in 0.1 N HCl at room temperature and centrifuging. $CHCl_3$ and pentanol were removed from the $CHCl_3$/pentanol extract *in vacuo* on a rotary evaporator, and salts were removed by dialysis.

A 0.2% solution of HMG2 was centrifuged and washed twice with water. The clear supernatant layer was decanted each time and the gel layer retained. This treatment removed water-soluble components of HMG2. The water-extracted gel components were freeze-dried (HMGG).

Chemical Analyses. Oil was determined by Butt extraction. Uronic acids were determined according to the procedure of Blumenkrantz and Asboe-Hansen (1973). Galacturonic acid was used as the standard. Amino acid analysis was done at

the University of Illinois Protein Sequencing Laboratory (Urbana, IL). Metal cation analysis was performed on a Perkin-Elmer 400 ICP apparatus using NIST standards. β-Glucan was measured with a Biocon assay kit, which is based on degradation with β-glucosidase and measurement of the glucose generated. Fiber analyses (Table 2) were performed according to AOAC standard methods (AOAC, 1980).

Sugar Analyses. Sugars, soluble polysaccharides, and gels were present in several samples. Removal of excess reagents, such as borate after $NaBH_4$ reduction, from polysaccharides was by dialysis. Borate was removed from sugars by treatment with AG 50-X8 acid form ion-exchange resin and then repetitive distillation with added methanol.

The basic analytical procedure for sugars consisted of $NaBH_4$ reduction of aldoses in the presence of Na_2CO_3 to protect uronic acids (Lehrfeld, 1987), activation of uronic acids with 1-[(3-dimethylamino)propyl]-3-ethylcarbodiimide (DMECDI) at pH 4.75, and then reduction of uronic acids at pH 7 with $NaBD_4$ (Taylor and Conrad, 1972).

Uronic acid residues in polysaccharide samples (20 mg) were also treated with DMECDI and reduced with $NaBD_4$ as above. Excess $NaBD_4$ was decomposed with 6–8 drops of 25% acetic acid in water, and the solution was dialyzed. Reduced polysaccharide samples were divided, and half was hydrolyzed with 2 N trifluoroacetic acid (TFA) at 120 °C for 30 min. The other half was hydrolyzed in 6 N HCl under N_2 for 24 h at 110 °C. TFA was removed, and a phenyl β-glucopyranoside internal standard was then added. The hydrolysate was reduced with $NaBH_4$, excess borohydride decomposed, and the sample passed through AG 50-X8 ion-exchange resin. Unhydrolyzed residue was lost on the resin. Excess borate was removed by evaporation with methanol. Dry samples were acetylated with acetic anhydride (1 mL) in pyridine (1 mL) at 95 °C for 1 h. Excess anhydride was reacted with methanol, and the alditol acetates were recovered in a $CHCl_3$ layer washed three times with water. Alditol acetates were analyzed using a 0.75 mm × 30 m SP 2330 column programmed from 220 (6-min hold) to 240 °C at 5 °C/min. Controls of guar, gum arabic, xanthan gum, cellulose (cotton), and xylan were analyzed according to the same methods along with the samples. Samples hydrolyzed in 6 N HCl were treated similarly to TFA hydrolysates.

To assess reaction losses, galactose, arabinose, and xylose were heated in 2 N TFA at 120 °C for 30 min, internal standard added, reduced with $NaBH_4$, and acetylated. The percent of sugars recovered after the hydrolysis procedure was compared to that of the same sugars that had not been heated with TFA, and the amounts of sugars recovered from hydrolyzed polysaccharides were increased to compensate for reaction losses.

RESULTS AND DISCUSSION

Mechanical Separations. Pin milling and air classification of hexane-defatted meal resulted in five fractions as shown in Table 1. One fraction (38% of the meal weight, >30-μm particle size) enhanced viscosity best. This fraction was further subdivided by silk screen sieving into six fractions (Table 1). The second refinement yielded fractions in the 44–63-μm range (12.3% of the original seed meal) which had a viscosity of about 470 mPa·s at 1% concentration in 2% NaCl solution saturated with $CaCO_3$. It would be possible to set a desired viscosity for a product and to adjust grinding and classification to maximize the recovery of the product.

Since the major portion of the gum is on the surface of the seed, whole seeds were fractured and aspirated to test possible oil, protein, and gum separation. The fractions collected are listed in Table 2. The viscosity in 2% NaCl solution did not develop immediately on dispersion in a blender or after 2 h of swelling, because the capsular gel remained attached to the seed coat. After swelling for 2 h and restirring for 20 s in a blender,

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Table 3. *L. fendleri* Gum[a] Yields, Analysis, and Solution Viscosities

	SSG	HMG1	HMG2	PCG
yield, %	21	47	35	45
N, %	1.56	3.2	2.08	2.62
ash, %	7.02	7.72	9.93	6.54
polymer, %	82.6	80	54	86
viscosity,[b] mPa·s	800	280	420	380
oil, %	1.05	1.96		3.14

[a] SSG, gum from the surface of whole seed, separated from seed residue at 22100*g* in a high-speed centrifuge. HMG1, gum from hexane-extracted meal, separated at 800*g*. HMG2, gum from hexane-extracted meal, separated at 12780*g* in a pilot plant centrifuge. PCG, gum from presscake also separated at 12780*g*. [b] 1% gum in a 2% NaCl solution, saturated with CaCO$_3$.

however, the viscosity increased rapidly. This behavior contrasted with that of the air-classified fractions from hexane-extracted meal, which developed viscosity in the first minute of blending and after 2 h did not change viscosity with restirring. Some separation of gums, oil, and protein was achieved by aspiration of fractured whole seed. Lower density fractions, eluted with 7.5–12.5% of fully open air intake or less, yielded 34% of the seed weight. These fractions increased the viscosity of salt solution to the same level as obtained with air-classified fractions >30 µm. Yields were comparable with those from hexane-extracted meal. However, 20% of the oil, a high-value coproduct, is also contained in the low-density fractions from fractured seed. Consequently, further refinements need to be made in aspiration of fractured whole seed.

Laboratory-Scale Aqueous Separations. The capsular seed surface layer was not removed either by shaking on a rotating table in water at 25 °C for 6 h (Bailey, 1935) or by squeezing through layered cheesecloth after swelling and decanting in 25 °C water over several hours. Fracturing the swollen seed surface layer with a Waring Blendor was necessary before it could be separated from seed particles by squeezing through cheesecloth. Characteristics of freeze-dried seed surface gums (SSG) are listed in Table 3. The gum-free residue (73.2%) from 60 g of seed contained 33.8% oil on a dry basis compared to 24.8% oil in the original seed. A low level of oil (1.05%) was found in the recovered SSG (21% of the seed weight). When the swollen seed was washed with water before the gelatinous surface layer was disrupted, 13.1% of the seed weight was recovered as gel and 7.9% as water-soluble components. This result is important because the four water washes removed most metal salts and low molecular weight components while leaving gel components (92% undialyzable) having 4.18% ash content (dry basis). In the water-soluble extract only 58.6% is retained on dialysis and 11.7% is ash. Oil analyses of starting seed and recovered seed revealed little or no oil extraction into the gums.

SSG best exemplifies the polysaccharides extractable from the seed surface alone and is useful for comparison to gels and soluble components extracted from presscake and hexane-extracted meal.

Gums extracted from presscake or hexane-extracted meal will also contain seed endosperm components, some of which would be expected to contribute to the viscosity. Therefore, the component gums from the meal would be expected to differ from those obtained from the seed surface. This result is evident for mechanically separated gum isolates by comparison of Tables 1 and 2 to SSG in Table 3, but it will also be shown for gums extracted with water from the meal or presscake.

HMG1, the gum isolated from hexane-extracted meal (Figure 2), was gelatinous in a 1% solution that had a viscosity of 280 mPa·s. The dried gum amounted to 47% of the starting meal, and when dialyzed against water, 80% was retained (polymeric components). Dried gum contained 20.6% crude protein (3.2% N), 2.0% oil, and less than 1% β-glucan.

Color is important for many cosmetic applications and may require bleaching. For oil-drilling applications or thickening of acidic foods, stability over a wide pH range or in the presence of salts may be important. Solubility and stability of 1% dispersions of HMG1 were tested in mild acid or base or in H$_2$O$_2$ for color bleaching. A significant portion (>80%) of every sample was not hydrolyzed or degraded by 1 h of treatment with 1% H$_2$O$_2$ (T$_1$), 0.1 N NaOH at 80 °C (T$_2$), 0.1 N HCl at 80 °C (T$_3$), 1% H$_2$O$_2$ at pH 10 (T$_4$), or HCl to pH 4 (T$_5$). Viscosities of the 1% solutions after these treatments were 180, 200, 130, 140, and 160 mPa·s, respectively, compared to a control value of 250 mPa·s with no treatment. A brown seed residue remained (14%) in the control and after T$_1$, T$_4$, or T$_5$ when centrifuged at 21000*g* but not after T$_2$ or T$_3$. After each of the last four treatments, 26, 40, 26, and 33%, respectively, of HMG1 was dialyzable permeate compared to 20% with no chemical treatment. In general, this gum isolate retains thickening ability over a wide range of pH or after peroxide bleaching.

Hydrolysis of the gels, after the above treatments, with 1 N TFA for 6 h at 100 °C left unhydrolyzed residues (27–31% of the starting gel weight). The nondialyzable components of HMG1 were also unhydrolyzed to the extent of 27% by the same treatment. Hydrolyzed gums were analyzed for sugars, and the unhydrolyzed residue was analyzed for nitrogen content and by FTIR.

On the basis of earlier papers, the unhydrolyzed residue might be microcrystalline cellulose, protein, or resistant polyuronides. Nitrogen content of insoluble, water-washed HMG1 residues after TFA hydrolysis was 2.96%; after T$_2$, dialysis, and TFA hydrolysis was 2.49%; and after T$_3$, dialysis, and TFA hydrolysis was 3.19%. Infrared spectra of the insoluble residues are compared in Figure 4 to a microcrystalline cellulose spectrum. In the original and treated material there is an infrared carbonyl absorbance at 1650–1670 cm^{-1}, indicative of protein, and a carbonyl absorbance at 1620–1635 cm^{-1}, indicative of ionically bonded uronic acid carboxylates. In addition, several sharp absorbances in the 1000–1200-cm^{-1} region closely resemble the spectrum for microcrystalline cellulose. The sharp absorbances in the 1000–1200-cm^{-1} region are not evident in spectra of samples taken before TFA hydrolysis, which are more typical of noncrystalline polysaccharides, as shown in spectra (Abbott et al., 1993) of the seed surface cells of *L. fendleri* and the water-insoluble polymers from the seed surface. These spectra show the predominantly carbohydrate nature of the gums and the bonded uronic acid carboxylates. It is apparent from these analyses and the sugar analyses discussed below that published values of cellulose content for gums of this type are higher than the actual cellulose content. The gels, which swell greatly in water but are resistant to acid and base hydrolysis, appear to be microcrystalline cellulose and acidic polysaccharides associated with protein. However, more detailed analyses of the components and their linkages are needed.
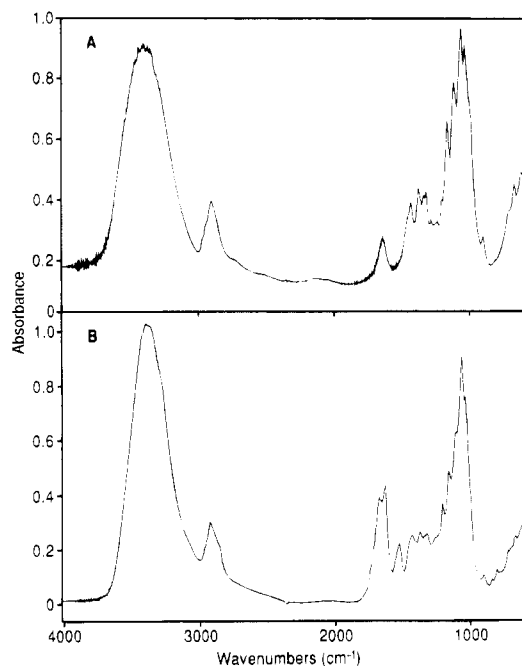
Figure 4. Infrared spectra of (A) microcrystalline cellulose, 1 mg in 300 mg of KBr, pressed disk, and (B) insoluble gum fraction after 1 N TFA hydrolysis, in a KBr disk as in (A).

Figure 5. Viscosity of *L. fendleri* gum isolate from hexane-extracted meal (HMG2) at different concentrations.

Table 4. Stability of *Lesquerella* Gum Viscosity[a] at pH 3

	viscosity, mPa·s					
	0 h	1.8 h	3.7 h	6.5 h	22 h	120 h
AC > 30[b]	230	100	110	100	130	130
HMG2	260	140	160	150	210	210
HMGP	650	410	490	440	480	690
guar	730	420	400	390	430	360

[a] Labline rotary spindle viscometer (spindle 3, 30 rpm, 25 °C); 0.54% gum concentrations in water. [b] Air-classified meal of particle size >30 μm as in Table 1.

Pilot-Plant-Scale Separations. *Of Hexane-Extracted Meal.* Stirring 2 kg of hexane-extracted meal at 2% concentration in water with an air stirrer only slightly increased the viscosity to 80 mPa·s. Higher shear achieved with the Cowles dissolver rapidly increased the solution viscosity as the gelatinous gums were released from denser, opaque seed particles. Samples taken after 2.5 and 5 min of shearing revealed that the longer stirring time did not significantly increase the amount of gums recovered. After centrifugation, 19 kg of the supernatant was drum-dried to give 103 g of recovered product. The remaining supernatant was freeze-dried to yield 600 g of water-soluble components (HMG2) for an overall yield of 35.2%. HMG2 contained less seed residue than HMG1 because the pilot-plant centrifuge operates at a much higher centrifugal force (see Figure 2). Less seed residue is reflected in the lower yield, lower nitrogen content, and higher viscosity for a 1% solution of HMG2 compared to HMG1. Drum-drying enhanced viscosity by 16% and demonstrated that the gums are sufficiently heat stable for drum-drying. The increase in viscosity may be caused by protein denaturation or more stable bond formation between components during the hot drum-drying process. HMG2 is 54% macromolecular compared to 83% for SSG. The higher ash content of HMG2 is due to water-soluble salts from the endosperm and the fact that SSG was prepared from cleaned, washed seeds, whereas hexane-extracted meal was from unwashed seed processed in commercial equipment.

Isolation of a gum with much lower ash content (4.5%) and fewer low molecular weight components (17%) was accomplished by washing the meal with water before using high-shear mixing to release the gel component. Yields of the gel component by this two-step extraction were 17–20% of the meal weight. Apparent viscosity of the gel component was 1630 mPa·s in a 1% aqueous solution.

In addition to the test results already given, HMG2 viscosity at various concentrations is shown in Figure 5. The viscosity of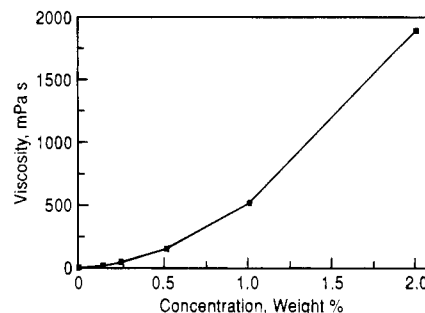 2% HMG2 in water (1850 mPa·s) was not appreciably affected by added NaCl up to 10%. A 4% dispersion of HMG2 gave an apparent viscosities of 37 000 mPa·s at 55 °C and 26 000 at 95 °C but darkened on heating. Rotating viscometers are not adequate for measuring the true rheological properties of dispersed lesquerella gums at 4% concentration. Table 4 compares the viscosity of HMG2, the air-classified fraction >30 μm (described in Table 1), and the high molecular weight portion of HMG2, HMGP, to guar gum at 0.54% concentration. Guar gum levels used in oil drilling are reported to be used at concentrations of 0.14–0.57%. Stabilities in acid seem comparable over the time tested, and the viscosity-enhancing effects of HMGP appear to be similar to those of guar in this test. Guar gum dissolves into a smooth, ungelled solution that enhances viscosity by its hydrodynamic volume in solution. Lesquerella gum is a combination of soluble components and swollen gel that enhances viscosity and would be expected to exhibit a higher yield point in rheological measurements. The higher yield point of a gel component enhances the ability of the gum to maintain suspensions against settling. Yield point is the amount of shear stress needed to initiate flow. Rheologically, guar and lesquerella gum are not comparable, but in use tests their relative stabilities in acidic solutions is important. Exhaustive dialysis of HMG2 at 4 °C for 30 days against deionized water reduced the ash content of the freeze-dried retentate to 2.52%. Analyses for metals (mg/g) in the freeze-dried retentate gave Ca (4.48), Mg (1.21), Fe (0.353), K (0.254), Na (0.214), Zn (0.033), Mn (0), and Cd (0).

Of Presscake. Presscake (11.1% oil, 5.04% ash, and 3.90% N) that was finely ground was separated in the same way as HM except that viscosity was followed as a function of stirring time with the Cowles dissolver. Viscosity rose from 330 mPa·s at 1 min to 550 mPa·s at 5 min, but fell to 390 mPa·s after 10 min. Viscosity of the effluent from the centrifuge varied from 390 to 420 mPa·s compared to 209–234 mPa·s for the effluent from the HMG2 separation. The effluent was partially drum-dried and partially freeze-dried as presscake gum (PCG). Yield of PCG was higher than HMG2 and 86% was nondialyzable, but this value is deceiving because the smaller initial particle size of the ground presscake

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Table 5. Compositions of *Lesquerella* Gums and Gum Fractions[a]

fraction	mole ratios of component sugars							% GalA	crude protein,[c] %	protein from AA,[c] %
	Rha	Ara	Xyl	Man	Gal	Glc	GalA			
HMG1	0.2	1.0	0.2	0.1	1.6	0.6			20.0	13.9
HMG2	0.6	1.0	0.1	0.1	2.0	0.7			13.0	6.56
HMGP	0.5	1.0	0.1	0.1	1.4	0.5	0.74	15.7[b]	12.7	14.7
F1 permeate				1.0	1.1	7.3				1.33
F1 retentate	1.1	1.0	0.2	0.1	2.6	0.9			10.3	8.18
F2	0.9	1.0	0.1	0.1	2.2	0.8			22.6	5.14
F3	0.3	1.0	0.1	0.1	1.6	0.7			20.7	5.30
F4	0.2	1.0	0.1	0.1	1.5	0.3			10.0	6.89
P-1	0.3	1.0	0.1	0.1	1.1	0.2		6.51	33.3	29.1
P-2	0.3	1.0	0.1	0.1	1.6	0.4			25.0	21.9
P-3	0.8	1.0	0.1	0.1	2.3	0.6			5.69	4.65
P-4								17.2	4.75	4.50
HMGG	0.3	1.0	0.1	0.1	1.5	0.4		10.0	15.1	7.20
P-4s	3.7	1.0	0.2	0.2	5.4	2.7				
P-4i	0.3	1.0	0.1	0.1	1.4	0.4				
arabic	0.5	1.0			1.3	0.3		22.2		

[a] See Figures 1–3 for description of gums and isolates. [b] 13% protein, 4% ash. [c] Crude protein = % N × 6.25. Protein from amino acid analysis (AA) was calculated from moles of amino acid residues per unit sample weight.

left more seed residue in the gum isolate. More seed residue is evidenced in the higher nitrogen level and lower viscosity of PCG compared to HMG2 (Table 3). Lower oil content, 3.1% in PCG, than in the original presscake indicates that the major portion of the oil was retained in the centrifuged solids. It should be noted that color and optical clarity of the gum isolates in Table 3 depended on the amount of seed residue remaining in the gum and, in a 2% dispersion, ranged from tan-brown opaque to water white translucent in the order HMG1, PCG, HMG2, SSG.

There is a strong possibility that the lowest cost process would be mechanical because the expense of drying large quantities of water adds considerably to the overall cost of the product. However, wet processing and solar drying in the desert southwest would also be possible. Of the three starting materials, the advantage of using whole seed is that after the gum is removed and the seed is dried, it contains more oil than before gum removal, which would improve pressed-oil yields. If industry finds the oil to be valuable for industrial products, then hexane-extracted meal is the likely starting material for gum recovery. For cosmetics use, cold pressed oil is sometimes preferred, and presscake will be the likely starting material. Because these decisions have not yet been made for lesquerella we investigated each of the possible starting materials and isolated gums from all three.

Fractionation. The fractionation of HMG2 with EDTA and NaOH yielded (as percentages of HMG2) seed residue, 3.1%; F1 permeate, 4.1%; F1 retentate, 12.5%; F2, 8.5%; F3, 12.0%; and F4, 14.3%. The total recovery (54.5%) agrees with dialysis of HMG2 against water to yield 54% retentate and indicates that no apparent hydrolysis occurred during the separation, which suggests that endogenous polysaccharidases are not a problem in this meal.

The second fractionation of HMGP based on protein extraction and solubilization with HCl yielded seed residue, 4.3%; first protein extract (P-1), 14.5%; second protein extract (P-2), 5%; clear supernatant (P-3), which also contained Na₂CO₃ and pentanol, 21.5%; and deproteinized gel layer (P-4), 36.9%. Fraction P-4 was subsequently divided into 0.1 N HCl extractable, P-4s (34.3%), and residual gel, P-4i (48.0%), components. In summary, 82.2% of HMGP was recovered in the seed residue and fractions P-1–P-4; P-4 was apparently hydrolyzed to the extent of 17.7% by treatment with HCl.

Table 6. Metal Cations (Milligrams per Gram) in Gums and Isolates

	Ca	Zn	Na	Cd	Fe	Mn	Mg	K
HMG1	5.86	0.033	0.193	0	0.191	0	2.76	3.58
HMG2	6.99	0.014	0.546	0	0.196	0	2.30	5.00
HMGP	7.30	0.029	0.102	0	0.298	0	2.34	0.304
HMGG	3.19	0.024	0.302	0	0.960	0	0.960	1.03
P-3	2.24	0.013	11.9	0	0	0	0.497	0.039

Component Analyses of Gums and Isolates. Sugar, protein, and uronic acid analyses of lesquerella gums and their components are listed in Table 5. In the water-extracted gum, HMG2, not all of the nitrogen is accounted for by the amino acid analysis. Low molecular weight salts likely account for the difference, because the nondialyzable portion of HMG2, HMGP, has only protein nitrogen. The overall neutral sugar compositions of HMG2 and HMGP differ slightly in galactose and glucose. These are among the three sugars found in the low molecular weight dialysis permeate F1—mannose, galactose, and glucose. Further differentiation of HMG2 polymeric components into water-soluble polymers, F1 retentate, and water-washed gel, HMGG, indicates that the water-soluble gums are higher in rhamnose, galactose, and glucose and slightly lower in arabinose. The gelled component is lower in rhamnose, galactose, and glucose and higher in arabinose.

Uronic acid was higher in the F1 retentate than in HMGG. Galacturonic acid was shown by GC/MS of alditol acetates in a hydrolysate of deuterioreduced gum to be the only uronic acid present. Alditol acetate from reduced galacturonsyl residues is probably the source of the higher galactose value in the sugar analysis of F1R. Protein was lower in the water-soluble fractions than in the total gums. If a mixture of polymers is present, the results based only on extractability with water and dialysis suggest that the soluble polysaccharides in HMG2 are composed in part of a polygalacturonate and that increased amounts of rhamnosyl residues are associated with higher uronic acid content extracts. Insoluble arabinans, uronates, and some proteins occur in the water-insoluble gels.

HMGG ash content is 4.61% compared to 3.95% in HMGP after 3 days of dialysis and 2.52% for HMGP exhaustively dialyzed for a month. Dialyzable salts from HMG2 are mainly Na and K (Table 6). Calcium appears to be retained in equal amounts in the dialyzed (HMGP) and original extract, HMG2. These results

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Table 7. Amino Acid Analysis (Mole Percent of Total Amino Acids) of Proteins in Gums and Isolates

sample	Asx	Glx	Ser	His	Gly	Thr	Ala	Arg	Tyr	Cys	Val	Met	Phe	Ile	Leu	Lys	Pro
HMG1	10.2	14.1	8.38	1.84	12.7	5.59	8.66	5.75	2.08	0.42	4.47	0.36	3.82	3.51	6.22	4.33	7.68
HMG2	11.6	13.9	11.5	1.49	15.2	5.7	10.1	4.58	3.35	0.15	3.85	0.47	3.45	3.49	4.53	2.41	4.37
HMGP	10.9	11.8	11.8	1.45	16.3	6.44	10.9	4.58	1.36	0.28	4.11	0.22	3.65	3.0	4.83	2.68	5.73
F1 permeate	17.9	25.2	4.15	1.58	8.58	2.48	6.24	5.22	4.52	0.07	2.47	1.64	2.24	2.59	1.49	11.1	12.5
F1 retentate	11.53	8.99	17.0	0.87	18.6	7.13	12.8	3.39	1.2	0.39	3.5	0.27	3.54	2.72	2.64	1.67	3.74
F2	11.1	8.83	15.2	1.05	18.8	7.16	13.0	4.22	1.09	0.32	3.69	0.25	3.58	2.68	2.85	1.74	4.45
F3	10.3	13.1	11.2	1.6	16.9	5.73	10.3	4.95	1.39	0.15	3.91	0.21	3.93	3.3	4.92	2.05	6.06
F4	11.0	14.2	8.63	1.78	12.3	5.63	8.84	4.5	2.21	0.08	5.07	0.28	4.23	4.47	7.71	2.66	6.34
P-1	10.1	11.1	12.2	1.26	16.1	6.73	11.1	4.94	1.47	0.49	4.23	0.2	3.74	3.01	5.02	2.11	6.31
P-2	10.3	12.0	11.0	1.31	16.5	6.52	10.4	4.65	1.25	0.39	4.34	0.21	3.65	3.13	5.58	2.53	6.27
P-3	12.1	9.85	16.0	0.94	19.9	6.83	12.0	2.65	1.22	0.51	3.68	0.3	3.38	2.48	2.88	1.19	4.08
P-4	11.7	11.7	12.9	1.15	17.0	6.09	10.4	3.48	1.32	0.58	4.45	0.71	4.09	3.74	4.64	1.66	4.38
P-4i	11.2	12.9	12.0	1.35	15.2	6.22	10.7	3.36	1.27	0.21	4.17	0.18	3.82	3.36	5.52	1.72	6.73
HMGG	10.5	14.0	9.05	1.73	14.0	5.69	9.1	4.95	1.69	0.28	4.36	0.32	3.92	3.69	6.38	3.59	6.82

suggest that uronic acid residues cross-linked by Ca^{2+} and Mg^{2+} contribute to stability of the gel fraction. The water-washed gel, however, is lower in Ca and Mg but higher in Fe, Na, and K than is HMGP. This result is unexpected, but a cellulose component or insoluble arabinans would contribute less to the metal ion content than would soluble uronates.

Attempted chemical fractionation of the gel components, first with EDTA and then with NaOH after water extraction, led to soluble polysaccharides with, on the basis of N and amino acid analysis (Table 5), bound EDTA that could not be displaced on dialysis even in the presence of NaOH. It is, therefore, surprising that gel not extracted into water, EDTA, or NaOH (F4) retained a compositional profile very similar to that of the unfractionated HMGP and the water-extracted gel (HMGG). However, a substantial unhydrolyzed residue, which may be cellulose, remained after hydrolysis of F4 with 2 N TFA.

Extraction of protein from HMGP according to the Sevag procedure reduced protein in extracted fractions to less than 6%. The large amount of Na_2CO_3 used in the separation appears to disrupt some of the bonding with proteins. However, washing the $CHCl_3$ extract with additional Na_2CO_3 gave an apparent protein extract that was still largely carbohydrate (33% protein), which suggests that the protein is covalently linked to the polysaccharides isolated with it. Except for the lower galacturonosyl content, the polysaccharide bound to the proteins appears to be the same as seen in other gel fractions from this gum. P-2, the second extracted protein component, is similar to P-1 but has more carbohydrate. Once the protein is largely extracted, the soluble polysaccharides remaining, P-3, are similar to the soluble polysaccharides in F1R. Separating the remaining gel with 0.1 N HCl into extractable (P-4s) and gel (P-4i) components gives some indication of a galacturonosyl—rhamnosyl polysaccharide derived from hydrolytic loss of arabinosyl side chains. The resistant gel, P-4i, cannot be cellulose alone, as evidenced by the remaining hydrolyzable sugar and protein components.

Amino acid analyses of the gums and isolates (Table 7) indicate that the proteins found in various fractions were not very different from one another, with the exception of the dialysis permeate, F1P, which was high in proline, glycine, and aspartic acid/asparagine. Proline often is higher in the gels than in the soluble isolates.

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