Chemical and Physical Studies of Mustard and Rapeseed Coats

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ABSTRACT

The chemical and physical properties of seed coats derived from a number of species and varieties of mustard and rapeseed were examined. Seed coat polysaccharides were fractionated by a sequential solvent extraction procedure and partially purified by ethanol precipitation. Hydrolyzed polysaccharides were assayed for sugars by gas-liquid chromatography of their TMS derivatives. Unique thickening properties were observed with wet-milled yellow mustard hulls. These were largely a result of the mucilage fraction of the yellow mustard seed coats. This mucilage yielded a far more viscous solution at a 1% w./v. concentration than that obtained from other mustard and rape varieties. Defatted hulls of yellow mustard contained 20 to 25% by weight of mucilage, compared with 3 to 4% in *Brassica juncea* mustard hulls, and an average of 1% in rapeseed hulls. Refluxing with 2N H_2SO_4 for 4 hr. completely hydrolyzed the polysaccharide fraction obtained from the cold-water extract of rape and *B. juncea* mustard hulls. Only 35% of this polysaccharide fraction of yellow mustard hulls was hydrolyzed by this treatment; the residue proved to be cellulose. Hence, the mucilage of yellow mustard appeared to be unique in containing a naturally "solubilized" form of cellulose.

There are two major commercial species of mustard, namely, *Brassica juncea* (varieties Canadian Brown, Stoke, and Oriental) and *Sinapis alba* (also referred to as *B. hirta,* white or yellow mustard). There are two major species of rapeseed, *B. campestris* (Polish-type rape) and *B. napus* (Argentinian-type rape).

Although they are botanically similar, mustard seed is grown largely for use as a condiment (1), while rapeseed is primarily produced for its oil content. In the case of mustard, removal of the seed coat (dehulling) is an important preliminary step in the preparation of flours designed for incorporation into a number of foods. The seed coats (hulls) obtained by this operation also have a number of applications in the manufacture of food. On the other hand, dehulling of rapeseed is not practiced commercially at present, although it has been suggested that the nutritional quality of the rapeseed meal could be improved by removal of the hull before oil extraction (2).

The objective of this study is to evaluate some of the chemical and physical properties of the hulls obtained from a number of mustard and rapeseed varieties in order that the full commercial potential of these materials be realized.

MATERIALS AND METHODS

The mustard hulls were obtained from four different varieties of mustard, namely, *B. juncea* variety Canadian Brown, *B. juncea* variety Stoke, *B. juncea* variety Oriental, and *S. alba* (yellow mustard, *B. hirta*) variety Gisilba. The rapeseed hulls were obtained from *B. campestris* and *B. napus.*

The hulls were isolated by drying and then tempering the seed before cracking in a roller mill, followed by air separation of the hulls from the rest of the seed. All samples of hulls used in this study were subjected to repeated air-classification procedures in order to remove any contaminating seed cotyledon fragments.

The extraction procedures utilized for this study were based on those used by Aspinall et al. (3) for the isolation of polysaccharides from soybean hulls. Hull samples (100 g.) were extracted in a Soxhlet apparatus with the following

solvents: acetone (8 hr.), hexane (24 hr.), and ethanol:water, 4:1 v./v. (48 hr.). The defatted hulls were then micromilled through a U.S. No. 40 mesh sieve, and the hull flour was extracted with the following series of solvents: water at 20° C. (3 × 500 ml.); water at 60° C. (2 × 500 ml.) and 0.5% ammonium oxalate at 80° C. (3 × 500 ml.).

The hulls were next delignified by the chlorite method of Wise et al. (4). A lyophilized sample of the ammonium oxalate-extracted hull residue was suspended in 800 ml. hot water containing 3 ml. glacial acetic acid. Technical-grade sodium chlorite was then added; delignification was allowed to proceed for 1 hr. at 70° C. The process was then repeated by fresh addition of acetic acid and sodium chlorite. After 3 hr., the white residue was collected by filtration and washed with 2 liters cold water. The washed sample was recovered by centrifugation and lyophilized.

The resulting holocellulose was then extracted with 2% disodium ethylenediaminetetraacetate (EDTA) adjusted to pH 4.5 at 90° C. (3 × 500 ml.). The residue was extracted with 10% potassium hydroxide (3 × 500 ml.) at room temperature, and finally with 10% sodium hydroxide containing 4% boric acid (3 × 500 ml.). The cellulose residue was washed with 2 liters water, collected by centrifugation, and lyophilized.

The various extracts were concentrated under reduced pressure at a bath temperature not exceeding 45° C.

Isolation of Polysaccharide Components

The two preliminary aqueous extractions of micro-milled defatted hulls (at room temperature and at 60° C.) were concentrated to 200 ml. and acidified to pH 4.0 with dilute hydrochloric acid to precipitate any solubilized protein. Any precipitate was removed by centrifugation. The supernatant was neutralized with sodium hydroxide and poured into 4 volumes of ethanol. The precipitated polysaccharides were then washed with ethanol, dispersed in water, frozen to −20° C., and lyophilized.

Pectin-type polysaccharides were obtained by addition of 10% aqueous calcium chloride solution to the concentrated ammonium oxalate extract. Calcium pectate was collected by centrifugation and recovered by heating in 0.3% ammonium oxalate solution for 30 min. at 90° C. Calcium oxalate was removed by centrifugation. The polysaccharide was obtained by precipitation in 4 volumes ethanol.

The EDTA extract was concentrated to 200 ml., dialyzed against distilled water (48 hr.), and poured into ethanol (4 volumes); the precipitate was washed with ethanol, dispersed in water, and lyophilized.

The alkaline extracts were both acidified to pH 4.0 by addition of concentrated hydrochloric acid. The material that precipitated at this pH was collected by centrifugation, washed in water, and lyophilized. The acidified solutions were concentrated to 200 ml., dialyzed against distilled water (48 hr.), and mixed with 4 volumes ethanol. The precipitated alkali-soluble polysaccharides were then redispersed in water and lyophilized.

Analytical Methods

Viscosity studies of the fractionated hull polysaccharides were made using an Ostwald bulb viscometer. The viscosity assays of wet-milled bran samples (using

a Morehouse stone mill) were made using a Brookfield Synchro-lectric Viscometer, Model RVT.

Sugar analyses were made by first hydrolyzing samples in 2N sulfuric acid (refluxed for 4 hr.). The hydrolyzed polysaccharides were neutralized with saturated barium hydroxide solution, centrifuged and the supernatant passed through an Amberlite IR 120 (H+) ion-exchange column. The neutral sugars were eluted with water, concentrated, and trimethylsilyl (TMS) derivatives prepared (5). The TMS-sugar derivatives were identified by gas-liquid chromatography on a 3% SE 30 Gaschrome Q column (from Applied Science Laboratories, P.O. Box 440, State College, Pa.).

The uronic acid content of the polysaccharides was assayed by adding 1 ml. of sample to 6 ml. concentrated sulfuric acid containing 2.5% 1N sodium borate. This mixture was heated at 100° C. for 20 min., cooled to room temperature, and mixed with 0.2 ml. of a 0.1% carbazole solution in ethanol. The solution was heated to boiling for 10 min. Absorbancy was read at 530 nm. on a Bausch and Lomb Spectronic 20 colorimeter (6).

Crude fiber, Kjeldahl nitrogen, total ash, and acid-insoluble ash were all assayed by standard AOAC procedures.

RESULTS AND DISCUSSION

Yields of seed coats derived from mustard seed ranged from 12 to 20% by weight; 16 to 19% yields were noted in the case of rapeseed. The yellow mustard seed (*S. alba*) proved to be the easiest material to dehull; seeds of this variety were also the largest in diameter. It was also apparent that the yellow-colored varieties of both *B. juncea* and rapeseed proved to dehull easier than the brown-seed varieties.

Fixed Oil

The hulls of both mustard and rapeseed contained varying amounts of fixed oil depending on the degree of contamination with cotyledon. Following exhaustive cleaning of the hulls by aspiration, the hulls of all varieties of mustard and rape tested were shown to contain 8 to 15% oil. Lowest levels of oil were consistently found in the yellow mustard hulls, possibly due to the relative ease with which yellow mustard seed can be mechanically dehulled and freed from contaminating cotyledon fragments. The results indicated that removal of the hulls would lead to a 4 to 8% loss in oil yield. This amount of oil loss is economically significant. Hence, dehulling of rapeseed could only be justified if the resultant meal, following dehulling and oil extraction, was sufficiently increased in nutritive value by virtue of its decreased fiber content.

Crude Fiber

The fiber content of the Brown *B. juncea* seed hulls was 15 to 16% on an oil-free basis. The yellow-coated *B. juncea* hulls contained 17 to 20% crude fiber. Both the rape species (*B. campestris* and *B. napus*) and yellow mustard (*S. alba*) hulls contained 26 to 30% crude fiber. The fiber content of these oilseeds was virtually all contained in the hulls. Whole ground yellow seed contained 7 to 8% crude fiber; consequently, the yellow hulls contain approximately 80% of the total seed fiber content.

Protein

Solvent-extracted hulls from all varieties of rape and mustard examined, with the exception of the *B. juncea* Oriental mustard, were shown to contain 14 to 16% protein (N% × 6.25). Similarly treated hulls from the Oriental mustard contained 21% protein. Meal produced from defatted whole seed contained 40 to 46% protein. The process of dehulling therefore may involve a loss of about 6 to 8% of the total seed protein. These results are supported by data presented by Youngs (2).

Total Ash and Acid-Insoluble Ash

The total ash content of whole mustard and rapeseed varied from 4 to 5%; the hulls also were shown to contain 3.5 to 5% ash indicating a fairly even distribution of ash throughout the seed. The acid-insoluble ash levels of both whole seed and the hulls ranged from 0.25 to 0.40%.

Polysaccharide Analyses

The main commercial value of mustard hulls lies in their functionality in terms of water absorption, emulsification, and thickening properties, in addition to their useful flavor components.

It is apparent, however, that these properties are not shared equally between the different varieties of mustard and rapeseed coats. Data in Table I indicated some of the major differences in thickening properties among the various mustard and rapeseed hull samples examined in this study. Slurries made from wet-milled yellow mustard hulls yielded the most viscous preparations. Similar treatment of Brown mustard seed coats yielded slurries one-half to one-third as viscous as those prepared from the yellow mustard seed coats. Oriental mustard hulls, and hulls derived from both rapeseed species, conferred negligible thickening properties following wet-milling. The wet-milled Stoke mustard bran exhibited thickening properties lying between the Oriental and Brown mustard varieties.

The data in Table II quantitate the various amounts of polysaccharide material extracted by the sequential procedure as outlined above. These data are of particular interest as they clearly demonstrate the important quantitative differences between the various polysaccharide components of yellow mustard seed coats and those obtained from the other mustards and rapeseeds examined. It is also important to recognize that in addition to the quantitative differences

TABLE I. VISCOSITIES OF STONE-MILLED 5% w./v. AQUEOUS DISPERSIONS OF MUSTARD AND RAPESEED HULLS

Species	Viscosity cp.[1]
B. juncea (Canadian Brown)	1,845
B. juncea (Stoke)	218
B. juncea (Oriental)	82
S. alba (Yellow mustard)	5,200
B. campestris	55
B. napus	45

[1]Viscosities expressed in centipoise (cp.) units were taken using a Brookfield Viscometer Model RVT using appropriate spindles at 20 r.p.m. at room temperature.

TABLE II. POLYSACCHARIDE FRACTIONS FROM DEFATTED RAPE AND MUSTARD HULLS OBTAINED BY SEQUENTIAL SOLVENT EXTRACTION[1]

	Lyophilized Solvent Extractives, %								
	I	II	III	IV	VA	VB	VIA	VIB	VII
S. alba (Yellow mustard)	22.6	1.1	6.2	10.4	1.7	4.4	1.5	1.2	17.7
B. juncea (Brown mustard)	3.7	2.2	8.6	6.4	6.4	8.8	trace	4.0	21.9
B. juncea (Stoke mustard)	3.2	1.4	18.5	9.6	9.3	7.6	0.4	4.2	27.9
B. juncea (Oriental mustard)	2.2	0.6	5.1	8.9	5.6	8.9	trace	2.7	18.4
B. campestris (Polish rape)	1.2	1.0	7.5	9.2	3.2	1.0	trace	3.4	12.8
B. napus (Argentinian rape)	0.9	1.3	6.5	12.8	2.3	7.7	trace	1.8	10.3

[1]Extraction steps: I, Cold water; II, hot water (60°C.); III, ammonium oxalate (80°C.); IV, EDTA; VA, KOH (acidified precipitate); VB, KOH; VIA, NaOH borate (acidified precipitate); VIB, NaOH borate; VII, insoluble residue.

TABLE III. VISCOSITIES OF 1% w./v. AQUEOUS SOLUTIONS OF CERTAIN POLYSACCHARIDE FRACTIONS FROM MUSTARD AND RAPE HULLS

	Viscosity (seconds through bulb viscometer)		
Sample	Fraction I	Fraction III	Fraction IV
Water (control)	85	85	85
B. juncea (Brown)	510	487[1]	95
B. juncea (Oriental)	148	456[1]	375[1]
B. juncea (Stoke)	111	170	210
S. alba	3,720	372[1]	150
B. campestris	155	186	475[1]
B. napus	135	170	102

[1]A significant thickening or gelling occurred following acidification with HCl.

between these polysaccharide fractions from the various sources of hulls, there are a number of chemical and physical differences. Some of these differences will be explored in the following sections.

Hull Polysaccharide Fraction I

Yellow mustard seed coats appeared to be unique in their functional properties, and these in turn could be readily related to their novel chemical composition. Many of the properties exhibited by dispersions of yellow mustard seed hulls are a result of the mucilaginous polysaccharide material present in the cold water-soluble fraction I. This material was shown to constitute 22% of the total hull weight (Table II). On the other hand, the *B. juncea* hulls yielded only 2.2 to 3.7% of this fraction I polysaccharide. Hulls from the rapeseed species contained only about 1% of this fraction I component.

Data in Table III indicate that there are significant quantitative variances between the different fraction I polysaccharides. They demonstrate the differences in viscosity between 1% aqueous solutions of fractionated hull polysaccharides. It is most evident that yellow mustard hull fraction I polysaccharide has a far greater thickening capacity than the equivalent fraction isolated from other varieties of mustard and rapeseed.

In addition, the yellow mustard hull fraction I is 10 to 20 times greater in

TABLE IV. URONIC ACID CONTENT OF THE VARIOUS POLYSACCHARIDE FRACTIONS FROM MUSTARD AND RAPE HULLS[1]

	Percent Uronic Acid				
	I & II	III	IV	V	VI
B. juncea (Brown)	1.8	52.0	0.0	0.0	0.0
B. juncea (Oriental)	10.2	60.0	52.0	0.0	53.0
B. juncea (Stoke)	0.0	22.0	7.0	0.0	0.0
S. alba (Yellow mustard)	29.0	43.0	9.0	0.0	0.0
B. campestris	6.0	22.5	53.0	0.0	0.0
B. napus	7.0	32.0	0.0	0.0	0.0

[1]Uronic acid assays were made by the modified carbazole procedure (5).

quantity than the similar fractions obtained from the other Brassica seeds examined. A detailed study of the functionality of this component of yellow mustard hulls has recently been published by our laboratory (7).

The chemical nature of the yellow mustard cold water-soluble polysaccharide also appeared to be unique among the varieties examined. Refluxing with 2N H_2SO_4 for 4 hr. completely hydrolyzed the polysaccharide fraction obtained from the rapeseed and *B. juncea* mustard hulls. This same treatment only hydrolyzed 35% of the polysaccharides of yellow mustard mucilage. The insoluble fraction proved to be cellulose. Similar results have been reported by Grant et al. (8) who studied the cold water-extracted polysaccharide from yellow mustard and noted that it contained crystalline bundles of cellulose chains solubilized by association with, or perhaps encapsulation by, other polysaccharides. Our results indicated that this mucilage was not present in the *B. juncea* mustard or either of the rapeseed species examined. The neutral sugars, as identified by gas chromatography of their TMS derivatives, of the fraction I polysaccharides from *B. juncea* mustard and rapeseed hulls consisted of arabinose (40 to 50%), xylose (18 to 25%), glucose (10 to 14%) and traces of fructose, galactose, and rhamnose. Uronic acids were also present at about 6 to 12% concentration (Table IV).

The type of sugars in the hydrolyzed fraction I polysaccharides from yellow mustard differed from those found in the other varieties examined. This fraction contained 60 to 70% native cellulose which yielded β-glucose following hydrolysis. The remaining polysaccharide consisted of galacturonic acid (30%), arabinose (20%), glucose (20%), xylose (6%), fructose (6%), and rhamnose (6%), with traces of galactose and mannose.

Hull Polysaccharide Fraction II

The fraction II polysaccharide proved to have a similar sugar profile to the fraction I material, and also had similar viscosity properties.

Hull Polysaccharide Fraction III

The ammonium oxalate-extracted fraction III was present at 5 to 8% concentration in all the hulls examined except the *B. juncea* variety Stoke which contained 18.6% fraction III material. This fraction was shown to contain about 60% uronic acid residues in both the Oriental and Brown mustard. The yellow mustard fraction III material was found to be intermediate in uronic acid content

at 43% (Table IV). The major neutral sugars present in this fraction III were arabinose (15 to 20%) and xylose (15 to 20%) in all the hulls tested with the exception of the yellow seeded *B. juncea* Stoke which contained 52% arabinose. Other sugars present were glucose (13 to 20%), rhamnose (8 to 10%) and traces of fructose and galactose.

The data in Table III indicate that this pectin-like material contributed to the thickening properties of both the yellow mustard and the *B. juncea* varieties Brown and Oriental, but was less significant in both Stoke mustard and the rape species examined. Acidification of the Stoke mustard pectin with HCl slightly increased the viscosity (from 170 to 428 sec. to pass through a bulb viscometer). A more significant thickening occurred following acidification of the yellow mustard and rapeseed fraction III pectin, while the Brown and Oriental mustard pectins yielded a rigid gel after acidification of 1% aqueous solutions with dilute HCl (Table III), but not with acetic acid.

Hull Polysaccharide Fraction IV

The neutral sugars derived from the fraction IV polysaccharides consisted primarily of arabinose (35 to 50%), xylose (18 to 28%), fructose (10 to 15%), galactose (5 to 10%) and glucose (5 to 10%). Traces of uronic acid residues were noted in the Brown and Stoke varieties of *B. juncea, S. alba,* and *B. napus*. The Oriental mustard and *B. campestris* hull fraction IV polysaccharide contained 52 to 53% uronic acid residues. Reference to Table III indicates that 1% solutions of both Oriental mustard and *B. campestris* rapeseed hull fraction IV polysaccharide had significantly higher viscosities than those from the other varieties examined. These two samples were also unique in forming rigid gels following acidification with dilute hydrochloric acid. The content of fraction IV polysaccharide in the hull samples examined did not show much variation from a mean of about 10%.

Hull Polysaccharide Fraction VA & VB

The potassium hydroxide-extracted polysaccharide constituted 14.5 to 17.0% of the *B. juncea* mustard hulls, 6% of the yellow mustard hulls, 4% of the *B. campestris,* and 10.5% of the *B. napus* hulls. This fraction appeared to have no significant contribution to the thickening properties of the samples examined.

The major neutral sugars comprising this fraction were fructose (20 to 30%), arabinose (15 to 20%), glucose (10 to 15%), xylose and galactose (both about 10%).

Fructose was particularly evident in the Oriental mustard hull fraction V material (about 50%). No uronic acid residues were observed.

Hull Polysaccharide Fraction VIA & VIB

The NaOH/borate extracted material constituted only a minor fraction among the various hull polysaccharides examined, ranging from 2 to 4% by weight. The major constituent neutral sugars were glucose (30 to 40%), fructose (20 to 30%), arabinose (especially in the yellow mustard hull fraction at 35% concentration) and galactose. No uronic acid residues could be detected in this fraction, with the exception of the Oriental mustard hull fraction VI which contained 53% uronate.

Hull Polysaccharide Fraction VII

The insoluble residue remaining after the completion of the sequential solvent extraction of the hulls was found to consist of cellulose, and yielded β-glucose following acid hydrolysis. The content of this fraction was found to be lowest in the rape hulls (10 to 13%) and highest in the *B. juncea* hulls (18 to 28%).

CONCLUSION

Mustard hulls are currently utilized in a variety of food applications largely based on the valuable functionalities observed upon hydration of the ground hulls (7). These properties have now been examined and found to be largely due to the unique nature of the polysaccharide fraction in certain varieties of mustard. It is apparent that while rapeseed hulls lack some of the useful functional properties found in mustard hulls, both types of hull have a relatively high fiber content. Dehulling of both rapeseed and mustard is therefore likely to improve the nutritional value of the remaining meal.

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