Polysaccharides and Proteins of Glandless Cottonseed Flour

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ABSTRACT

Cottonseed polysaccharides were concentrated by removing proteins, sugars, lipids, and color components from cottonseed flour by five successive extractions. The polysaccharide fraction (28.6% of flour) still contained 38% protein, which had an essential amino acid composition similar to that of skim milk solids. After the removal of starch by α-amylase digestion, the polysaccharide-rich fraction was subfractionated into four nonstarch polysaccharide fractions: pectic substances, two hemicellulose fractions, and celluloses. Each fraction was analyzed to identify and quantify the monosaccharides present. The pectic substances contained 56% polysaccharides, which on hydrolysis yielded predominantly arabinose and uronic acids. The first hemicellulose fraction consisted of 62% polysaccharides and contained predominantly uronic acids, glucose, and arabinose. The second hemicellulose fraction contained 49% polysaccharides, which on hydrolysis yielded primarily glucose, xylose, and uronic acids. The cellulose fraction contained 72% polysaccharides, which on hydrolysis yielded 95% glucose. The nonstarch polysaccharides were responsible for the high water absorption of glandless cottonseed flour, which absorbed about 2 g of water per gram of flour. However, the nonstarch polysaccharide fraction absorbed over 19 g of water per gram of material.

Glandless cottonseed is currently incorporated in numerous snack foods, and is regarded as a potential source of human nutrients (Simmons 1980). Cottonseed flour contains protein with desirable food functional and nutritional characteristics (Cherry et al 1978). Flour from glandless cottonseed also contains 6.0–7.0% nonstarch polysaccharides and 0.5–0.8% starch. The starch content is dependent upon the maturity of seeds used to prepare flour, being found mainly in immature seeds (Dollear and Markley 1948). The nonstarch polysaccharides may be beneficial both as dietary fiber and as a functional ingredient in foods.

The role of fiber in the diet has received much favorable attention recently among nutritionists and members of the medical community (McConnell et al 1974). The National Cancer Institute has recommended that the American population double its current intake of dietary fiber (Anonymous 1985). Dietary fiber consists principally of pectic substances, hemicelluloses, and lignins. Glandless cottonseed flour contains about twice the amount of pectic substances and hemicelluloses found in wheat flour, and therefore could be considered a source of dietary fiber.

Nonstarch polysaccharides also serve as functional ingredients in foods because of their high water absorption capacity. Water absorption is an important functional property in baked goods and other food products, because it allows for increased product yield and increases shelf life through reduction of moisture loss during food storage (D'Appolonia and Kim 1976). Apparently, different nonstarch polysaccharides absorb water in different quantities (McConnell et al 1974). For example, wheat flour pentosans, which constitute only 1.5% of the total flour solids, are responsible for absorbing 23.4% of the total water in bread dough (Bushuk 1966).

Several studies (Zarins and Cherry 1981, Dieckert et al 1981, Zarins et al 1984) describe the isolation and characterization of cottonseed proteins, but the cottonseed nonstarch polysaccharides have not been thoroughly investigated. This study was designed to isolate and characterize the nonstarch polysaccharides of glandless cottonseed flour.

MATERIALS AND METHODS

Materials

Hexane-defatted glandless cottonseed flour, prepared at the Southern Regional Research Center, was used for the polysaccharide isolation. The enzymes pepsin and α-amylase were purchased from Sigma Chemical Co. Skim milk solids (Carnation) were obtained from a grocery shelf.

Isolation of Polysaccharides

Figure 1A summarizes five solvent extraction steps using a method described by Blouin et al (1982). The hexane, chloroform, and 2-propanol extractions were carried out for 24 hr at ambient temperature. The aqueous and 10% NaCl extractions were performed for 1 hr at ambient temperature, to minimize enzymatic action. Extractions performed at lower temperatures were incomplete because several of the cottonseed proteins are cryoprecipitable (Rossi-Fanelli et al 1964, Zarins et al 1984). All extractions were performed using round-bottom flasks equipped with a glass sleeve through which a mechanical stirrer was inserted. The residues were lyophilized before each extraction.

The residue after 10% NaCl extraction (RSP) was further fractionated as shown in Figure 1B. The pepsin digestion and phytate removal were accomplished by suspending the RSP at 1 g/100 ml in water adjusting the pH to 2.0 at 37° C with HCl. Pepsin (2% based on the weight of RSP) was added to the suspension and

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digestion performed for 20 hr.

The pepsin digest was analyzed for phytic acid by adjusting it to pH 7.0 with 1*N* NaOH. At that time, a white precipitate was formed. The precipitate was removed by centrifugation and washed with water. It was identified by infrared spectroscopy as a salt of phytic acid.

The residue after pepsin digestion was suspended in water at 1 g/100 ml and the pH adjusted to 6.9, 3% α-amylase was added and digestion performed at ambient temperature for 7–8 hr.

The nonstarch polysaccharide extractions were all performed at a polysaccharide-to-solvent ratio of 1:70 (w/v) in an atmosphere of nitrogen. The pectic substances were extracted with an aqueous solution of oxalic acid-ammonium oxalate (0.25% of each), for 1 hr at 80°C. The first hemicellulose fraction (B.1E in Fig. 1) was obtained by two successive extractions of the pectin-free residue with aqueous 1.25*N* NaOH for 24 hr. After the two lengthy extractions, the residue (R.1B in Fig. 1) still contained hemicelluloses. An attempt was made to complete the extraction with 4*N* NaOH at ambient temperature for 4 hr. After this extraction only about 3% hemicelluloses remained in the final residue (R.2B).

Extraction of Residual Lipids

The hemicellulose fractions (B.1E and B.2E) were washed with chloroform at ambient temperature for 1 hr and filtered. The chloroform was evaporated under nitrogen to yield a lipid-like substance. Further analysis of this material by thin-layer chromatography, using the method of Neher (1969), showed this fraction consisted of steroid type compounds.

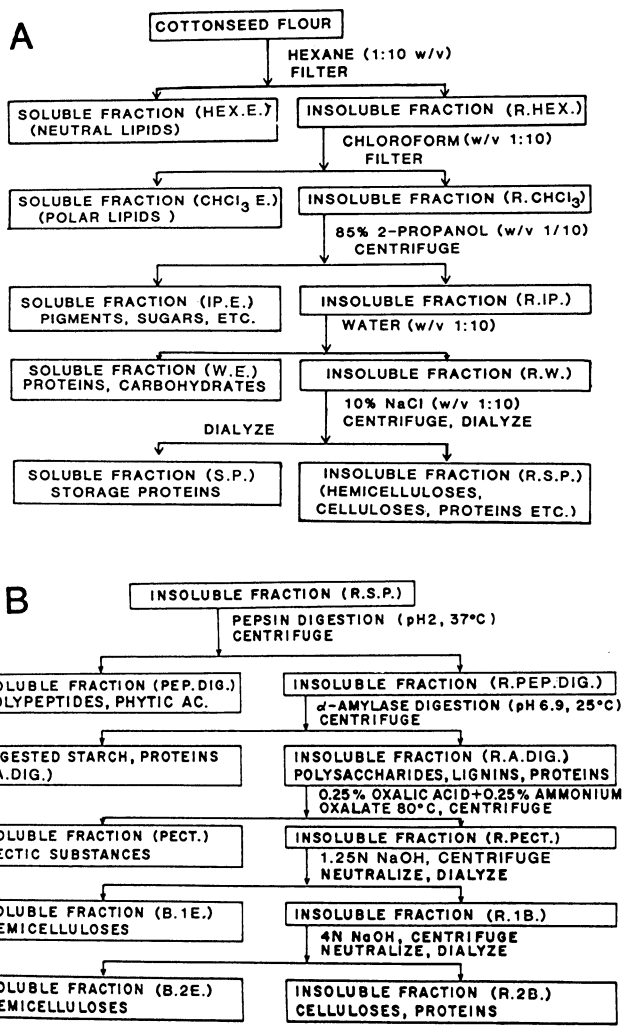

Fig. 1. Isolation and fractionation of cottonseed flour polysaccharides. **A,** solvent extractions; **B,** enzyme digestions, followed by fractionation into pectic substances, hemicelluloses, and celluloses. **PEP.DIG.** = pepsin digest, **R.PEP.DIG** = residue after pepsin digestion, **R.PECT.** = pectin-free residue.

Determination of Water Hydration Capacity

Water hydration capacity, or water absorption was determined by AACC method 88-04 (1983).

Determination of Sugars in Nonstarch Polysaccharides

The different polysaccharide fractions obtained from RSP were hydrolyzed and derivatized to alditol acetates by the method of Albersheim et al (1967). Myo-inositol was added to the polysaccharides prior to hydrolysis as an internal standard, because it was not present in any of the polysaccharides. The alditol acetates were dissolved in chloroform and analyzed by gas liquid chromatography (GLC). GLC analysis was performed on a Hewlett Packard 5700A gas chromatograph using an SP-2330 capillary column, 30 m long, and 0.32 mm i.d. (Supelco, Inc., Bellfonte, PA). The chromatograph was equipped with a flame-ionization detector. The column temperature was programmed to hold 4 min at 220°C, then to 240°C at the rate of 4°C/min, then held 8 min. The alditol acetates were identified and quantified by comparison of their retention times and peak areas with those of known standards.

The final residue (R.2B) did not hydrolyze in 0.2*N* trifluoroacetic acid. This fraction was hydrolyzed by a modified Southgate (1969) procedure. The sample was suspended in 72% sulfuric acid and stirred at ambient temperature for 1 hr, then stored at 4°C for 16 hr. The mixture was then diluted to a 5% sulfuric acid concentration and refluxed for 3 hr. Sulfuric acid was removed by passing the hydrolysate through a Dowex 1-X8 (formate form) column. Formic acid was removed on a rotary evaporator at 45°C. Reduction, acetylation, and GLC analysis were performed in the same manner as for pectic substances.

Uronic acid content of the polysaccharides was determined by the method of Wardi et al (1974).

Determination of Protein Composition

Essential amino acid analysis was accomplished as described by Zarins and Cherry (1981). Protein content of cottonseed flour and its fractions was determined from Kjeldahl nitrogen using a factor of 5.3. A factor of 6.38 was used to determine the protein content of skim milk solids.

The amino acids of the proteins, associated with the nonstarch polysaccharides, were identified by thin-layer chromatography (TLC) on cellulose plates by the method of von Arx and Neher (1963).

RESULTS AND DISCUSSION

Proteins were extracted from cottonseed flour and polysaccharides concentrated in fraction RSP (Fig. 1A). This fraction normally is discarded after protein isolation, even though it contains 96% of the polysaccharides present in cottonseed flour. The essential amino acid composition of cottonseed flour, its fractions, and skim milk solids is listed in Table I. The storage proteins have the poorest amino acid composition as compared with the skim milk proteins. The RSP in its protein content and essential amino acid composition closely resembles skim milk solids. Analysis of pepsin digest (Table I) indicates that more than two thirds of the RSP proteins would be utilized by the human digestive system.

The yields and protein contents of the various fractions (Table II) show that the RSP fraction accounts for about one third of cottonseed flour, and contains more protein than the water extract. The RSP fraction also contains condensed flavonoids, tannins, and lignins. These phenolic compounds give the RSP fraction a tan color (Blouin et al 1981).

Water hydration capacities of cottonseed flour and its fractions are listed in Table III. These data show that the high water absorption of cottonseed flour is attributable mostly to the nonstarch polysaccharides; the water-soluble fraction and storage protein have low water absorption capacities.

TABLE I
Essential Amino Acid and Protein Content of Cottonseed Flour, Its Fractions, and Skim Milk Solids
(g of amino acid/100 g dry weight of protein)

Amino Acid	Flour	Water Extract	10% NaCl Extract	Residue-10% NaCl	Skim Milk Solids	Pepsin Digest	Residue-Pepsin Digest
Lysine	5.30	7.43	3.49	7.76	7.30	5.47	3.42
Threonine	3.05	2.96	2.79	4.61	4.03	5.50	5.19
Valine	5.18	2.10	5.23	6.75	6.60	6.21	5.94
Methionine	1.14	1.29	1.28	1.50	2.32	1.18	1.91
Isoleucine	3.43	1.25	3.80	4.95	5.21	5.04	4.72
Leucine	6.25	2.40	6.70	9.61	9.78	12.18	10.82
Phenylalanine	6.38	2.22	7.06	4.97	4.57	5.19	5.08
Tryptophan	1.42	0.93	1.31	1.55	1.34	...	...
Protein	51.1	34.7	93.7	38.2	36.7	59.6	20.5

TABLE II
Extraction Yields from Glandless Cottonseed Flour and Protein Contents of Isolated Fractions

Fraction	Yield from Flour (%)	Protein Content (%)
Cottonseed flour fractions		
Cottonseed flour	100	51.1
Hexane extract	1.6	0
Chloroform extract	1.9	0
2-propanol extract	7.8	0
Water extract	27.1	34.7
10% NaCl extract	33.0	93.7
Residue-10% NaCl	28.6	38.2
Residue-10% NaCl fraction		
Pepsin digest	17.0	59.6
Amylase digest	1.2	32.8
Pectic substances	2.2	20.4
Hemicelluloses 1	3.5	18.5
Hemicelluloses 2	0.9	9.7
Cellulose fraction	2.6	20.8
Loss during dialysis	1.2	...

TABLE III
Water-Hydration Capacity of Cottonseed Flour Fractions[a]

Cottonseed Flour Fraction[b]	Water-Hydration Capacity (g water/100 g solids)
Wheat flour	95
Cottonseed flour	204
Residue after lipid extraction	205
Water extract	50
Residue-water	210
10% NaCl extract	70
Residue-10% NaCl	450
Residue after pepsin digestion	1,070
Residue after amylase digestion	1,920

[a]The water hydration capacity was determined by AACC method 88-04.
[b]For a detailed description of each fraction, refer to Fig. 1.

Several proteases were tried, such as trypsin, to remove proteins from the RSP fraction (unpublished results). Enzymes acting at neutral pH were found to be ineffective. Pepsin digestion alone accomplished as much as trypsin digestion followed by pepsin digestion. The low pH during pepsin digestion also solubilized phytic acid, which is present in cottonseed flour as phytin (Ca, Mg salt); this enabled the removal of proteins and phytic acid in one step.

The sugar composition of the nonstarch polysaccharide fractions is listed in Table IV. The pectic substance fraction in its sugar composition greatly resembled the first hemicellulose fraction. After the pectic substance extraction most of the color remained in the residue. The polyphenolic substance-hemicellulose linkages were saponified during the long alkali extractions, and most of the color was lost during dialysis (about 1% of the flour), as

TABLE IV
Sugar Composition of Nonstarch Polysaccharide Fractions[a]

Sugar	Pectic Substances (%)	Hemicelluloses B.1E (%)	Hemicelluloses B.2E (%)	Cellulose Fraction (%)
Rhamnose	2.0	1.9	1.0	0.3
Fucose	trace	1.0	1.5	>0.1
Arabinose	20.0	14.3	4.0	1.0
Xylose	4.1	7.8	7.9	1.0
Mannose	Not detected	1.0	2.8	0.3
Galactose	1.7	3.1	4.5	0.4
Glucose	7.4	14.5	18.5	69.0
Uronic acids	20.9	18.4	8.0	trace

[a]The polysaccharides were hydrolyzed, derivatized to alditol acetates, and analyzed by gas-liquid chromatography.

evidenced by the yellow color outside the dialysis tubing in the dialysate.

TLC indicated that the hemicellulose fractions also contained steroid-type compounds and lipoproteins. The cellulose fraction still contained some lignin type compounds, as indicated by its color. This color disappeared when the fraction was treated with sodium hypochlorite.

When the hydrolyzed fractions of the nonstarch polysaccharides were analyzed by two-dimensional TLC, the chromatograms showed that the proteins associated with the nonstarch polysaccharides were made up of predominantly from the hydrophobic amino acids: leucine, arginine, alanine, tyrosine, and isoleucine. The cellulose fraction also contained a considerable amount of valine.

A more detailed examination of the fractions will be necessary before carbohydrate-protein, or carbohydrate-lignin linkages can be identified. In the solid state, a polysaccharide matrix, held together by covalent linkage with minimum hydrogen bonding, will have a large capacity to form hydrogen bonds with water through free hydroxyl groups (Preston 1974). The nonstarch polysaccharides differ from other oilseed polysaccharides, i.e., soybean (Aspinall and Cotrell 1971) by their high pentosan and low galactose content.

CONCLUSION

Cottonseed flour polysaccharides contain relatively high proportions of pentosans and uronic acids, which enables them to absorb and retain large amounts of water. The polysaccharides are also associated with protein high in essential amino acid content. These properties should make the RSP fraction a desirable ingredient in certain food products.

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