Carbohydrate Composition of Soybean Flours, Protein Concentrates, and Isolates

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Total carbohydrate content and the amounts of each sugar present in commercial samples of soybean protein products were determined by hydrolysis and gas–liquid chromatography of the alditol acetate derivatives of the sugars. Dehulled, defatted soybean flours contained the following mean sugar content: rhamnose, 0.6%; fucose, 0.1%; ribose, 0.1%; arabinose, 2.4%; xylose, 1.0%; pinitol, 0.9%; mannose, 0.9%; galactose, 7.6%; and glucose, 8.1%. The same sugars were found in soybean protein concentrates; however, the amount of each was less. Soybean protein isolates contained mannose, 0.8%; galactose, 0.5%; and glucose, 0.5%, with traces of the other five sugars.

Compared to oil and protein, carbohydrates of soybeans have received comparatively slight attention by chemists. This neglect is a result of the greater economic importance of the oil and protein constituents in foods and feeds. It is generally assumed that the carbohydrate content of soybeans and defatted meal is equal to the nitrogen-free extract. This quantity is determined by difference, i.e., 100% – percent moisture – percent protein – percent fat – percent crude fiber – percent ash. Since the crude fiber determination is an empirical method, the accuracy of the carbohydrate determinations of soybeans, soy flours, and derived products is questionable. A number of investigators have studied the nitrogen-free extract of soybean meal from various points of view (Street and Bailey, 1915; MacMasters et al., 1941; Kawamura, 1967; Aspinall et al., 1967b), but no complete separation or analysis of the carbohydrate components in soybean protein fractions has been published.

The purpose of this study, therefore, was to gain more direct information on the total carbohydrate content and the amount of each sugar present in soybean flours, concentrates, and isolates.

MATERIALS AND METHODS

Samples. Three dehulled, defatted soybean flours were prepared in the laboratory from two varieties of soybeans. In addition, six samples of commercial defatted soybean flours were obtained. Four commercial samples of soybean concentrates (products containing a minimum of 70% protein) from three different manufacturers were also analyzed. Three processors each use a different procedure for the preparation of their concentrate (Circle and Smith, 1972). Five soybean protein isolates (products containing a minimum of 90% protein) from two different manufacturers were likewise analyzed.

Trade names and sources of the samples are given in Table I. All samples were ground to pass a 60–80 mesh screen, and moistures were determined by heating at 130 °C for 2 h (AACC, 1967).

Hydrolysis of Samples and Preparation of Alditol Acetates. To measure the total neutral carbohydrate, the procedure of Sloneker (1971) was modified slightly. The samples (40–70 mg) were weighed into 15 × 125 mm Teflon-lined screw-cap test tubes. Sulfuric acid (0.3 mL of 72% concentration, w/w) was added to each tube, and the contents were stirred with a small glass rod, after which the glass rod was broken off, leaving the wetted portion in the test tube. After heating 1 h at 30 °C, 8.4 mL of water was added to each sample to dilute the acid to 1 N.

Table I. Identity of Samples Used in Study

sample	trade name or description	source[a]
flours		
A	hexane defatted, Kanrich variety, 1971 crop	1
B	hexane defatted, Amsoy variety, 1971 crop	1
C	hexane defatted, Amsoy variety, 1974 crop	1
D	Nutrisoy 7B	2
E	toasted Nutrisoy	2
F	Baker's Concentrate	3
G	unflavored, minced TVP	2
H	uncooked, flavored, Crumbles	4
I	soybean meal, starting material for sample G	2
concentrates		
J	Promsoy 100 (aqueous alcohol leach process)	3
K	undenatured GL-301 (dilute acid leach process)	5
L	denatured Patti-Pro (dilute acid leach process)	5
M	food protein concentrate (steaming, water leach process)	6
isolates		
N	Promine D	3
O	Edi-Pro N	7
P	Edi-Pro A	7
Q	Supro 700	7
R	Supro 610	7

[a] 1, Northern Regional Research Center; 2, Archer-Daniels-Midland Co.; 3, Central Soya Co.; 4, General Mills, Inc.; 5, Griffith Laboratories, Inc.; 6, Swift and Co.; 7, Ralston Purina Co.

The diluted samples were then hydrolyzed 2 h at 120 °C in an oil bath. After the first 10–15 min, the samples were stirred on a Vortex stirrer to disperse the material in the acid. After the 2-h hydrolysis, the samples were cooled in tap water and an internal standard of 2-deoxy-D-glucose was then added. After mixing thoroughly, the hydrolyzate was transferred to a beaker and neutralized with lead carbonate. The lead salts were removed by centrifugation, and the aldoses and ketoses in the supernatant were reduced by adding 10–20 mg of sodium borohydride. After 1 h, excess borohydride was destroyed by adding acetic acid

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Table II. Sugar Analysis of Hydrolyzed Carbohydrates of Nine Defatted Soy Flours (Percent)[a]

sugar	flours A	B	C	D	E	F	G	H	I	mean	SD[b,c]
rhamnose	0.48	0.62	0.69	0.37	0.61	0.46	0.61	0.61	0.60	0.56	0.171
fucose	0.10	0.27	0.20	0.12	0.04	0.04			0.06	0.09	0.079
ribose	0.13	0.18	0.19	0.17	0.12	0.08	0.11	0.08	0.13	0.13	0.067
arabinose	2.32	2.40	2.35	2.13	2.01	1.81	2.08	2.01	2.09	2.37	0.194
xylose	0.84	0.98	0.98	0.95	0.76	0.70	0.91	0.90	0.86	0.97	0.098
pinitol	0.54	0.64	0.88	0.88	0.79	0.65	1.06	0.71	0.89	0.87	0.171
mannose	0.86	0.75	1.08	0.86	0.78	0.81	1.01	0.65	0.84	0.94	0.331
galactose	7.10	7.92	9.42	7.85	7.34	6.28	7.83	7.14	7.50	7.60	0.433
glucose	7.97	9.50	10.90	7.97	7.83	6.40	7.57	6.73	8.36	8.14	0.731
total	20.34	23.26	26.69	21.30	20.28	17.23	21.18	18.83	21.33	21.67	1.013
monosaccharides	0.43	0.54	0.49	0.59	0.60	0.43	0.64		0.59	0.47	*d*
sucrose	7.49	9.02	9.39	6.52	7.17	6.46	6.17		6.36	7.32	*d*
raffinose	1.18	0.54	0.62	1.11	0.92	0.75	0.96		0.97	0.88	*d*
stachyose	4.92	4.57	4.93	4.34	4.78	4.09	4.96		3.95	4.57	*d*

[a] Uronic acids were not determined and fructose is destroyed during hydrolysis. [b] SD, standard deviation. [c] Calculated from 19 determinations. [d] Not replicated.

(1 N) until effervescence of hydrogen ceased.

Each reduced sample was passed through a small column (2–4 mL) of AG 50 × 4 cation-exchange resin (200–400 mesh) in the H^+ form (Bio-Rad Laboratories, Richmond, CA). After the columns were washed with water, the effluents and washings were evaporated to dryness. Borate ions produced during the reduction were removed as trimethyl borate by adding methanol and again evaporating three times. The residual alditols were acetylated for 16 h at 100 °C with 0.3 mL of pyridine–acetic anhydride (1:1).

Gas Chromatography of Alditol Acetates. The alditol acetates were separated by direct injection onto a 200 × 2 mm glass column packed with 3% ECNSS-M on 100–120 mesh Gas-Chrom Q (Applied Science Laboratories, State College, PA). The column was preconditioned overnight at 210 °C and operated at 195 °C. Carrier gas flow rate was maintained at ~40 mL/min. The chromatograph was a Packard Model 7409 equipped with a dual-flame ionization detector. The detector response was recorded on a magnetic tape recorder (Model CRS-43RAI, Infotronics Corporation, Houston, TX).

Response factors (R) were determined for known sugars based on the internal standard 2-deoxy-D-glucose. From these response factors, the sugar composition of the starting materials was calculated using a computer program.

The sugars occur as oligosaccharides and polysaccharides in soy flour and primarily as polysaccharides in concentrates. Practically all of the sugars therefore exist in glycosidically linked forms. Hence the amount of each sugar present in a product has been calculated as the glycosidically linked form.

Identification and Determination of Pinitol. An authentic sample of pinitol isolated from sugar pine (courtesy of Dr. L. Anderson, University of Wisconsin, Madison, WI) was acetylated as in the preparation of the alditol acetates of the sugars and used to measure GLC retention time and response factor. Mass spectrum of the derivative was determined on a DuPont 21-491 instrument.

Determination of Oligosaccharides. Analysis of the oligosaccharides (sucrose, raffinose, and stachyose) in the samples was by the high-performance liquid chromatography (LC) procedure of Black and Bagley (1978).

RESULTS AND DISCUSSION

Figure 1 shows a typical gas–liquid chromatography (GLC) pattern of the alditol acetates from a hydrolysate of hexane-defatted soybean meal to which has been added an internal standard, 2-deoxy-D-glucose. Observed were

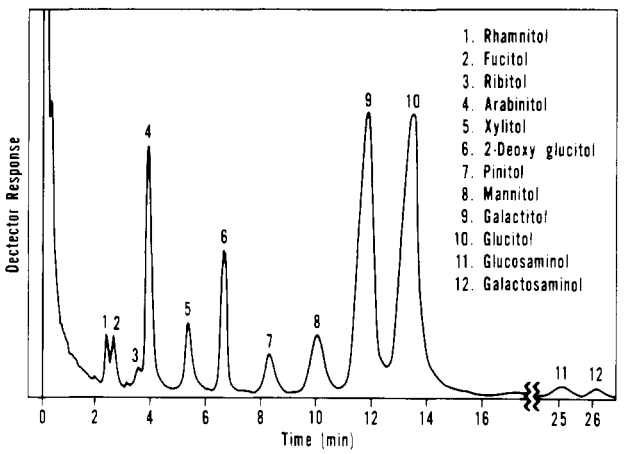

Figure 1. Separation of acetylated alditols and amino alditols from defatted soybean meal by GLC.

alditol acetates with retention times corresponding to eight sugars plus glucosamine and galactosamine. An additional peak between 2-deoxy-D-glucitol and mannitol (peak 7) did not correspond to any known sugar, but was tentatively identified as pinitol (5-*O*-methyl-D-inositol) because it had a retention time and mass spectrum indistinguishable from this derivative of inositol. Pinitol has been reported in soybeans (Schweizer et al., 1978), full-fat and defatted soybean flakes (Honig et al., 1971; Nielson, 1960), and in the cotyledons and the hulls (Kawamura et al., 1977). We noted, however, that quebrachitol has the same retention time and mass spectrum as pinitol and may also exist in soybeans as suggested recently (Schweizer et al., 1978). As shown in Figure 1, the separation of rhamnitol from fucitol and ribitol from arabinitol is incomplete. Since the concentration of three of these sugars in soybeans is low, an inaccurate value would not introduce a large error in the analysis for total carbohydrates.

Table II gives the percentages of various sugars found in soybean flours. Also included are mean values and the standard deviation of the analysis of each sugar. Fucose and ribose were minor sugars, whereas glucose and galactose accounted for over 70% of the total sugars. Table II also shows a mean value for pinitol of 0.87% for defatted soy flours. This value compares with a total pinitol (free pinitol plus pinitol from galactopinitol) value of 0.86% reported by Schweizer et al. (1978) for soybeans grown in the United States.

There are greater variations in total sugar in laboratory-prepared samples (A, B, C) than with commercial soy

Table III. Sugar Analysis of Hydrolyzed Carbohydrates of Four Soybean Protein Concentrates (Percent)[a]

sugar	concentrate J	K	L	M	mean	SD[b,c]
rhamnose	0.12	0.78	0.59	0.78	0.40	0.374
fucose		0.12	0.15	0.37	0.09	0.136
ribose		0.23	0.09	0.10	0.05	0.086
arabinose	1.98	2.39	2.27	2.05	2.18	0.463
xylose	0.62	1.07	0.96	1.01	0.92	0.237
pinitol		0.16	0.13		0.08	0.124
mannose	0.38	1.04	0.86	0.59	0.74	0.381
galactose	5.05	5.68	5.42	6.11	5.69	1.058
glucose	3.72	6.28	5.00	5.25	5.06	0.599
total	11.87	17.75	16.30	16.26	15.55	3.857
monosaccharides	0.00	0.11	0.00	0.27	0.10	*d*
sucrose	1.35	0.22	0.70	1.40	0.92	*d*
raffinose	0.11	0.00	0.00	0.08	0.05	*d*
stachyose	1.80	0.00	0.50	0.54	0.71	*d*

[a] Uronic acids not determined and fructose is destroyed during hydrolysis. [b] SD, standard deviation. [c] Calculated from nine determinations. [d] Not replicated.

Table IV. Sugar Analysis of Hydrolyzed Carbohydrates of Five Soybean Protein Isolates (Percent)

sugar	isolates N[a]	O	P	Q	R	mean	SD[b,c]
rhamnose		0.01	0.12		0.04	0.04	0.033
fucose		Tr		0.11		0.03	0.099
ribose	0.20	0.24	0.22		0.38	0.19	0.176
arabinose	0.13	0.04	0.18	0.09	0.29	0.13	0.060
xylose	0.03	0.04	0.04	0.11	0.16	0.07	0.105
pinitol	0.07	0.03				0.01	0.019
mannose	0.74	0.59	0.68	0.87	0.86	0.75	0.158
galactose	0.63	0.41	0.57	0.48	0.55	0.51	0.158
glucose	0.50	0.43	0.53	0.43	0.67	0.50	0.178
total	2.23	1.76	2.34	2.09	2.95	2.27	1.414

[a] Isolate N contained 0.1% sucrose. Oligosaccharides were absent from all other samples. [b] SD, standard deviation. [c] Calculated from 12 determinations.

flours (samples D, E, G, I). The reason for this is unknown. The laboratory prepared samples contained fewer hulls (seed coats) than the commercial preparations, yet sample C had the highest carbohydrate content. The higher total sugar value for samples B and C may be caused by seasonal differences as noted by earlier workers (MacMasters et al., 1941), or varietal differences such as in the amount of starch present (Wilson et al., 1978).

The total carbohydrate contents reported in Table II are somewhat lower than values reported by others. Street and Bailey (1915), for example, reported a nitrogen-free extract of 31.1% for soybeans. MacMasters et al. (1941) reported total carbohydrate in soybeans by difference after moisture, protein, fat, and ash had been determined. Their values ranged from 31.1 to 43.9%, with an average of 35.4%. Kawamura (1953) reported a nitrogen-free extract of 34.7% for defatted meal (apparently not dehulled because crude fiber content was 5.8%) but was able to account for only 20% of the meal in terms of reducing sugars after hydrolysis.

Our low carbohydrate values for soy flours as compared to nitrogen-free extract values can be attributed largely to destruction of D-fructose during hydrolysis (Sloneker, 1971). This destruction is appreciable because fructose constitutes one-half of the sucrose, one-third of the raffinose, and one-fourth of the stachyose found in soy flours. To make a correction for this loss, contents of sucrose, raffinose, and stachyose were determined by LC for eight of the nine soy flours listed in Table II. Based on the mean values for the oligosaccharides, the amount of fructose not accounted for in our alditol acetate analysis is approximately 3.7 + 0.3 + 1.1 = 5.1%. Additional corrections are necessary for galacturonic acid found in the polysaccharides (Kikuchi et al., 1971) and saponins (Gestetner et al., 1966) and glucosamine that occurs in the 7S glycoprotein (Koshiyama, 1969). The largest of these corrections is for the galacturonic acid in the acidic polysaccharides. Kikuchi et al. (1971) isolated the cell wall polysaccharides from dehulled, defatted soybean meal and found them to contain 16% galacturonic acid. Based on a yield of 15.8% of cell wall polysaccharides, the galacturonic acid content of defatted meal is about 2.5%.

We obtained evidence for glucosamine and galactosamine in the GLC patterns (Figure 1) but did not include them in the calculations because the slow eluting peaks were often diffuse. Ignoring the amino sugars, the total carbohydrates of defatted soy flour thus amount to approximately:

sugars as alditol acetates	21.7%
fructose destroyed by hydrolysis	5.1%
galacturonic acid	2.5%
	29.3%

Table III gives the results obtained from the analyses of four soy protein concentrates (70% protein). Even though three of the concentrates were prepared by different processes (Table I), they contained approximately the same amounts of the individual sugars. As in the flours, fucose and ribose were minor sugars and glucose plus galactose were the major sugars. Pinitol was present in detectable amounts in only two of the concentrates. Analyses of the concentrates for the oligosaccharides revealed greatly reduced amounts as compared to the flours, but there were large variations between the different preparations. These variations likely reflect differences in the extraction efficiencies of the processes used in preparation of the concentrates.

Table IV presents carbohydrate analyses of five commercial soy protein isolates (90–95% protein). As expected, the sugars occurred in greatly reduced amounts as compared to their levels in flours and concentrates. Traces of fucose suggest the presence of small amounts of polysaccharides, since this sugar has been reported to occur in the terminal position of side chains of the acidic polysaccharide complex (Aspinall et al., 1967a). The other minor sugars—rhamnose, xylose, and arabinose—likewise are polysaccharide constituents, but they also occur in soybean saponins (Gestetner et al., 1966; Wolf and Thomas, 1971) which are found in protein isolates (Nash et al., 1967; Eldridge and Wolf, 1969). Ribose is likely to originate from a ribonucleoprotein fraction that exists in

acid-precipitated soybean proteins (Koshiyama and Iguchi, 1965). Glucose, galactose, and mannose were the major sugars found in the isolates. Mannose was expected since it occurs in the 7S globulin glycoprotein (Koshiyama, 1969).

CONCLUSIONS

Sugars in soybeans exist in a diversity of forms including monosaccharides, oligosaccharides, polysaccharides, saponins, sterol glucosides, glycolipids, and isoflavones. By hydrolyzing the complex mixture and analyzing the resulting neutral sugars by the alditol acetate GLC procedure, we have obtained a greater insight than previously available about the individual sugars that comprise the carbohydrate fractions of soybean protein products. Our results confirm discrepancies noted earlier between the neutral sugar content obtained on hydrolysis of defatted soybean meal and the nitrogen-free extract (Kawamura, 1953). However, separate analyses for uronic acids and the oligosaccharides (to correct for fructose destruction) should give a fairly complete analysis of the total sugars and account for most of the nitrogen-free extract.

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Relationship among Maize Endosperm Characteristics of Normal and *Sugary Opaque-2* Kernels during Development

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A normal endosperm and its double mutant *sugary opaque-2* counterpart of a maize synthetic were compared for physical and chemical characteristics during 7 weeks at weekly intervals beginning 7 days after pollination. Nitrogen, potassium, magnesium, and phosphorus in the endosperm were determined and correlated with fresh weight, dry weight, and water content. Data indicated that nitrogen concentration is similar in both endosperms with the exception at 7 weeks after pollination (WAP). However, N content per endosperm was different in both endosperms from 3 to 7 WAP. Normal endosperms were consistently higher than *sugary opaque-2* endosperms in N content with maximum differences reaching 57% at late maturity. With a different pattern, potassium, magnesium, and phosphorus were significantly higher in concentration and content per endosperm in *sugary opaque-2* from 3 to 6 WAP. Nitrogen is highly correlated with endosperm dry weight for both normal and *su o2* endosperms. Potassium, Mg, and P showed high correlation with water content in both *su o2* and normal endosperms.

Genes affecting maize endosperm have been studied extensively in recent years. Special attention, however, has been given to *opaque-2* mutation since it increases in lysine content in the mutant endosperm (Mertz et al., 1964; Misra et al., 1972). Higher concentrations of K, P, Mg, Fe, and Zn have been found in *opaque-2* kernels than in normal seeds (Arnold et al., 1977a,b; Goodsell, 1968). *Opaque-2* endosperm shows also higher water content than normal endosperm from early stages of kernel development to maturity (Elmore, 1971). The *o2* effect on mineral content of the endosperm is still not well understood.

It was also shown elsewhere (Silva et al., 1978) that the *su* gene when combined with the *o2* gene enlarges the differences between normal and *opaque-2* endosperms for water-soluble polysaccharide and water content resulting

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