Sunflower Carbohydrates

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The total carbohydrate contents of the flour, protein concentrate, testa, hull, and head of Commander sunflower were 29, 21, 42, 48, and 71%, respectively. The yields of simple sugars in the ethanol extracts from flour, hull, and head were 11, 2, and 34%, respectively, while flour, testa, and head contained 1–3% of water-soluble arabinogalactans. Galacturonans extracted by dilute ammonium oxalate constituted 11% of the testa and head material but only negligible amounts were found in other head components. The concentrations of alkali-soluble hemicelluloses, arabinans and arabinogalactans, were 9 and 13% in the flour and protein concentrate, respectively, while the hull and head contain 6 and 1%, respectively, of an alkali-soluble xylan. The cellulose contents of the insoluble residues were 6% in the flour and protein concentrate and were 24, 39, and 31% of the testa, hull, and head residues.

Sunflower is an important source of vegetable oil and there are excellent prospects for the utilization of the meal proteins and head pectins in food applications. In previous investigations the chemical characteristics of the seed proteins (Sabir *et al.*, 1973) and phenolic constituents of the flour and protein concentrate (Sabir *et al.*, 1974) have been determined. The present study was designed to characterize the seed, hull, and head carbohydrates which may be of economic importance in food and nonfood products.

Although the polysaccharides in sunflower kernels and hulls have not been investigated, Mikolajczak *et al.* (1970) reported that sunflower meal contained 9.7% of di- and oligosaccharides, mainly sucrose, trehalose, and raffinose. Pomenta and Burns (1971) found that the alcohol-soluble sugars constituted 4.4–6.3% of the kernel weight in ten sunflower varieties.

The pectins (Bishop, 1955; Zitko and Bishop, 1965, 1966) and hemicelluloses (Baqai *et al.*, 1972) in sunflower heads have been isolated and characterized but the high proportions of simple sugars and other polysaccharides in heads were not identified.

The objectives of the present study were to isolate, identify, and quantitate the simple sugars and constituent sugars in polysaccharides of the head components—flour, protein concentrate, testa, hull, and head (after removal of seeds) of Commander sunflower. The ethanol-soluble mono- and oligosaccharides and acid hydrolysates of polysaccharides, in water, oxalate, and sodium hydroxide extracts, and of residue were fractionated and characterized by paper chromatography and gas–liquid chromatography (glc).

EXPERIMENTAL SECTION

Dried heads (after removal of seeds), hulls, defatted flour, protein concentrate (Sosulski *et al.*, 1973), and defatted testa from the sunflower cultivar, Commander, were ground to 65 mesh and extracted three times with boiling water according to the general scheme of Robinson (1963). The aqueous extracts were pooled and adjusted to 80% (v/v) ethanol and centrifuged before analyses for mono- and oligosaccharides in the supernatant and water-soluble polysaccharide in the precipitate. The sediments from the boiling water extractions were then extracted three times with oxalate solution (0.25% ammonium oxalate–0.25% oxalic acid) at 90°. After centrifugation, the soluble polysaccharides were precipitated by adjusting to 50% (v/v) ethanol and dried by solvent exchange with ethanol and ether. The sediments from the oxalate extractions were then extracted three times with 2% sodium hydroxide at room temperature and centrifuged and the polysaccharides precipitated from the supernatant by adjusting to 50% (v/v) ethanol, the pH of the mixture being maintained between 4 and 5 with acetic acid. These alkali-soluble polysaccharides were washed with acidified ethanol–water (1:1, v/v, 1 l. of the solution containing 4 ml of glacial acetic acid) before drying by the solvent exchange method. The water-, oxalate-, and alkali-soluble polysaccharides were hydrolyzed into their constituent sugars with 1 *N* HCl at 100° for 6 hr under vacuum in sealed tubes. The hydrolysates were neutralized to pH 6.5 with dilute NaOH solution and evaporated to dryness under vacuum at 40°. The dried material was redissolved in distilled water for further analyses by paper chromatography and glc. The residue from alkali extractions was washed with water, dried by solvent exchange, and stirred to solution in 70% H_2SO_4. An aliquot of this solution was quantitated for cellulose by the anthrone–H_2SO_4 method using methyl cellulose as standard (Vomhof *et al.*, 1966). Nitrogen in the water-, oxalate-, and alkali-soluble fractions and residue was determined by the micro-Kjeldahl procedure (AOAC, 1970).

The uronic acids are decarboxylated during acid hydrolysis of the polysaccharide fractions (Robinson, 1963). Therefore, authentic galacturonic acid samples were hydrolyzed and decarboxylated under the same conditions to construct a standard curve for the estimation of galacturonic acid in the hydrolysates of sunflower polysaccharides.

Preliminary identification of sugars in the ethanol extracts and hydrolysates of the polysaccharides was obtained by descending paper chromatography using ethyl acetate–pyridine–water (10:4:3) as the solvent system. The visualization of sugars on the paper chromatograms was achieved with $AgNO_3$ reagent according to the method of Trevelyan *et al.* (1950).

Final identification of sugars in hydrolyzed and nonhydrolyzed carbohydrate fractions was achieved by glc in which the relative retention times of the TMS derivatives of sunflower sugars were compared with those of the authentic sugars and by cochromatography with TMS derivatives of authentic sugars. Aliquots of the ethanol extracts and hydrolyzed fractions were combined with inositol as an internal standard before evaporation to dryness under nitrogen at 40°. To decrease the number of tautomeric forms the reducing sugars in the mixture were derivatized to their oxime forms by heating with 1 ml of hydroxylamine hydrochloride (25 mg/ml) in pyridine at 70–80° for 30 min. Pyridine was removed under a gentle stream of nitrogen and the last traces of water were removed by evaporation with benzene (Davison and Young, 1969). The reducing sugar oximes and nonreducing sugars in the mixture were silylated with 1 ml of TRI-SIL-Z (Pierce) by incubation at 65° for 1 hr. The TMS derivatives were chromatographed on a F&M Model 402 chromatograph

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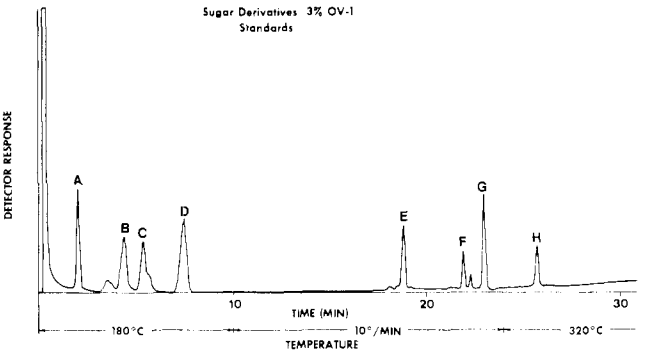

Figure 1. Chromatogram of oxime TMS derivatives of authentic reducing and TMS derivatives of authentic nonreducing sugars. Oven temperature program: isothermal at 180° for 10 min; programmed at 10°/min to 320°; isothermal at 320° for 10 min. The peak identities are: (A) arabinose; (I) galacturonic acid; (B) fructose; (C) galactose; (D) inositol; (E) sucrose; (F) maltose; (G) melibiose; (H) raffinose.

equipped with a hydrogen flame detector and peak area integrator. The 6-ft glass column, 0.25 in. o.d., was packed with 3% OV-1 on 80–100 mesh Chromosorb W (HP) conditioned with silyl-8 (Pierce) at 180°. The flow rate for the carrier gas, helium, was 30 cm^3/min and the sample injected was 1 μl. The injection port temperature was 340° and the oven was operated at a combination of isothermal-programmed-isothermal temperatures as shown in Figure 1. After identification, the TMS derivatives of sugar in sunflower flour, concentrate, testa, hull, and head (after removal of seeds) were quantitated from the integrated peak areas and weight ratios of TMS derivatives of authentic sugars and the internal standard, inositol (Mason and Slover, 1971). A typical chromatogram for eight reference sugars which were found in the sunflower samples is illustrated in Figure 1.

As a check on the efficiency of the glc column, the recovery of added raffinose was evaluated by analyzing the ethanol extracts from sunflower flour before and after the addition of graded levels of the known sugar (Table I). The calculated recoveries of raffinose ranged from 99 to 101%.

RESULTS AND DISCUSSION

The plants of Commander sunflower used in the present study yielded about 15% of seeds and 30% of heads (after removal of seeds) while the stalks constituted 55% of the plant material. The seeds contained 42% hulls and the meats contained 55% oil. Testa constitutes about 3% of the dehulled kernels and was sifted from the defatted flour on a 65-mesh screen to yield about 6% of the flour weight. Another 15% of the flour solids were removed during the aqueous continuous diffusion process in which the chlorogenic acid and other low molecular weight compounds were extracted from the dehulled kernels by stirring in hot water (60°) for 2 hr (Sosulski *et al.*, 1973). After defatting the diffused kernels, the resulting meal was a protein concentrate containing 70% protein.

Flour. The total amount of solids in the ethanol extracts was 10.6% of the flour weight (Table II) and the TMS derivatives of the mono- and oligosaccharides were resolved into ten peaks including the internal standard (D) as shown in Figure 2. The total monosaccharides constituted only 0.6% of the flour and were identified as arabinose, fructose, and glucose while the total oligosaccharides—sucrose (4.4%), maltose (0.9%), melibiose (2.0%), and raffinose (2.5%)—represented nearly 10% of the flour solids (Table II). The two unknown sugar components with long retention times between those of the triose, raffinose (25 min), and the tetrose, stachyose (33 min), were not quantitated. These results support the findings of Mikolajczak *et al.* (1970) that Armavirec sunflower meal contained 4% of sucrose, 2% of raffinose, and 1% of an unknown component (probably maltose). However, the reducing oligosaccharide, melibiose, constituted 2% of the flour solids in the present study while Mikolajczak *et al.* (1970) reported the same quantity of α,α'-trehalose, a nonreducing sugar.

Table I. Recoveries of Added Raffinose from Sunflower Flour

Raffinose ($5H_2O$) added, mg/g	Total raffinose, %	Yield of anhydrous raffinose, %
0.0	2.6	
2.5	4.7	99.1
5.0	6.8	99.1
10.0	11.2	101.2

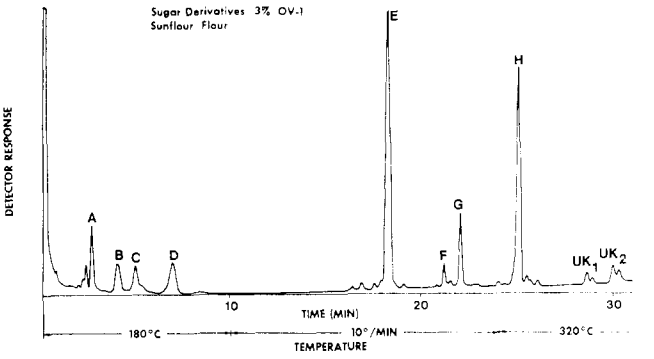

Figure 2. Chromatogram of oxime TMS derivatives of reducing and TMS derivatives of nonreducing sugars in ethanol extracts from sunflower flour. The peak identities are: (A) arabinose; (B) fructose; (C) galactose; (D) inositol; (E) sucrose; (F) maltose; (G) melibiose; (H) raffinose; UK_1; UK_2.

Polysaccharides constituted only a small portion of the water-soluble compounds in sunflower flour, with proteins being the major water-soluble constituent (Table II). The principal sugars in the water-soluble polysaccharides were arabinose, galactose, and glucose, with rhamnose and galacturonic acid occurring in trace quantities. Arabinans and arabinogalactans are major water-soluble polysaccharides in soybean and mustard (Aspinall and Cottrell, 1971) and in rapeseed (Siddiqui and Wood, 1972). The sugars in the soybean arabinogalactans occurred in a weight ratio of 1:4 while the rapeseed arabinogalactans consisted of arabinose, galactose, and glucuronic acid in the ratio of 1:1.2:0.2. In the present study, the principal polysaccharides in water-soluble fraction from sunflower flour appeared to be arabinogalactans in the weight ratio of 1:2.

There were almost no pectic materials in the small oxalate-soluble fraction of sunflower flour but over 63% of the dry flour solids were extracted with dilute alkali (Table II). Proteins were the major constituent of the alkali fraction and the extracted hemicelluloses accounted for only 10% of the flour weight. The two major sugars in the hydrolysate were arabinose and galactose in the weight ratio of 8:1, indicating the presence of arabinans and arabinogalactans. Traces of xylose, rhamnose, and galacturonic acid were found in the chromatograms. The alkali-soluble polysaccharides in mustard had xylose and arabinose as the major sugars with glucose, galacturonic acid, and mannose being the minor constituents (Hirst *et al.*, 1965). The presence of glucose in the water-soluble arabinogalactans might have arisen from the hydrolysate of cellulose-containing mucilages in sunflower flour or contamination with testa that was high in cellulose. Smith and Montgomery (1959) have demonstrated the presence of cellu-

Table II. Composition of Simple Sugars, Oligosaccharides, and Polysaccharides in Sunflower Flours, Concentrates, Testa, Hulls, and Heads (g/100 g of Head Component)

Head component and solvent	Dry solids in extract	Simple and constituent sugars							Total oligosaccharides	Hydrolyzed cellulose	Total carbohydrate	Crude protein
		Ara	Xyl	Rha	Fru	Galac. acid[a]	Gal	Glc				
Flour												
Ethanol	10.6	0.3			0.2			0.2	9.8		10.5	
Water	8.1	0.3		Tr		Tr	0.6	0.5			1.4	5.2
Oxalate	1.2	Tr				Tr	Tr					0.3
NaOH	63.4	9.2	Tr	Tr		Tr	1.1	Tr			10.3	49.0
Residue	15.6					Tr				6.3	6.3	4.3
Protein concentrate												
Ethanol	1.5							Tr	0.2		0.2	
Water	1.9					Tr		Tr				1.3
Oxalate	0.8	Tr				Tr						
NaOH	77.0	12.5	Tr	Tr		Tr	1.6				14.1	62.5
Residue	14.3									6.4	6.4	2.7
Testa												
Water	5.4	1.1		0.1		Tr	1.3	0.2			2.7	1.4
Oxalate	31.8	1.3		0.3		10.5	1.6	0.2			13.9	12.9
NaOH	10.8	1.1					0.4				1.5	3.3
Residue	51.1									24.1	24.1	2.1
Hull												
Ethanol	5.8		Tr			1.9	0.2	0.1			2.2	
Water	3.7					0.1					0.1	0.6
Oxalate	2.6		Tr	Tr		0.4	Tr				0.4	
NaOH	15.9	Tr	5.9	Tr		Tr	0.2	Tr			6.1	0.4
Residue	67.7		Tr			Tr				38.8	38.8	1.7
Head												
Ethanol	34.2	11.4					16.4		6.2		34.0	
Water	9.7	0.2	Tr			0.7	0.3	Tr			1.2	0.6
Oxalate	15.3	0.4	Tr			10.6	0.7	Tr			11.7	1.1
NaOH	2.8	Tr	0.7	Tr		Tr	Tr	Tr			0.7	0.1
Residue	34.3					Tr				23.2	23.2	2.2

[a] Abbreviations used are: Ara, arabinose; Xyl, xylose; Rha, rhamnose; Fru, fructose; Galac. acid, galacturonic acid; Gal, galactose; Glc, glucose.

lose-containing mucilages in the seed of flax and white mustard. Grant *et al.* (1969) claimed that the solubilization of cellulose-containing mucilages is possible by noncovalent encapsulation.

The flour residue consisted of 6% cellulose, 4% protein, and 5% of other constituents including lignins. The crude fiber of sunflower flour is reported to be 6% (Sosulski *et al.*, 1972).

Protein Concentrate. The protein concentrate was prepared by aqueous extraction of the kernels and most soluble sugars were removed during the diffusion process. Therefore, no simple sugars were isolated in the ethanol extract and the total dry solids of 1.5 g contained only 0.2% of the disaccharide, sucrose. The yields of water- and oxalate-soluble solids were also very low and the hydrolysates contained only traces of arabinose, galacturonic acid, and glucose. Most of the dry solids in the protein concentrate were extracted with dilute alkali and, like the flour, an arabinose to galactose ratio of 8:1 was obtained in the analysis of the constituent sugars. The cellulose content of the residue was similar to that of the flour.

Two important properties of pentosans in aqueous systems are high viscosity and water-binding capacity, which has resulted in their use as thickeners, adhesives, and stabilizers. The high content of pentosans in protein concentrate and flour suggests their utilization as thickening agents and in adhesive and stabilizer manufacture.

Testa. The dry solids in water- and oxalate-soluble fractions constituted 5 and 32% of testa, respectively (Table II). About half of the dry solids were polysaccharides while the remaining portions were mainly proteins, bound polyvalent metallic ions, and other minor constituents. The ethanol-soluble fraction was discarded because the solvent was used to separate the testa from the cut kernal and was contaminated with flour constituents. The sugars in the water-soluble polysaccharides were arabinose, galactose, and glucose in the weight ratio of 1:1.2:0.2 and traces of galacturonic acid and rhamnose were also detected. The proportion of arabinose to galactose in the water-soluble polysaccharides from testa is similar to that of the arabinogalactans from rapeseed (Siddiqui and Wood, 1972) but glucuronic acid was the third component in their study.

Galacturonic acid constituted 10.5% of the defatted testa with lower concentrations of arabinose, galactose, and glucose and traces of rhamnose being present (Table II). The oxalate-soluble pectins appear to be mainly a galacturonan, with branches of rhamnose, and arabinogalactans with a weight ratio of 1:1.2 for the constituent sugars. Because of their hydrocolloid properties these polysaccharides would contribute desirable functional properties such as fat and water absorption and fat emulsification if incorporated into the flour or protein concentrate. The insoluble testa residue consisted of 24% cellulose, 2% protein, and other indigestible constituents.

Hull. About 12% of the hull was soluble in ethanol, water, and oxalate including 3% carbohydrate and 0.6% protein (Table II). Galacturonic acid was the principal

constituent in these polysaccharides, indicating that pectins were present in each fraction.

The main polysaccharide in the alkali-soluble fraction was xylan which represented about 6% of the hull contents while cellulose constituted 39% of this fibrous plant part. Carbohydrates and proteins accounted for one-half of the hull weight and almost half of the hull material remained unaccounted for in the residue after cellulose determination.

Acidic polysaccharides also occur in the oxalate-soluble carbohydrates of soybean hulls (Aspinall *et al.*, 1967) while xylans were extracted with dilute alkali (Aspinall *et al.*, 1966). Pectins and xylans are also the principal carbohydrates in these two fractions from white mustard seed coats (Hirst *et al.*, 1965).

The hull constitutes 25% of the seed in oilseed varieties and 40% of the seed in the large-seeded confectionery type of sunflower. The contents of pentosans and cellulose in sunflower hulls are similar to those of rice hulls but are substantially lower than for oat hulls (Houston, 1972). Therefore, sunflower hulls would have more applications as feed ingredients, soil conditioners, litter and bedding, filter aids, and fuel than as a source of furfural and cellulose (Whistler, 1950).

Head (after Removal of Seeds). Over one-third of the head material was soluble in 80% ethanol and essentially all of the dry solids were simple sugars, arabinose and galactose, plus a significant amount of sucrose (Table II). An additional 25% of the head material was extracted with water and oxalate solution. The principal constituent sugar in these two fractions was galacturonic acid with lesser amounts of galactose and arabinose. Galacturonic acid alone accounted for over 11% of the head material. Zitko and Bishop (1965) and Riaz and Uddin (1972) have also reported that galacturonic acid constituted 66–80% of the water- and oxalate-soluble polysaccharides in sunflower heads. The acidic polysaccharide has been identified as pectin and contained galacturonic acid as the major sugar component with small amounts of galactose and arabinose (Zitko and Bishop, 1965) and xylose, as in the present study, plus rhamnose and 2-*O*-methylfucose (Riaz and Uddin, 1972). Yields of pectins have varied between 17 and 23% in these studies and, because of its hydrocolloid properties of high viscosity and gel formation, the polysaccharide is extracted commercially from sunflower heads in Eastern European countries for use in food products.

Only 3% of the head material was extracted with dilute alkali and one-fourth of this fraction appeared to be a xylan with similar constituent sugars to the xylan found in the sunflower hulls (Table II). Baqai *et al.* (1972) obtained a 9% yield of hemicellulose from three sunflower varieties but, unlike the present study, this xylan contained 12% of glucuronic acid which was not detected in the present study. Cellulose in the unextracted residue constituted 23% of the head material with the remainder being noncarbohydrates such as cutin, suberin, waxes, lignin, and mineral substances of the cell wall.

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