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Studies on Vegetables: Fiber Content and Chemical Composition of Ethanol-Insoluble and -Soluble Residues[1]

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The ethanol-insoluble residues from four vegetables were analyzed for moisture, nitrogen, starch, Klason lignin, ash, and uronic anhydride. The starch-free residues following enzymatic hydrolysis were recovered and analyzed for neutral sugars following acid hydrolyses and derivitization, as alditol acetates, by GLC. The sum of lignin, cellulose, and noncellulosic polysaccharides showed the dietary fiber contents of celery, parsnip, rutabaga, and squash to be 1.1, 4.7, 2.3, and 2.3%, respectively, on a fresh plant basis. The ethanol-soluble residues showed major amounts of glucose, myoinositol, and sucrose. The residue from celery in addition showed appreciable amounts of malic acid (butanedioic acid) and exorbitant amounts of mannitol. Fructose was not detected in any of the four residues.

Dietary fiber embraces largely those materials consisting of lignin/polysaccharide complexes (cellulose, hemicellulose, pectin) and associated substances resistant to digestion by the enzymes of the human gestrointestinal tract. The fiber originates mainly from the plant cell walls and is largely composed of carbohydrates. By definition it also includes some commercially available food additive types such as alginates, carrageenans, pectins, xanthan gum, xyloglucans (amyloids), exudate gums, dextrans, levans, and 1,3-β-D-glucans. Some of the cell wall polysaccharides often occur in association with proteins, including cell wall proteins (extensin), cuticular waxes, fats, and polyphenols, and carry esterified groups containing methanol, acetic acid, and phenolic acids. Phytate, oxalate, and inorganic constituents are sometimes also present (Theander and Aman, 1979).

Traditional gravimetric methods of analysis are not adequate. A critical review of available methods (Southgate et al., 1978) points to some of the difficulties inherent in the methodologies. The drawbacks associated with these methods have been adequately reported (Theander and Aman, 1979; Bittner et al., 1982; Selvendran and Dupont, 1984). Additional information has appeared in a book by James and Theander (1981) and a review by Asp and Johansson (1984). In recent years methods based on analysis of ethanol-insoluble residues with or without removal of starch by enzymatic digestion have been reported by the preceding authors and others (Englyst et al., 1982; Selvendran and Dupont, 1980; Selvendran, 1984). For more recent information on the analytical aspects, reference can be made to the Report of The Expert Advisory Committee on Dietary Fiber, Health and Welfare, Canada (1985) and a study of Theander and Westerlund (1986).

This paper describes an alternative approach to fiber analysis using ethanol-insoluble residues from four Canadian vegetables. A report on the ethanol-soluble lower molecular weight carbohydrates is also presented.

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MATERIALS AND METHODS

The method described in the following pages meets most of the requirements suggested by Southgate et al. (1978) for a procedure of dietary fiber analysis. The following outline summarizes the major steps:

Preparation and analysis of residues: preparation of 80% ethanol-insoluble residues in sufficiently large amounts to achieve proper sampling and to avoid possible variation in the values of percent dry matter; analysis of residues for moisture, protein, starch, Klason lignin, ash, and uronic anhydride.

Removal of starch: removal of starch by amyloglucosidase and analysis of starch-free residues for any residual starch by enzymatic hydrolysis using α-amylase and amyloglucosidase followed by determination of glucose by an automated glucose oxidase procedure.

Analysis of starch-free residue: hydrolysis under predetermined optimum conditions followed by addition of D-allose (internal standard) at the neutralization stage in both 1 M and 72% hydrolyses; determination of the relative percent composition (by weight) of neutral sugars (corrected for detector response factors) as alditol acetates.

Calculation of dietary fiber: calculation of total neutral polysaccharides in residues B (Table II) from 72% hydrolysis results, cellulose from the difference in the value of glucose between 72% and 1 M sulfuric acid hydrolyses, neutral noncellulosic polysaccharides by difference, and dietary fiber content by multiplying the sum of cellulose, noncellulose neutral polysaccharide, uronic anhydride, and Klason lignin with the weight of resides A (Table II) divided by 100.

Plant Material. Celery (*Apium graveolens*), parsnip (*Pastinica sativa*), rutabaga (*Brassica mapibrassica*), and squash (*Curcibita maxima*) were obtained fresh from a local distributor. Parsnips, rutabaga, and squash were peeled and diced prior to extraction while only the stalks of celery were used.

Chromatographic Methods. Descending paper chromatography was performed on Whatman No. 1 paper with ethyl acetate–pyridine–water (8:2:1). Paper electrophoresis was performed with Whatman No. 3MM paper with borate/calcium chloride buffer (pH 9.2) (Haug and Larsen, 1961) at 800 V for 4 h. Detection was effected with aniline hydrogen phthalate.

Ethanol-soluble residues were additionally analyzed by paper chromatography using butanol–pyridine–water (10:3:3) and with 0.2 M borate buffer (pH 10) and 0.2 M acetate buffer (pH 5) with

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detection using napthoresorcinol (Bryson and Mitchell, 1951) and alkaline silver nitrate spray reagents (Trevelyan et al., 1950).

Gas–liquid chromatography was performed with a Varian Vista 6000 gas chromatograph, with a flame ionization detector, DB-225 fused silica capillary column (0.25-μm film, 30 m × 0.32 mm (i.d.), cold-column injection). Initial temperature was 50 °C with a hold time of 2.5 min, programmed to 215 °C at a rate of 20 °C/min, and a helium flow rate of 1.5 mL/min with nitrogen as the makeup gas (30 mL/min). Injector temperature program (180 °C/min), 30–220 °C. Evaporations were carried out at 35 °C with a rotary evaporator.

Preparation of Ethanol-Insoluble Residues. Samples (800 g, wet weight) were extracted in a Waring blender for 20 min with 1.5 L of boiling 80% ethanol. The hot solutions were filtered on a Buchner funnel (medium porosity). Five more extractions of the residue in this manner yielded the 80% ethanol-insoluble solids, which were washed with acetone to give fluffy white materials that were air-dried on trays for 24 h, thoroughly blended, and stored in glass-stoppered bottles.

The percentage yields (moisture-free basis) of ethanol-insolubles, ethanol-solubles, and water contents were as follows: celery, 1.4, 2.9, 95.7; parsnip, 11.6, 9.5, 78.9; rutabaga, 2.8, 17.9, 79.3; squash, 4.5, 7.0, 87.5.

A total of 500 g of ethanol-insoluble residue from celery, 1200 g from parsnips, 600 g from rutabaga, and 1400 g from squash was prepared.

Analysis of Residues. Moisture contents were determined by drying in vacuo to constant weight at 56 °C. Ash and nitrogen contents were determined by the method of Steyermark (1961). Uronic acid analyses were carried out (Castagne and Siddiqui, 1975; Siddiqui and Morris, 1979) by decarboxylation of samples (30 mg) with 57% HI, 145 °C for 1 h. Starch content was determined with α-amylase (Type 1-A hog pancreas; Sigma Chemical Co., St. Louis, MO) and amyloglucosidase (*Aspergillus orysae*, Sigma) (Batey, 1982) and an automated glucose oxidase (glucose oxidase, *Aspergillus niger*; Boehringer, Mannheim, Quebec, Canada) method, based on the method of Lloyd and Whelan (1969), using the Technicon autoanalyzer II system (Method No. 42B-76A). Klason lignin was determined gravimetrically (Theander and Aman, 1979).

Removal of Starch. Enzymic hydrolyses of parsnips, rutabaga, and squash were performed; celery being very low in starch was not subjected to hydrolysis. The same hydrolysis procedure was applied to each commodity. Sample (30 g) was stirred with 0.5% sodium hydroxide (1300 mL) for 1 h. The suspension was adjusted to pH 4.5 (acetic acid), 400 mg of amyloglucosidase (*A. orysae*, Sigma) was added, and the surface was layered with toluene. After the suspension had been stirred for 48 h, at room temperature, it was dialyzed (Spectrapore membrane tubing, diameter 32 mm, molecular weight cutoff 12 000–14 000) for 48 h against running tap water and 4 h against distilled water. The sample was concentrated to a slurry (2 L), added to stirred ethanol (4 volumes), and allowed to settle. The clear supernantant was removed by syphoning and filtration, and the insoluble residue was washed with ethanol and acetone and air-dried for 24 h to yield a dry fluffy residue.

The percent recoveries of residues from parsnips, rutabaga, and squash were 49.7, 92.2, and 53.6%, corresponding by difference to starch contents of 50.3, 7.8, and 46.8%, respectively.

Analysis of Neutral Sugars. Hydrolysis of the residues (5 mg) was performed with (a) 1 M sulfuric acid (0.5 mL) for 3 h at 100 °C and (b) 72% sulfuric acid (0.2 mL) for 1 h at 5 °C and then dilution to 1 M acid for 2 h at 100 °C. The hydrolysates were neutralized with barium carbonate and filtered, and the solutions of the hydrolyzed sugars were decationized with Rexyn-101 (H^+) resin, filtered, and fractionated on columns (60 × 5 mm) of Dowex 1X-2 (CO_3^{2-}). Elution with water (5 mL) removed the neutral sugars, and elution with 0.5 M ammonium carbonate yielded the uronic acid material.

The neutral fractions were reduced with sodium borohydride. Excess borohydride was destroyed with a drop of acetic acid, and glycitol mixtures were evaporated with 5 × 3 mL of methanol on a rotary evaporator at 46 °C to remove boric acid. The glycitols were acetylated with acetic anhydride–pyridine (Swardeker et al., 1965), and the acetates were analyzed by GLC (general methods). Correction factors (to account for losses on neutralization, ion-exchange resin, derivitization, and also GLC detector response) for the sugars were applied (Slonekar, 1972) with D-allose as an internal standard and a Vista 402 data system.

The starch-free residue hydrolysates were also analyzed by paper chromatography and electrophoresis (general methods).

Table I. Results of Optimum Hydrolysis of Aqueous 80% Ethanol-Insoluble Residue of Celery

hydrolysis conditions	alditol acetate					
	Rha	Ara	xyl	Man	Gal	Glc
1 h, 72% H_2SO_4, room temp; 3 h, 1 M H_2SO_4, 100 °C	0.4	0.8	0.6	0.6	1.0	7.8
1 h, 72% H_2SO_4, 5 °C; 2 h, 1 M H_2SO_4, 100 °C	0.3	1.2	1.2	0.6	1.0	6.2
3 h, 72% H_2SO_4, 5 °C; 3 h 1 M H_2SO_4, 100 °C	0.3	0.9	0.8	0.5	1.0	6.4

Table II. Analysis of Hot 80% Ethanol-Insoluble Residues

sample	celery	parsnip	rutabaga	squash
yield of air-dried residue, %	1.6	12.7	3.2	5.7
moisture, %	11.6	9.0	11.5	4.8
Analytical Data (Dry Matter Basis) of Residue, %				
protein (N × 6.25)	9.8	9.9	7.1	4.5
starch	0.3	49.2	7.3	45.9
lignin	1.7	2.1	0.9	1.9
ash	8.8	2.8	4.5	2.0
uronic anhydride	25.5	18.2	25.0	12.4
Calculated Yield of Residues, %				
A: moisture, starch-free basis	1.4	5.6	2.7	2.5
B: moisture, protein starch, lignin ash, uronic anhydride free basis	0.9	4.2	1.7	2.3
C: moisture, protein starch, ash-free basis	1.2	4.9	2.3	2.4

RESULTS AND DISCUSSION

The four vegetable samples after being freed from sugars and other low molecular weight carbohydrates by extraction with boiling 80% ethanol were recovered as fluffy white residues. The conditions most suitable for hydrolysis were determined by treating the residue from celery as a standard, first with 72% sulfuric acid and then following dilution with 1 M sulfuric acid at 100 °C. The results (Table I) indicated the most appropriate conditions were 72% for 1 h at 5 °C and then 1 M sulfuric acid for 2 h at 100 °C. The conditions were milder than 12 M sulfuric acid, 0.5 h, room temperature, and then 1 M sulfuric acid, 1 and 3 h, 100 °C of Bittner et al. (1982) and 12 M sulfuric acid, 2 h, room temperature, and then 0.358 M sulfuric acid, 6 h, 100 °C of Theander and Aman (1979). In the context of hydrolysis it is imperative that the optimum conditions of hydrolysis be determined on the basis of the nature of the product to be hydrolyzed. It is obvious (Table I) that the hydrolysis conditions employed for cereal brans etc. may be too severe to be applied to vegetable fibers.

Analysis of uronic acids in fiber samples was carried out with use of hydroiodic acid as the decarboxylation agent. Unlike colorimetric methods, this procedure is not influenced by the nature of the uronic acid and its molecular environment. In this laboratory the technique has been successfully applied to the analysis of a number of acidic polysaccharides of plant and animal origin as well as those elaborated by microorganisms (Castagne and Siddiqui, 1975; Siddiqui and Morris, 1979; Hidirogluo et al., 1979a,b).

After a preliminary analysis of the 80% ethanol-insoluble residues (Table II), the next step in the procedure was complete removal of starch and for this purpose amyloglucosidase (*A. orysae*, Sigma) proved to be very effective. Large-scale digestion of residues from parsnip, rutabaga,

Table III. Relative Composition of Neutral Sugars[a] after Hydrolysis Expressed as Anhydro Sugars

	celery		parsnip		rutabaga		squash	
sugar	M	72%	M	72%	M	72%	M	72%
rhamnose	8.2	5.0	6.6	4.4	9.7	4.1	7.1	2.9
arabinose	26.3	10.7	37.7	26.6	30.0	11.4	6.0	2.2
xylose	17.3	8.9	6.6	5.6	14.8	7.1	13.6	6.0
mannose	3.9	6.0	4.5	5.4	4.7	5.5	13.0	7.7
galactose	35.3	13.1	36.9	21.0	32.5	12.8	48.5	16.4
glucose	9.0	56.4	7.8	37.1	8.3	59.1	11.4	64.9

[a] Negligible traces of ribose and fucose were also detected.

Table IV. Lignin, Cellulose, Noncellulosic Polysaccharide, and Dietary Fiber Content Expressed as Percent of 80% Ethanol-Insoluble Moisture and Starch-free Residues

			noncellulosic polysaccharides		total × starch-free residues	
	lignin	cellulose	neutral	acidic	total	dietary fiber
celery	1.7	25.9	28.5	25.5	81.6	1.1
parsnip	2.4	18.5	44.6	18.2	83.7	4.7
rutabaga	0.9	29.8	28.8	25.0	84.3	2.3
squash	1.9	44.8	31.8	12.4	90.9	2.3

and squash (celery residue being very low in starch was not digested) was carried out. The resultant loss for starch parsnip (50.3%), rutabaga (7.8%), and squash (46.8%) corresponded very closely to the values obtained by the automated method (Wood, personal communication). The digested or starch-free samples analyzed by the same procedure showed a very low or negligible starch content (~0.5%) for each sample. The enzyme showed no β-glucanase activity and produced 98.5% recovery of glucose from corn starch.

The residue from celery and the three enzyme-digested starch-free residues were hydrolyzed with 1 M sulfuric acid and 72% sulfuric acid (see the Experimental Section, Analysis of Neutral Sugars), and composition of neutral sugars was determined by GLC after conversion to glycitol acetates (Table III).

The sugar D-allose was used as an internal standard at the neutralization stage in all the hydrolyses. Corrections for losses and response factors for all the six sugars were also applied.

The procedure of calculation as applied to celery was as follows: The 80% ethanol-insoluble residue after subtraction of moisture, ash, protein, lignin, starch, and uronic anhydride gave a remainder (0.9; Table II) that was multiplied by the difference in the value of glucose between the 72% and 1 M hydrolyses (cellulose will not be hydrolyzed under the latter conditions). This was divided by the total moles of sugar in the 72% sulfuric acid hydrolysate (Table III), yielding a factor that was multiplied by 100 and divided by the moisture-free, starch-free residue (1.4; Table II) to give the cellulosic content. The total cellulosic and noncellulosic sugars calculated similarly in the 72% sulfuric acid hydrolysates after subtraction of cellulose gave the noncellulosic neutral polysaccharides. The sum of cellulose, noncellulosic polysaccharides, uronic anhydride, and lignin added up to 81.6% of 1.4, corresponding to a dietary fiber content of 1.1%. The data are summarized in Table IV.

The sugar profiles (Table III) indicate that only a small quantity of glucose in the samples originates from noncellulosic nonstarchy polysaccharides and arabinose, xylose, and galactose account for the majority of neutral sugars in the noncellulosic polymers. The amount of uronic acid (galacturonic acid by paper electrophoresis) coupled with the presence of significant amounts of rhamnose is indicative of the presence of polysaccharides of the pectic type. Arabinose, galactose, and xylose, which besides the pectic polysaccharide could occur alone or in combination in various other categories of polysaccharides, represent a complex picture requiring detailed fractionation studies to elaborate the nature of their structural associations. The same arguments apply to the presence of glucose and mannose.

Table V. Percent Dietary Fiber of Some Vegetables

sample	Theander and Aman	Selvendran	Bitner et al.	Siddiqui
broccoli			2.4	
brussels sprouts			3.0	
cabbage[a]	1.2	2.2	1.4	
carrot	2.6			
cauliflower			1.6	
celery			1.0[b]	1.1
corn			2.6	
cucumber			0.7	
green beans			2.1	
lettuce	1.5		1.1	
peas	4.4		4.0	
parsnip				4.7
potatoes	2.0[c]	1.8	1.7	
runner beans		4.0		
rutabaga				2.3
squash				2.3

[a] White. [b] Lignin excluded. [c] With skin.

The amount of dietary fiber for the four vegetables (Table IV) ranged between 1.1 and 4.7%, cellulosic content varied in the range 18.5–44.8%, noncellulosic neutral polysaccharide content was between 28.5 and 44.6%, uronic anhydride content was between 12.4 and 25.5%, and the lignin content was between 0.9 and 2.4%. The fiber value for celery of 1.1% compared to 1.0% (lignin excluded) reported by Bittner et al. (1982) is in excellent agreement with our value. However, our cellulosic and noncellulosic polysaccharide and uronic anhydride contents and total recovery figure for celery (Table IV) are considerably higher than their values. A considerable variation in the values of percent dry matter for celery (5.4) reported by Bittner et al. (1982) and our value (1.4) was also noted. Such variation could have resulted, to some extent, from differences in variety and maturity of the crop. The fiber values for a variety of vegetables determined by other workers using similar methods of analyses are listed in Table V.

An interesting finding that emerged from the present investigation is that the total fiber values (Table IV) are very close, for all four commodities, with the calculated values (Table II) obtained following subtraction of moisture, ash, starch, and protein from the original ethanol-insoluble residues. It means that the total dietary fiber in a fruit or vegetable residue may be evaluated quite precisely by determining its moisture, starch, protein, and ash contents and subtracting the sum of these from the ethanol-insoluble residue. Other associated substances such as fat, etc., not removed by ethanol extraction, if

Table VI. Identification of Lower Molecular Weight Components

constituent	rel retention time, min	commodity			
		celery	parsnip	rutabaga	squash
malic acid	0.21	5.5			
α-D-glucose	0.53	11.6	31.9	32.5	21.6
unknown	0.57	2.0	4.0	3.5	3.1
β-D-glucose	0.61	13.7	25.5	25.0	37.9
D-mannitol	0.66	46.3			
myoinositol	0.72	13.3	32.8	31.8	25.2
unknown	0.80	0.3	0.8	0.8	1.9
methyl arachidate	1.00				
sucrose	1.35	7.4	5.1	6.4	10.1

present is appreciable amounts, could also be similarly subtracted, giving values for fiber as acceptable as those obtained by the detailed analysis of alditol acetates. The simple subtraction procedure could be applied routinely to the analysis of a variety of fruits and vegetables before a detailed analysis of alditol acetates is carried out.

The 80% ethanol-soluble fractions from celery, parsnip, rutabaga, and squash yielded residues 2.9, 9.5, 17.9, and 7.0%, respectively. A portion from each residue was trimethylsilylated (Siddiqui and Rosa, 1983) with methyl arachidate as an internal standard and examined by GLC. The average molar percentage composition of constituents, uncorrected for detector response, is shown in Table VI.

These components were identified by their paper chromatographic and electrophoretic behavior, GLC retention times, and comparison of GLC–CI-MS patterns (Horton et al., 1974) with authentic trimethylsilylated samples. D-Mannitol was identified further, following crystallization from the mixture, by its melting point (166–168 °C) and by its $[\alpha]_D$ +23° (*c* 1, 1.3% borax solution) and following acetylation by GLC of mannitol hexaacetate.

None of the soluble residues showed fructose to be a sugar commonly present in fruits. Malic acid and exorbitant amounts of D-mannitol were present only in the residue from celery. The former compound acts as a catalyst in the interaction of sugars with amino acids, yielding *N*-glycosyl amino acids that then rearrange to form 1-amino-1-deoxy-D-fructose derivatives. Such Amadori compounds play a role in the browning and flavor of food. The presence of malic acid suggests that such compounds may also be present in celery, but their identification and characterization was beyond the scope of the present investigation. D-Mannitol is widespread in plants including seaweeds; being nonmetabolizable and sweet, it has been used in the diabetic food industry. Its presence in celery would have the effect of enhancing its dietary fiber characteristics on a fresh plant basis.

Celery also contained the lowest amount of glucose and myoinositol. Myoinositol occurs widely in nature in both free and bound forms (phytic acid), and its role in the biosynthetic pathways, as being a precursor of glucuronic acid and xylose, is now well-known.

Since no new lower molecular weight components were detected in this preliminary study, the work was not pursued any further.

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Registry No. Ethanol, 64-17-5; lignin, 9005-53-2; cellulose, 9004-34-6; starch, 9005-25-8; sucrose, 57-50-1; *myo*-inositol, 87-89-8; β-D-glucose, 492-61-5; α-D-glucose, 492-62-6; malic acid, 6915-15-7; D-mannitol, 69-65-8.

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