Studies on Dietary Fiber. 3. Improved Procedures for Analysis of Dietary Fiber

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Three related methods, A–C, are described for analysis of dietary fiber (DF). Homogenized and/or milled foodstuff is extracted with 80% ethanol and light petroleum ether. The extracted residue is analyzed directly for the DF and starch content (method B) or treated further with thermostable α-amylase at 96 °C and amyloglucosidase at 60 °C. Insoluble and soluble DF components were then separated by centrifugation and, respectively, dialysis and freeze-drying of the supernatant (method A). Alternatively, insoluble and soluble DF components were isolated together by precipitation of soluble fiber in 80% ethanol and subsequent centrifugation (method C). Neutral polysaccharide constituents were analyzed as alditol acetates, uronic acids by a decarboxylation method, and Klason lignin by gravimetric techniques. Starch was determined enzymatically. A satisfactory correlation between the different DF methods was observed for several food samples.

INTRODUCTION

The concept of dietary fiber (DF) refers in general to the plant polysaccharides and lignin that are not digested in the upper gastrointestinal tract of man (Trowell et al., 1976). The physiological importance of DF in human nutrition (Spiller and Kay, 1980) has resulted in an extensive development of various analytical methods. Several common DF methods, based on gravimetric, colorimetric or gas–liquid chromatographic (GLC) procedures for determination of the sugar constituents of the DF polysaccharides, were thoroughly discussed in the book *The Analysis of Dietary Fiber in Food* (James and Theander, 1981) and also in a recent review (Asp and Johansson, 1984). In order to obtain a detailed characterization of the neutral polysaccharide constituents in DF, utilization of somewhat comprehensive GLC methods (Theander and Åman, 1979, 1981, 1982; Englyst, 1981; Englyst et al., 1982; Englyst and Cummings, 1984) are necessary. High-performance liquid chromatography has occasionally been used for the same purpose (Laine et al., 1981; Slavin and Marlett, 1983). Disparities in the cited GLC methods are essentially a consequence of the varying approaches used for fractionation of DF, including enzymatic removal of starch and acid hydrolysis of nonstarch polysaccharides. Also, the Englyst method does not include a gravimetric lignin determination and uronic acid is determined colorimetrically, whereas we employ a decarboxylation method. Methods have also been designed (Selvendran and Du Pont, 1980a,b; Selvendran et al., 1981) for preparation of cell wall material for GLC analysis, but a general procedure fitted for analysis of different foods was not proposed.

The present paper deals with further development of our common methodology (Theander and Åman, 1979, 1981, 1982) for fractionation and GLC analysis of DF. The resulting methods, A (for water-insoluble and -soluble DF, respectively) and the faster methods B and C for total DF (Theander, 1983; Theander and Westerlund, 1986), are now described in detail, and the results on application to several food samples are reported.

MATERIALS AND METHODS

Samples and Sample Preparation. Whole wheat flour, low-extraction wheat flour, and peeled raw and french-fried potatoes were provided in a collaborative study (Varo et al., 1983). Samples of oat, potato, rice, soya isolate, wheat bran (AACC-certified food grade), high-extraction wheat flour, and nonvegetarian and vegetarian mixed diets were all from an interlaboratory AOAC study (Prosky et al., 1984). Samples of soya flour and lactovegetarian diet were from previous investigations at this laboratory (Theander and Åman, 1981; 1982). Wheat bran of the cultivar Drabant was prepared at the Department of Plant Husbandry of this University and milled in a Cyclotec mill (Tecator AB, Höganäs, Sweden) to pass a 0.5-mm screen. Carrots of different batches (1–3, marked in tables) were bought in a local supermarket. Wheat starch of the cultivar Folke was a gift from Stärkelsen (Kristianstad, Sweden), and the white wheat flour was of commercial quality (Nord Mills, Sweden). Sugar beet fiber was obtained from the Sugar Co. (Malmö, Sweden).

Enzymes. Amyloglucosidase (EC 3.2.1.3) isolated from *Aspergillus niger* (activity 140 EU/mL) was obtained from Boehringer, Mannheim, West Germany, or alternatively in the AOAC study a similar preparation (activity 1000 EU/mL) was obtained from BDH, Poole, England. The bacterial α-amylases Termamyl 60L and 120L were gifts from Novo A/S, Copenhagen, Denmark. The glucose oxidase reagent was manufactured by Kabi AB, Stockholm, Sweden, which also supplied a manual for the glucose determination.

General Methods. All results are expressed on a dry-weight basis, determined by separate oven drying at 105 °C for 18 h. Solutions were concentrated under reduced pressure at a water bath temperature not exceeding 40 °C or by freeze-drying. Dialyses were performed in regenerated cellulose tubes (Union Carbide) having molecular weight cutoff at 12 000–14 000 according to the manufacturer. This is also our experience from dialysis of a maltodextrin mixture. Centrifugations were performed at 2500*g*.

Gas–liquid chromatography (GLC) was conducted on a Packard 427 instrument fitted with a flame-ionization detector and a glass capillary column. Neutral polysaccharide constituents were analyzed as alditol acetates (Sawardeker et al., 1965) at 210 °C on an OV-225 column (15 m × 0.22 mm i.d., helium gas flow ~65 cm/s) and low molecular weight carbohydrates (if desirable) chromatographed as trimethylsilyl derivatives (Sweeley et al., 1963) on a Cp-Sil 5 column (25 m × 0.25 mm i.d.; helium gas flow ~35 cm/s) at 160–280 °C (6 °C/min) holding the final temperature for 5 min. A Hewlett-Packard integrator

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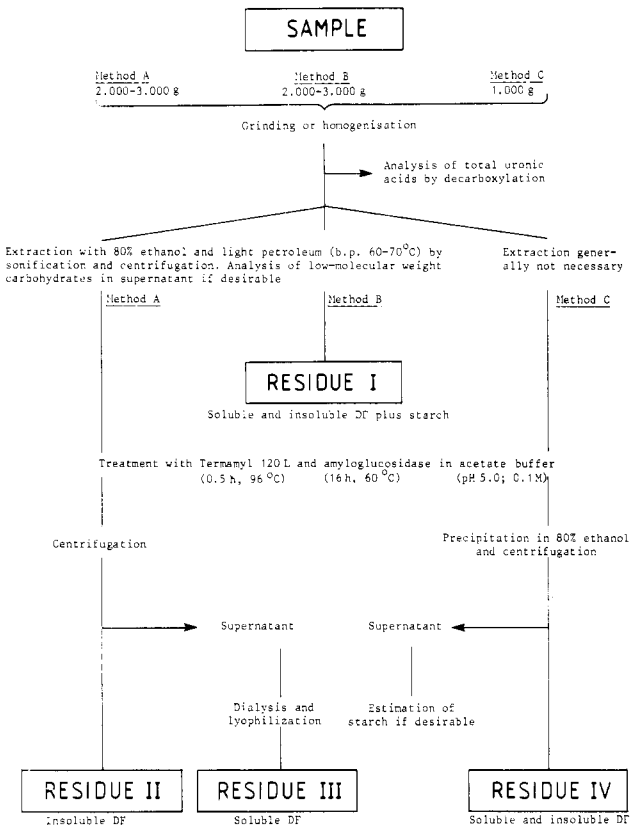

Analysis of Residues: Determination of neutral polysaccharide constituents as alditol acetates by GLC, uronic acid constituents (in residues II and III) by decarboxylation, starch (only in residue I) by enzymatic methods, and Klason lignin by gravimetric procedures.

Calculation of DF: Sum of neutral and uronic acid polysaccharide constituents calculated as polymers (less starch in method B), plus Klason lignin.

Figure 1. Analytical scheme for methods A–C for dietary fiber (DF).

3390A was used for quantitative evaluation of gas chromatograms.

Preparation of Dietary Fiber Fractions. A fractionation scheme is shown in Figure 1. In general, the samples (1.000–3.000 g) were extracted in screw-capped glass tubes (100 mL; Pyrex No. 0016) at ambient temperature in an ultrasonic bath (cf.: Salomonsson et al., 1984) using (a) 80 % aqueous ethanol (3 × 75 mL; 15 min) and (b) hexane (2 × 50 mL; 10 min). Each extraction was followed by centrifugation for 10 min and decantation of the supernatant. We are now using light petroleum ether (bp 60–70 °C), which is cheaper than hexane and also removes lipids to the same extent. The fat extraction may be omitted for foods containing less than 6 % fat (Asp et al., 1983). Samples with a high water content such as carrot were preferably disintegrated in a Sorwall homogenizator with 80% ethanol (75 mL, adjusted for the water content of the sample) for 5 min, and the resulting slurry was transferred quantitatively to a centrifuge tube and extracted further as described above. After the final decantation the insoluble residues were dried under a stream of warm air from a heat gun, air-conditioned, and weighed, and portions were analyzed directly for the DF constituents (method B) or treated further as follows.

In method A, a ground portion (1.500–2.000 g) of the extracted and dried residue was suspended in acetate buffer (75 mL, 0.1 M, pH 5.0) with α-amylase (100 L, Termamyl 120L). The suspension was kept in a screw-capped glass tube in a boiling water bath, and the tube was shaken three times during a period of 30 min. After cooling to 60 °C, the content in the tube was incubated with a suspension of amyloglucosidase (500 μL) and kept capped at 60 °C overnight in a water bath with a shaking device. The tube was then centrifuged for 15 min and the supernatant decanted and filtered into an evaporation flask. The insoluble residue in the tube was washed successively by suspension (ultrasonication, 5 min) and centrifugation in water (2 × 50 mL), ethanol (2 × 50 mL), and acetone (50 mL), and the supernatants were collected as above. The remaining insoluble residue (containing the water-insoluble DF fraction) was dried with warm air and weighed. The corresponding water-soluble DF fraction was obtained after concentration of the combined supernatants to approximately 100 mL and dialysis against running tap water (1 × 24 h) and distilled water (1 × 24 h) followed by freeze-drying.

In method C, particularly suitable for starch-rich samples, the ground sample (1.000 g) was treated with Termamyl and amyloglucosidase in acetate buffer (20.0 mL) as described for method A, but without previous extractions. In the case of samples with a high water content as for example vegetables, however, one homogenization with 80% ethanol (75 mL) followed by quantitative transfer to centrifuge tubes by washing, removal of the solvent, and drying as usual was performed before the enzyme treatment overnight. The water-soluble polymers were then precipitated by addition of absolute ethanol (80 mL) with stirring, and the tube was cooled by tap water and kept at +4 °C for 0.5 h. After centrifugation and decantation of the supernantant, the insoluble residue was thoroughly washed by suspension and centrifugation with 80% aqueous ethanol (2 × 80 mL) and acetone (50 mL) following the procedure described in method A. The final washed residue containing the water-soluble and water-insoluble DF polymers was dried with warm air.

Analysis of Neutral Polysaccharide Constituents and Klason Lignin Content. The water-insoluble fractions were subjected to acid hydrolysis essentially according to Sloneker (1971), although on a larger scale. A ground sample (150.0–250.0 mg) was weighed into a glass tube (100 mL), and 3.0 mL of 72% (12 M) sulfuric acid was added. The sample was dispersed for a few minutes with a round-melted glass rod. The tube with rod was capped and then shaken automatically in a water bath for 1 h at 30 °C with redispersion of the sample after 30 min. Next, distilled water (79.0 mL) and an internal standard solution of myoinositol (5.00 mL; 3.0 mg/ml) was added, and after the mouth of the tube had been covered with a beaker, the device was put in a preheated autoclave for 1 h at 125 °C.

While the resulting hydrolysis mixture still was warm, the insoluble material was quantitatively filtered off (Pyrex No. 2 glass filter) and thoroughly washed with hot water (3 × 5 mL). The glass filter was dried (105 °C, 18 h) and the filtered material, Klason lignin, determined gravimetrically (Bethge et al., 1971). The main part of the samples was derivatized as follows: Duplicate portions (10 mL) of the cooled filtrate were neutralized ($BaCO_3$) and reduced overnight (KBH_4), cations were removed with Dowex 50 H^+ and boric acid by evaporations with methanol (3 × 5 mL), and the residues obtained were acetylated with acetic anhydride/pyridine (Bethge et al., 1971). The resulting alditol acetates were quantified by GLC analysis (Sawardeker et al., 1965; Sloneker, 1971; Theander and Åman, 1979). A reference sample with a known weight and composition of pure monosaccharides was subjected to the hydrolysis procedure together with each sample batch.

The correction factors used for calculation of individual monosaccharides in food samples (taking into account hydrolysis/derivatization losses and also GLC response factors) were the mean values found by GLC analysis of 10 reference samples using myoinositol as an internal standard (Theander and Westerlund, 1986).

Portions (5.0–10.0 mg) of the water-soluble DF-containing fractions were hydrolyzed (cf.: Albersheim et al., 1967) with 2 M trifluoroacetic acid (2 mL, 96 °C, 16 h or 125 °C, 1 h) in screw-capped vials containing aqueous myoinositol (2.00 mL = 2.0 mg) as an internal standard. The hydrolysate was filtered and divided in two portions that were concentrated to dryness, first without and then with distilled water (2 × 5 mL) to remove traces of trifluoroacetic acid. The neutral sugars in the duplicate residues obtained were then quantified by GLC after the usual preparation of alditol acetates.

At present we are using the 1-methylimidazole catalyst method of Blakeney et al. (1983), essentially as modified by Englyst and Cummings (1984), for a simple and fast preparation of alditol acetates as follows. A part of the hydrolysate (1.0 mL) from the water-insoluble or -soluble DF fractions is made alkaline in a test tube with 12 M ammonium hydroxide (100 respectively 200 μL) and then reduced at 40 °C for 1 h in a thermostated water bath with 3 M aqueous ammonium hydroxide (100 μL) containing potassium borohydride (15 mg). After addition of glacial acetic acid (100 μL), parts (0.5 mL) of the resulting solution were mixed with 1-methylimidazole (0.5 mL) and acetic anhydride (5.0 mL) in screw-capped tubes and allowed to react for 10 min. Absolute ethanol (1.0 mL) was added next, and after 10 min the tubes were placed in a water bath at room temperature. Water (5.0 mL) was added followed by duplicate portions (5.0 mL) of 7.5 M potassium hydroxide with a few minutes interval. The tubes were vibromixed and left for 10 min, and the upper layer formed was transferred to a vial containing a small amount of anhydrous sodium sulfate and stored at +5 °C until GLC analysis. As we have not yet seen any discrepancies with the results from the previous derivatization method, we prefer and recommend the new method.

Analysis of Starch. This was performed in methods A and B by the procedure of Salomonsson et al. (1984). In brief, duplicate portions (40.0–100.0 mg) of the extracted and ground fractions were treated in 25.0 mL of acetate buffer (0.1 M, pH 5.0, 96 °C) and Termamyl 120L (50 μL) for 30 min in well-tightened screw-capped tubes (35 mL; Pyrex No. 9826). After incubation with amyloglucosidase, which for practical reasons is carried out overnight at 60 °C although complete hydrolysis of starch probably is achieved after 6 h (Åman and Hesselman, 1984), part of the resulting solution was diluted and analyzed for starch as glucose by the glucose oxidase method.

In method C, an estimate of starch can be performed as follows (see Figure 1) provided that low molecular weight sugars previously have been removed with 80% ethanol. The supernatants obtained after ethanol addition, centrifugations, and washings of the enzymatically hydrolyzed DF fraction were thus combined, the ethanol was evaporated, and the remaining solution was diluted to 250 mL in a volumetric flask. Duplicate portions (2.00 mL) were taken out and further diluted to 100.0 or 250.0 mL depending on the expected content of starch in the original sample. Aliquots (1.0 mL) of the resulting solution were then analyzed by the glucose oxidase method.

Analysis of Acidic DF Polysaccharides. The content of uronic acids (acid nonstarch polysaccharide constituents) was determined by a decarboxylation method (Theander and Åman, 1979) without previous extractions of the samples, except for carrot which was extracted by homogenization, to provide a representative sample.

Table I. Effect of Extraction with 80% Ethanol on Analysis of Starch[a]

matl anal.	extrctn method[b]	starch content	SD (n = 5)
wheat starch	A	95.9	1.1
	B	94.4	0.8
white wheat flour	A	78.2	0.6
	B	77.5	0.5
wheat bran	A	20.8	0.2
	B	20.6	0.4

[a] Starch is analyzed according to the procedure described in the Experimental Section, and all results are expressed as percentages of the original unextracted material (moisture-free basis). [b] A represents ultrasonification (3 × 15 min) at room temperature and B reflux for 45 min.

Table II. Distribution of Low Molecular Weight Sugars in 80% Ethanol Extracts of Carrot[a]

extrctn no.	low mol wt sugars			
	fructose	glucose	sucrose	total
1	10.4	12.3	19.5	42.2
2	3.0	3.4	5.1	11.5
3	0.8	1.1	1.7	3.6
4	0.3	0.3	0.5	1.1

[a] The results are expressed as percentages of the original sample (dry matter) and calculated from GLC analysis of silylated extracts.

Calculation of Total Dietary Fiber. In methods A and C, the total DF corresponds to the sum of neutral sugars and uronic acid constituents (all calculated as polysaccharides) and Klason lignin in the DF fractions. The contents determined of pentose/deoxyhexose and hexoses/uronic acids were converted to polysaccharides by multiplication with the factors 0.88 and 0.90, respectively. In the fastest method, B, the total DF is calculated as above except that the starch value (enzymatically determined) must be substracted from the DF glucan value obtained by GLC analysis.

RESULTS AND DISCUSSION

Extractions. In the original methods, A and B (Theander and Åman, 1979, 1982), the samples were extracted by reflux with 80% ethanol and then with chloroform in order to remove free sugars and lipids. The introduction of ultrasonic treatment in the present methods, A–C, has the advantage that the sample is kept in the same centrifuge tube throughout the extraction procedure. Also, the lower temperature used for ethanol extraction seemed to facilitate the enzymatic removal or analysis of starch in extracted samples. Using an enzymatic method developed in this laboratory (Salomonsson et al., 1984), we found that for samples of wheat starch and white wheat flour the analyzed starch content was slightly decreased by alcohol treatment and that the decrease was smaller with ultrasonic than with reflux extraction (Table I). This is in agreement with findings by Selvendran et al. (1981) that part of the starch (and also protein) may become unavailable to enzymes after alcohol treatment.

The extraction efficiency of low molecular weight carbohydrates by ultrasonic 80% ethanol treatment was investigated using a carrot sample, representing a very sugar-rich food. Three successive extractions were sufficient for removing most (98%) of the free sugars (Table II). If desirable, it is thus very convenient to combine the DF analysis with analysis of low molecular weight carbo-

Table III. Content of Neutral Polysaccharide Constituents (NPC) and Low Molecular Weight Sugars (LMWS) in Preextracted and Unextracted Residues Using Method C[a]

components	carrot 1		wheat flour	
	preextr	unextr	preextr	unextr
		NPC		
rhamnose	0.7	0.6	trace	trace
arabinose	1.5	1.5	0.7	0.7
xylose	0.4	0.4	1.0	1.1
mannose	0.7	0.7	0.2	0.2
galactose	1.8	1.7	0.2	0.2
glucose	7.1	7.1	0.7	0.6
sum	12.2	12.0	2.8	2.8
		LMWS		
glucose	<0.01	<0.01	<0.1	<0.1
sucrose	<0.01	<0.01	<0.01	<0.01

[a] The results are expressed on a dry matter basis of original material.

Table IV. Determination of Anhydrouronic Acid Content:[a] (A) without Extraction, (B) after Extraction with 80% Ethanol

sample	method	mean	SD (n = 5)
sugar beet fiber	A	19.2	0.8
	B	20.9	0.6
wheat bran	A	1.8	0.1
	B	1.6	0.1
soya flour	A	2.3	0.1
	B	2.2	0.1
lactovegetarian diet	A	1.2	0.1
	B	1.1	0.1

[a] All values are expressed as percentages of dry matter of original unextracted samples.

hydrates in the combined ethanol extracts.

In method C, a separate initial extraction with 80% ethanol is generally not necessary because low molecular weight carbohydrates and other extractives commonly interfering in analysis of DF have been found to be effectively removed during the starch removal and DF precipitation steps. Thus analysis of the DF fractions obtained from white wheat flour and carrot showed that no significant difference existed in content and composition of DF, compared with corresponding analysis of preextracted samples (Table III). This observation was further substantiated by GLC analysis of low molecular weight sugars in the DF fraction from carrot and also by enzymatic analysis of glucosaccharides in the DF fraction from wheat flour using the glucose oxidase method. The result obtained showed that the DF precipitation and washing procedure used in method C satisfactorily removed the 80% ethanol-soluble sugars since less than 0.1% remained, calculated as the percentage of dry matter in the original sample.

We have also found that determination of uronic acids is not essentially affected whether it is performed before or after removal of 80% ethanol extractives (Table IV). Analysis of uronic acids is therefore performed directly on ground or homogenized samples, but should a quantitation of water-soluble and water-insoluble uronic acid constituents be required, it can be performed on residues II and III (cf. Figure 1).

Enzymatic Removal and Analysis of Starch. It is important that starch and its enzymatic hydrolysis products are effectively removed from the samples in methods A and C and that the content of starch is accurately determined in method B, in order not to overestimate the DF glucan content. Treatment with a thermostable α-amylase (Termamyl 120 L) at 85 °C followed by incubation with amyloglucosidase at 60 °C removed starch efficiently (Theander and Åman, 1979). It was recommended, however, that starch-rich samples, particularly those thermally treated, should be analyzed for residual starch as a precaution. When participating in an interlaboratory study on the effect of heat treatment of dietary fiber (Varo et al., 1983), we increased the temperature to 96 °C for the Termamyl treatment to eliminate the residual starch that otherwise was around 1% in samples of extrusion-cooked wheat flour (Theander and Westerlund, 1981). Similar conditions for Termamyl treatment were also used independently by Asp et al. (1983) in the same interlaboratory study. Ultrasonication in combination with Termamyl treatment at 85 °C has also been tried as a means of eliminating the occurrence of residual starch (Neilson and Marlett, 1983) in various foodstuffs but seems to be less effective than the present procedure.

In consequence with our findings on removal of starch, we included incubation with Termamyl at 96 °C (see: Varo

Table V. Comparison of Acid Hydrolysis by Autoclaving (A) or Reflux (R) in Analysis of Neutral Polysaccharides and Klason Lignin (All Figures Given as Percentages of Dry Matter)

food sample[a]	hydrol method[b]	neutral polysaccharide constituents						Klason lignin	total		
		rhamnose	arabinose	xylose	mannose	galactose	glucose		SD	mean	range
carrot 2	A	0.6	1.8	0.4	0.8	3.1	7.9	0.3	0.7	15.0	14.1–16.0
	R	0.6	1.7	0.4	0.7	3.0	7.8	0.4	0.9	14.3	13.4–15.5
wheat bran	A	0.1	7.7	15.2	0.4	0.7	11.1	4.6	1.1	39.8	38.2–41.0
	R	0.1	7.8	14.6	0.4	0.7	11.3	4.2	1.1	39.1	37.5–40.2

[a] Five determinations on each sample. [b] Autoclaving at 125 °C for 1 h or reflux for 6 h, using 0.4 M sulfuric acid.

Table VI. Precision of Determination of Total DF and Individual DF Constituents by Method C[a] (All Figures in Percentages of Dry Matter)

DF component	whole wheat flour			wheat bran			carrot 2		
	SD (n = 5)	mean	range	SD (n = 5)	mean	range	SD (n = 5)	mean	range
rhamnose	trace		<0.1	0.1	0.1	<0.1	<0.1	0.6	0.6–0.7
arabinose	0.1	2.4	2.3–2.5	0.1	7.7	7.6–7.9	0.1	1.8	1.7–2.0
xylose	0.1	4.2	4.1–4.3	0.5	15.2	14.7–15.8	<0.1	0.4	0.4–0.5
mannose	<0.1	0.3	0.3	<0.1	0.4	0.4	<0.1	0.8	0.7–0.8
galactose	<0.1	0.4	0.4	<0.1	0.8	0.7–0.8	0.2	3.1	2.9–3.5
glucose	<0.1	3.0	3.0–3.1	0.8	11.1	9.7–11.8	0.5	7.9	7.4–8.5
uronic acids	0.1	0.8	0.7–0.9	0.1	1.6	1.5–1.7	0.4	13.1	12.6–13.6
Klason lignin	0.1	1.2	1.2–1.4	0.3	4.6	4.2–4.9	0.2	0.3	0.1–0.5
total DF	0.1	12.4	12.2–12.5	1.1	41.5	39.9–42.6	0.9	28.1	27.2–29.2

[a] Values for polysaccharide constituents are expressed on an anhydro sugar basis.

et al., 1983) in the enzymatic method previously developed in this laboratory (Salomonsson et al., 1980) for analysis of starch. The modified method (Salomonsson et al., 1984) showed high reproducibility and precision when tested on samples of wheat flour and wheat bran (Table I) and also when applied to samples of cereal grains (Åman and Hesselman, 1984). Other workers (Baur and Alexander, 1979; Batey, 1982) have also included treatment with thermostable α-amylase (at 85 °C) in their procedures for starch analysis.

Analysis of Neutral Polysaccharide Constituents. Treatment with 12 M sulfuric acid followed by secondary hydrolysis in dilute acid is a common procedure for hydrolysis of lignocellulosic materials (cf. Saeman et al., 1963). Water-insoluble DF fractions are treated likewise, and in order to decrease the total time for analysis of released monosaccharides, conditions of secondary hydrolysis for 6 h under reflux (Bethge et al., 1971; Theander and Åman, 1979) were substituted by autoclaving for 1 h at 125 °C (cf. Sloneker, 1971). Autoclaving procedures have also been used by other workers (Saeman et al., 1954; 1963; Krull and Inglett, 1980; Borchardt and Easty, 1982). A comparison of reflux and autoclaving conditions applied to samples of carrot and wheat bran, showed that the procedures were essentially equivalent (Table V) when the appropriate GLC correction factors were used (Theander and Westerlund, 1986). It is therefore reasonable to assume that sulfate esters formed during the pretreatment with 12 M sulfuric acid (Bethge et al., 1971; Theander and Westerlund, 1986) are sufficiently hydrolyzed not only by the reflux but also by the autoclaving conditions used.

The subsequent analysis of monosaccharides as alditol acetates in the acid hydrolysate is considerably faster and simpler if 1-methylimidazole is used instead of pyridine as a catalyst for acetylation. The methylimidazole is effective even in the presence of borate (Blakeney et al., 1983), which is formed by borohydride reduction of sugars and known to retard acetylation (Albersheim et al., 1967). The time-consuming removal of borate by repeated coevaporation with methanol when pyridine is used as catalyst is thus not necessary. Although in this paper the figures accounting for the neutral polysaccharide content were mainly obtained by a pyridine acetylation procedure (Theander and Åman, 1979), we are now using essentially the methylimidazole procedure of Englyst and Cummings (1984).

The subsequent quantification of the resulting alditol acetates was performed by capillary GLC analysis on OV-225, but Silar 10C (Klok et al., 1981), OV-275 (Blakeney et al., 1983), and BP-75 (Blakeney et al., 1982) can also be used for the same purpose. We are now using (Theander and Westerlund, 1986) a short capillary column of OV-275 (8 m) since the enhanced resolution of alditol acetates compared to packed columns allowed a high carrier gas flow (~1 m/s) resulting in reduced time for GLC analysis (12 min). The problems with reproducibility previously observed (Anderson and Clydesdale, 1980; Bethge et al., 1971) when using myoinositol as internal standard and packed GLC columns were lessened, possibly due to reduced tailing of the myo peak on OV-275.

Investigation of the Reproducibility of Method C. The reproducibilities of methods A and B were reported earlier (Theander and Åman, 1979, 1982). In our latest method, method C (Theander, 1983), which is particularly useful for samples with high starch and low DF contents, the water-soluble DF components are precipitated in 80% ethanol after enzymatic starch hydrolysis. In order to check for completeness of polysaccharide precipitation, the

Table VII. Comparison of Content of DF Components and Starch in Various Food Samples Using Methods A–C[a]

sample	anhydro sugar constituents						uronic acids[c]	Klason lignin	total DF	starch	method
	rhamnose	arabinose	xylose	mannose	galactose	glucose					
raw potatoes	0.1	0.3	0.1	0.1	1.4	2.1	1.0	0.1	5.2	73.0	A
	0.1	0.3	0.2	0.1	1.6	1.8	1.0	0.4	5.5	71.6	C
french fried	0.1	0.3	0.1	0.1	1.5	3.9	1.0	0.8	7.8	69.7	A
potatoes	0.1	0.3	0.1	0.2	1.5	3.7	1.0	0.6	7.5	68.6	C
low-extrctn	trace	0.7	1.4	0.1	0.2	1.0	0.2	0.1	3.7	69.3	A
wheat flour	trace	0.8	1.3	0.2	0.2	0.9	0.2	0.2	3.8	69.7	C
wheat bran	0.1	7.2	15.0	0.3	0.7	10.2	0.9	5.0	39.4	23.9	A
	0.1	7.5	15.0	0.5	0.7	9.2	0.9	4.6	38.5	23.6	C
carrot 3	0.6	1.3	0.4	0.4	1.5	7.2	11.5	1.1	24.0	trace	A
	0.4	1.7	0.4	0.5	2.2	7.5	11.5	1.3	25.5	trace	C
potatoes	0.2	0.3	0.2	0.2	1.7	5.2	1.3	0.4	9.5	66.7	B
(AOAC)	0.2	0.3	0.2	0.4	1.7	6.0	1.3	0.1	10.2	ND	C
rice (AOAC)	trace	0.3	0.3	0.1	0.1	1.1	trace	0.7	2.6	77.3	B
	trace	0.2	0.2	0.3	0.1	1.5	trace	0.4	2.7	ND	C
soya (AOAC)	0.1	0.2	0.1	0.9	0.3	0.4	0.5	3.2	5.7	1.4	B
isolate	trace	0.2	0.1	1.0	0.3	0.3	0.5	2.2	4.6	ND	C
oats (AOAC)	trace	1.0	1.2	0.3	0.4	3.0	0.4	3.9	10.2	52.3	B
	trace	1.0	1.4	0.4	0.3	3.9	0.4	1.7	9.1	ND	C
whole wheat	trace	2.4	3.7	0.3	0.6	0.6	0.6	2.2	10.4	59.5	B
flour (AOAC)	trace	2.2	3.5	0.4	0.3	2.6	0.6	1.2	10.8	ND	C
vegetarian mixed	0.1	1.0	0.8	0.3	0.6	1.8	0.9	0.7	6.2	25.1	B
diet[b] (AOAC)	0.1	1.0	0.9	0.4	0.5	2.6	0.9	0.6	7.0	ND	C
nonvegetarian mixed	0.1	0.9	0.8	0.4	0.8	2.0	0.8	1.1	6.9	31.7	B
diet[b] (AOAC)	0.1	0.8	0.8	0.5	0.5	2.5	0.8	0.7	6.7	ND	C

[a] Values expressed as percentages of dry matter. All samples analyzed at least in duplicate. [b] Defatted by ultrasonic extraction with hexane in method C. [c] Determined on original unextracted samples except for carrot, which had been homogenized.

Table VIII. Content and Relative Composition of Soluble and Insoluble Neutral DF Polysaccharide Components in Some Foods Analyzed by Method A[a]

component	potatoes raw	potatoes french-fried	low-extrctn wheat flour	wheat bran	carrot 3
		Soluble Fraction			
polysacch components	0.3	0.9	1.3	0.9	0.6
rel compn					
rhamnose	7	4	3	2	5
arabinose	14	12	27	30	29
xylose	4	1	50	54	4
mannose	4	1	2	1	2
galactose	61	67	15	10	56
glucose	11	13	2	2	4
		Insoluble Fraction			
polysacch components	3.8	5.1	2.2	32.6	10.9
rel compn					
rhamnose	2	1	trace	trace	5
arabinose	7	4	18	21	11
xylose	3	2	32	45	4
mannose	2	2	5	1	4
galactose	33	17	2	2	11
glucose	54	74	43	31	66

[a] The results are expressed as percent of the original material (dry matter content), analyzed for dietary fiber.

ethanolic supernatants obtained in the procedure were combined, subjected to dialysis, and freeze-dried. The residue was analyzed by GLC after hydrolysis with trifluoroacetic acid (16 h, 96 °C) and preparation of alditol acetates. In no case did the total content of nonstarchy polysaccharides exceed 0.1% of the original sample when low-extraction wheat flour, raw potato, carrot, and wheat bran were investigated. This result is in good agreement with previous findings (Asp et al., 1983; Nyman et al., 1984) on the efficiency of polysaccharide precipitation with 80% ethanol. It should be borne in mind, however, that in some cases highly branched polysaccharides, e.g., arabinans, may remain soluble in 80% ethanol (Larm et al., 1975).

The precision of DF analysis with method C was evaluated on samples of whole wheat flour, carrot, and bran (Table VI). The standard deviations observed indicate that the magnitude of methodological errors is within reasonable limits.

Application of Methods A–C. The methods have been applied on several different foodstuffs, and a comparison of the results obtained (Table VII) showed good agreement between methods A and C and also, particularly if the starch content is low, between methods B and C. Method A is recommended if a separate analysis of water-insoluble and water-soluble DF components is required (Table VIII). In many instances the extra information thus obtained does not warrant the extension of analysis time. Methods B and C, which are faster and more straightforward, were therefore developed. Method C is more suitable for analysis of starch-rich samples, for instance wheat flour, than method B in which the necessary subtraction of a high starch value from a high total glucan value (determined by GLC) automatically lowers the accuracy of DF glucan determination. On the other hand, method B works well on samples with low and moderate starch values (Table VII). In an interlaboratory study on total dietary fiber in foods and food products (Prosky et al., 1984), method B correlated satisfactorily with the enzymatic gravimetric method of Asp et al. (1983) used in the same study.

All three methods give valuable information on the total sugar and Klason lignin content and on the composition of the neutral polysaccharide DF constituents.

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Registry No. Starch, 9005-25-8; klason lignin, 8068-04-0; fructose, 57-48-7; glucose, 50-99-7; sucrose, 57-50-1; xylose, 58-86-6; mannose, 3458-28-4; galactose, 59-23-4; rhamnose, 3615-41-6; arabinose, 147-81-9.

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Products Identified from Photosensitized Oxidation of Selected Furanoid Flavor Compounds

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Singlet oxygen was generated photochemically and used to oxidize furfural, 2-ethylfuran, furfuryl acetate, and furfurylacetone. Major products were identified and reaction pathways were proposed. In all reactions, singlet oxygen adds into the furan ring by 1,4-cycloaddition to form an unstable ozonide. This then reacts with the solvent and rearranges to form the identified products. The exocyclic double bond of furfurylacetone does not appear to be involved in the reaction.

INTRODUCTION

Furfural occurs naturally in essential oils, roasted coffee, and rum (Furia and Bellanca, 1975). Maga (1979) reviewed the extensive occurrence of this and many other furanoid flavor compounds in such varied foods as alcoholic and nonalcoholic beverages, fruits, meat and poultry products, milk products, oil seed products, and vegetables.

Many of these foods contain photosensitizers like chlorophyll and hemoglobin that can generate singlet oxygen (Clements et al., 1973). Singlet oxygen thus generated may oxidize furanoid flavor compounds and affect the quality of food. First, the total flavor of the food may become unbalanced due to the loss of furanoid flavor compounds. More importantly, the oxidation products may impart undesirable flavor notes to the food.

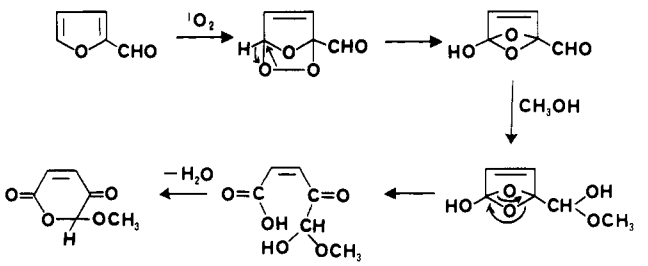
In this work, products from singlet oxygen reactions with selected furanoid flavor compounds were identified to propose reaction pathways.

EXPERIMENTAL SECTION

Materials. Reagent-grade furfural, furfuryl acetate, and ethylfuran were purchased from Aldrich Chemical Co. (Milwaukke, WI). Reagent-grade furfurylacetone was obtained from Pfaltz and Bauer, Inc. (Stamford, CT). Chlorophyll (99%) from spinach was purchased from Sigma Chemical Co. (St. Louis, MO). Methanol was UV spectroscopic grade (J. T. Baker Chemical Co., Phillipsburg, NJ).

Photosensitized Oxidation. Photosensitized oxygenation was carried out in a 150-mL reaction tube (4 cm i.d. × 20 cm) containing a given furanoid flavor compound and chlorophyll dissolved in methanol at 10^{-2} and 10^{-4} M concentrations, respectively. The tube was suspended in a trough of ice-cold water. Oxygen was bubbled through the solution at a flow rate of 30 mL/min under illumination from two 150-W lamps placed about 5 cm from reaction tube. The reaction was stopped after 60 min, and the solution was concentrated 10-fold by vacuum distillation. The reaction conditions were chosen to ensure adequate formation of products for NMR and/or GC-MS identification (Mensah, 1984).

Scheme I. Furfural Reaction Pathway



GC-MS Analysis. The concentrated samples were analyzed by GC-MS using a Finnigan 3300 mass spectrometer coupled with a Varian 3700 gas chromatograph. The GC was fitted with a 30 m × 0.32 mm i.d., 1-μm film, DB-1 fused silica capillary column (J&W Scientific, Inc., Rancho Cordova, CA). The column oven temperature was held at 100 °C for 1 min and then programmed to 300 °C at 8 °C/min. Mass spectra were obtained at 70 eV and a source temperature of 200 °C.

Proton NMR Analysis. A packed 10% OV-17 column (12 ft × $^1/_8$ in. o.d.) was used to collect major peaks from a Hewlett-Packard 5700 gas chromatograph for NMR determination. The peaks were dissolved in $CFCl_3$ and identified at ambient temperature on a Varian SL-100 NMR spectrometer at 40 rps using 1500-Hz sweep width and 3-s sweep time.

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