very crude extract. These and present studies suggest the possible usefulness of Folin–Ciocalteau reagent as a postcolumn derivatization procedure in high-performance liquid chromatographic systems.

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Registry No. Uracil, 66-22-8; cytosine, 71-30-7; isocytosine, 108-53-2; hypoxanthine, 68-94-0; adenine, 73-24-5; adenine N^1-oxide, 700-02-7; 2-hydroxypurine, 2308-57-8; 2-aminopurine, 452-06-2; xanthine, 69-89-6; xanthine N^3-oxide, 13479-29-3; uric acid, 69-93-2; guanine, 73-40-5; 2-(dimethylamino)-6-hydroxypurine, 1445-15-4; isoguanine hemisulfate, 49722-90-9; isoguanine N^1-oxide, 7593-46-6; 2,6-diaminopurine, 69369-16-0; 1-methylxanthine, 6136-37-4; 3-methylxanthine, 1076-22-8; 7-methylxanthine, 552-62-5; theophylline, 58-55-9; 1,7-dimethylxanthine, 611-59-6; theobromine, 83-67-0; caffeine, 58-08-2; 1,3,9-trimethylxanthine, 519-32-4; pterin, 2236-60-4; lumazine, 487-21-8; xanthopterin, 119-44-8; leucopterin, 492-11-5.

LITERATURE CITED

Box, J. D. "Investigation of the Folin–Ciocalteau Phenol Reagent for the Determination of Polyphenolic Substances in Natural Waters". *Water Res.* **1983**, *17*, 511–525.

Elving, P. J.; O'Reilly, J. E.; Schmakel, C. O. "Polarography and Voltammetry of Nucleosides and Nucleotides and Their Parent Bases as an Analytical and Investigative Tool". In *Methods of Biochemical Analysis*; Glick, D., Ed.; Wiley: New York, 1973; Vol. 21, pp 287–465.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. "Protein Measurement with the Folin Phenol Reagent". *J. Biol. Chem.* **1951**, *193*, 265–275.

Mosley, S.; Ikawa, M.; Sasner, J. J., Jr. "A Combination Fluorescence Assay and Folin–Ciocalteau Phenol Reagent Assay for the Detection of Paralytic Shellfish Poisons". *Toxicon* **1985**, *23*, 375–381.

Peterson, G. L. "Review of the Folin Phenol Protein Quantitation Method of Lowry, Rosebrough, Farr and Randall". *Anal. Biochem.* **1979**, *100*, 201–220.

Shepherd, G. R.; Hopkins, P. A. "Determination of Protein Contamination in Deoxyribonucleic Acid by the Folin-Lowry Method". *Anal. Chem.* **1963**, *35*, 1548–1549.

Subramanian, K. N.; Padmanaban, G.; Sarma, P. S. "Folin-Ciocalteau Reagent for the Estimation of Siderochromes". *Anal. Biochem.* **1965**, *12*, 106–112.

Varadi, M.; Feher, Z.; Pungor, E. "Determination of Purine Bases by Chromatovoltammetry". *J. Chromatogr.* **1974**, *90*, 259–265.

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Comparison of Gravimetric and Chemical Analyses of Total Dietary Fiber in Human Foods

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Dietary fiber in five foods, which have significant compositional differences, was determined by the gravimetric procedure recently receiving AOAC first action and compared to values determined by chemical analysis of the fiber components in extractive free residue (EFR) of the same foods. The gravimetrically determined total dietary fiber value was within 10% of the chemically determined value for three of the five foods analyzed: peas, soy polysaccharide, wheat bran. The gravimetric method overestimated the fiber content of apple by 14% and of a food composite of a typical daily intake by 18%. Chemical analyses of the gravimetrically obtained residue for neutral and acidic sugars and Klason lignin content were generally similar to the amounts of these fiber components measured in the EFR. Samples of apple and food composite obtained by the AOAC procedure contained slightly more neutral sugars and the wheat bran more Klason lignin than did the EFR samples. Following analysis of the fiber residues for crude protein and starch, recoveries of 97–104% were obtained. Fiber fractions of peas obtained by both methods contained 8–9% starch.

A knowledge of the dietary fiber content of human foodstuffs in needed for several reasons. Identifying mechanisms of action of dietary fiber within the gastrointestinal tract requires detailed information about the composition of the fiber. Food industries are interested in a total dietary fiber value appropriate for quality control and nutrition labeling. Dietitians and nutritionists want a database of food fiber values that can be used to estimate daily fiber intakes of individuals and populations. Thus, there is a need for the characterization of the kind of fiber in a foodstuff as well as for total dietary fiber values. Two approaches to dietary fiber analysis are being pursued to meet these diverse needs: a detailed procedure similar to the methods of Theander, Englyst, or Southgate that will provide information about the kinds and composition of the fiber in foods and a more rapid gravimetric method that will yield a single value for total dietary fiber. To meet the latter objective, Asp, DeVries, Furda, and Schweizer developed a gravimetric procedure that was submitted for an interlaboratory study in early 1982; the interlaboratory study was coordinated by the Food and Drug Administration (FDA) (Prosky et al., 1984). The results of two interlaboratory studies have been reported (Prosky et al., 1984, 1985), and the method has been given first-action approval by AOAC ("Total Dietary Fiber in Foods", 1985).

In the first interlaboratory study the reproducibility of the method, assessed by calculating a coefficient of variation of data of 32 laboratories who participated, was greater than 15% for 8 of the 13 food samples (Prosky et

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al., 1984). In the second collaborative study, nine laboratories measured total dietary fiber in nine foods, all grain products, using a modification of the original procedure that had been designed to reduce the coefficient of variation (Prosky et al., 1985). In this study the coefficient of variation exceeded 10% for only two samples that contained small amounts (<1.5%) dietary fiber on a dry-weight basis. Comparisons by the same laboratories of the original and modified methods were not included in the second study. It has been difficult to compare the results obtained by this procedure with those generated by other methods of fiber analysis. The fiber values obtained by the gravimetric method in the first interlaboratory study were compared to fiber values obtained by other methods in a graph (Prosky et al., 1984). The actual dietary fiber values obtained with the various methods can be only estimated with such an approach to data presentation.

There were four objective of the research report. First, total dietary fiber of five foods, representing all classes of fiber-containing foods, was determined by both gravimetric procedures to determine if, in fact, reproducibility was improved with the modifications that have been incorporated into the method. Second, the gravimetric yields of total dietary fiber were compared to the fiber content obtained by chemical analysis of fiber components of the same foods using a method based on the procedure of Theander (Theander and Aman, 1981; Theander and Westerlund, 1986). Third, the method was further validated by chemically analyzing the gravimetrically recovered fiber and comparing the yields of neutral sugars, uronic acids, and Klason lignin with those obtained by the modified Theander method (Neilson and Marlett, 1983; Marlett and Chesters, 1985; Shinnick et al., 1988). The intralaboratory reproducibility of the total dietary fiber analysis and of the blank that is part of the gravimetric procedure also were determined. Last, essentiality of the blank that is used to correct the gravimetric yield of total dietary fiber was also evaluated.

METHODS

Sample Preparation. Wheat bran (AACC-Certified Food Grade Soft White Wheat Bran, St. Paul, MN) was ground to 30 mesh in a Thomas-Wiley mill. Canned peas (Del Monte), fresh apples (Washington State Red Delicious) with the cores removed, and a food composite previously described (Slavin and Marlett, 1983) were each blended with distilled water and lyophilized. The soy polysaccharide (Ross Laboratories, Columbus, OH) was analyzed without modification. Dry weights of prepared samples were determined by AOAC procedures (1980).

Fiber Analysis. Gravimetric analyses were performed by both the original method (Prosky et al., 1984) and its more recently published modification ("Total Dietary Fiber in Foods", 1985). The modifications were an increase in the concentration of ethanol used to wash the fiber residue after filtration from 74 to 78%, decreases in the concentrations of the base (sodium hydroxide, from 0.285 to 0.171 N) and acid (phosphoric acid from 0.329 to 0.205 M) that are used to adjust the pH at various steps in the procedure, and a decrease in the phosphate buffer concentration from 0.083 to 0.05 M. In all instances the duration of the Termamyl incubation was sufficient to assure the temperature of the samples reached 100 °C for 15 min ("Total Dietary Fiber in Foods", 1985). Each analysis consisted of 6–10 aliquots (1 g) of a food and 3–4 blank samples. Six food fiber residues obtained by the modified method were subsequently used for duplicate analyses for nitrogen, starch, and ash in the fiber residue; the remaining four residues were used for duplicate analyses of uronic acids, neutral sugars, and Klason lignin. Nitrogen was measured in two of the blanks, and the remaining one to two blanks were used to determine ash. Nitrogen was measured by the Kjeldahl method and converted into crude protein with use of the correction factor 6.25. The completeness of starch removal was assessed by determining the starch content of two residues from each food by an enzymatic procedure of the American Association of Cereal Chemists (1976). The enzyme was checked for the presence of fiber-hydrolyzing enzymes by incubation with cellulose (Sigma) and pectin (Sigma), each of which had been precipitated with 80% ethanol.

Two residues prepared by the modified gravimetric procedure were hydrolyzed by the Blake and Richards modification (1970) of the Saeman procedure in use in this laboratory (Neilson and Marlett, 1983; Marlett and Chesters, 1985). Erythritol (10 mg) was added after the seconary hydrolysis as an internal standard. Material insoluble in 72% sulfuric acid was washed free of acid with water, filtered, vacuum-dried, weighed, and reported as Klason lignin. Hydrolysates were neutralized at 60 °C with barium carbonate and centrifuged (1000*g*, 10 min); the supernatant was added to cation-exchange resin (Bio-Rad AG 50W-X8, 200–400 mesh) for barium ion extraction and then filtered sequentially through a Waters Millipore Sep-Pak C18 cartridge and a 0.2-μm filter. The neutral sugar composition of the hydrolysates was determined by HPLC as previously described (Neilson and Marlett, 1983; Marlett and Chesters, 1985). Fucose present in the soy polysaccharide was measured by gas chromatography. The amounts of sugars present were computed by a reporting integrator after calibration data were obtained with vacuum-dried standard sugars (Sigma). Monosaccharides were expressed as polysaccharides (×0.9) following correction for hydrolysis losses and residual starch if present.

Polysaccharides containing uronic acids were solubilized with 72% sulfuric acid (Neilson and Marlett, 1983). Uronic acids were then determined colorimetrically by adapting the 3-hydroxybiphenyl method of Blumenkrantz and Asboe-Hansen (1973) using D-galacturonic acid (Sigma) as the standard. Uronic acids were expressed as a polysaccharide.

Total dietary fiber was also determined by a modification (Neilson and Marlett, 1983; Marlett and Chesters, 1985; Shinnick et al., 1988) of the Theander procedure (Theander and Aman, 1981; Theander and Westerlund, 1986), as the sum of the soluble and insoluble fiber fractions generated by this method. All foods were analyzed in duplicate. Briefly, extractive free residue (EFR) was prepared from 5-g food samples (dry weight) by duplicate sequential extractions under sonication with 80% ethanol and light petroleum ether (bp 60–70 °C). EFR was treated sequentially with Termamyl (Nova-120L) and amyloglucosidase (from *Aspergillus niger*, Sigma A3514) to hydrolyze starch. Following starch hydrolysis, samples were separated by centrifugation (10444*g*, 10 min) into operationally defined soluble and insoluble fiber fractions. Filtrate was dialyzed (12 000–14 000 MW cutoff) against distilled water (4 L/12 h for 48 h at 4 °C) and freeze-dried. The pellet was dried under vacuum (40 °C, P_2O_5, >4 h). Each of these fractions were analyzed for nitrogen, starch, neutral sugars, lignin, and uronic acids described above. Starch and crude protein (N × 6.25) were determined in order to calculate recoveries.

RESULTS AND DISCUSSION

The modifications incorporated into the AOAC method as a consequence of the first interlaboratory study did improve the reproducibility of the method (Table I). The

Table I. Reproducibility of the Gravimetric Analysis of Total Dietary Fiber[a]

	% of orig dry wt			
	orig method (n = 6)		modified method (n = 10)	
	mean ± SD	range	mean ± SD	range
peas	23.4 ± 1.3	21.2–24.6	24.9 ± 0.5	24.2–25.7
food composite	9.6 ± 1.2	8.5–11.7	8.8 ± 0.3	8.4–9.3
apple	11.4 ± 0.7	10.4–12.4	13.8 ± 0.2	13.5–14.1
wheat bran	40.5 ± 3.4[b]	35.3–44.4	47.4 ± 0.2[c]	47.1–47.8
soy polysaccharide	68.1 ± 2.3	66.1–71.0	75.0 ± 0.6	74.0–75.7

[a] All determinations corrected for weight of blank. [b] AACC standard soft white wheat bran purchased before 8/84. [c] AACC standard soft white wheat bran purchased after 8/84.

Table II. Gravimetric vs Chemical Analysis of Total Dietary Fiber

	% dry wt				
		AOAC first action			
	modified Theander	gravimetric	chemical	gravimetric[a,b]	gravimetric[a,c]
peas	24.0[d]	25.1	21.4	25.7	21.8
food composite	7.7	9.1	9.2	9.8	8.4
apple	12.2	13.9	14.9	14.3	*e*
wheat bran	45.9	47.7	50.1	48.1	47.1
soy polysaccharide	78.9	75.7	77.3	76.3	*e*

[a] Yield of the two residues used for neutral sugar analysis. [b] Not corrected for mean blank residue weight. [c] Corrected for residual starch content. [d] Mean, n = 2. [e] No starch detected.

range of fiber values obtained for each sample decreased. This was particularly true for the two concentrated fiber sources, wheat bran and soy polysaccharide, in which the intralaboratory coefficients of variation decreased from 8.4 to 0.4% and 3.4 to 0.8%, respectively (Table I).

The gravimetric method measured slightly more dietary fiber than the modified Theander method using direct analysis of fiber components for two of the five foods, 4.6% more fiber in peas and 3.9% more fiber in wheat bran (Table II). When expressed on the basis of fresh weight or in amounts typically consumed, the difference in the amount of fiber in a serving obtained by the two methods for peas or wheat bran was negligible. The gravimetric yield of dietary fiber from the food composite was 15% higher and from apple 13.9% higher than the values obtained by the modified chemical analysis method (Table II). Since apple is 85% water (Watt and Merrill, 1963), the difference in fiber content obtained by the two methods would be insignificant when expressed on a fresh-weight basis. If one assumes a daily dry-weight food intake of 400 g, the daily fiber intake determined by AOAC gravimetric analysis would be 36.4 g and by the Theander method 30.8 g. The difference between the two values for the food composite would produce significantly different daily fiber intakes. The gravimetrically determined fiber content of soy polysaccharide was 4.2% lower than the value determined with the direct analysis (Table II). The difference between the two values in the fiber provided by a 25-g serving would be 0.8 g.

One of the objectives of the AOAC method was to provide a relatively simple method of analysis that also required significantly less time than other methods. Because determination of a blank with every set of samples requires additional time, we evaluated the need for determining the blank and the variability in repeated determinations of the blank. Although the correction of the fiber values with the blank was modest, the net effect on the values for four of the five foods was to bring the value closer to the value determined by direct analysis of the fiber constituents (Table II). Determination of the blank in our hands ranged from 3.0 to 8.4 mg (Table III). The variation appeared to be more dependent on differences in the measurement of crude protein than in the determination of the ash content. Further, the high reproducibility we obtained in the fiber analyses with the modified method suggests the variability in the blank is representative and not a consequence of analytical technique (Table I vs Table III). The variability was sufficiently different between batches to suggest conservatively that at least two blanks should be included with each set of analyses (Table III).

Table III. Reproducibility of Blank Determination of the AOAC First Action Method of Total Dietary Fiber Analysis

residue, mg	% of blank			blank, mg
	N	crude protein	ash	
26.0			50.3	6.9
17.7	3.0	18.5		5.5
16.4	4.5	27.9		3.6
37.1			52.4	7.5
37.0			46.0	6.9
34.7	3.0	18.9		8.4
37.7	3.4	21.3		7.9
13.2			58.3	3.0
13.1			56.5	3.0
21.2	2.7	16.8		4.9
18.2	3.6	22.3		4.2
21.8			61.0	4.3
22.1			57.9	4.4
21.4	3.1	19.3		4.3
22.9	3.5	22.0		4.6
24.7			56.7	5.8
24.0			55.0	5.6
21.7	3.3	20.8		5.1
23.4				5.5
23.9 ± 7.6[a]	3.3 ± 0.5[a]	20.9 ± 3.2[a]	54.9 ± 4.6[a]	5.3 ± 1.6[a]

[a] Mean ± SD.

Correction of the AOAC values for residual starch lessened the difference between the two fiber values of wheat bran and food composite (Table II). The difference in daily fiber intake from 400 g of dry food would then be 33.6 g vs 36.4 g obtained by the modified Theander method, or ~8% lower compared to the 15% difference between the AOAC gravimetrically determined value not corrected for starch and the value obtained by direct analysis of the fiber components. Correction for starch content of the gravimetrically determined fiber value for peas increased the difference between the results obtained by the two methods. The fiber content determined by the AOAC method was 9.6% less than that determined by direct analysis (Table II). Recently, there has been considerable discussion about this "residual" starch, with some investigators concluding that starch not removed by an analytical procedure should be included as part of the dietary fiber complex. This view, which incorporates aspects of a physiological definition of dietary fiber, assumes that the extractions or enzymatic steps in an analytical procedure have the same net effect as the digestive activity in the upper gastrointestinal tract. Digestive activity in the stomach is more vigorous than what is used in most fiber analysis procedures, and this gastric activity most likely influences the availability of food substrate for small-bowel digestive activity. Until data are available to substantiate or refute this assumption, our laboratory has taken the position that the amount of residual starch should be determined and removed from the dietary fiber. If the amount of residual starch is known, it could be added back to the fiber value at a later time if physiological

Table IV. Neutral Sugar Composition of Dietary Fiber Recovered by Two Methods

	% of neutral sugars						
	Clb	Glc	Xyl	Gal/Rha	Ara	Man	Fuc
peas							
AOAC first action	10.6[a]	58.6	6.8	4.0	18.3	1.6	[b]
Theander	6.4	70.4	7.4	0.7	14.6	0.5	
food composite							
AOAC first action	8.9	45.5	11.4	13.5	17.9	2.8	
Theander	7.3	46.2	13.0	12.0	16.5	5.0	
apples							
AOAC first action	7.3	42.1	11.9	11.6	23.4	3.8	
Theander	6.2	41.2	11.1	12.5	25.0	3.8	
wheat bran							
AOAC first action	4.6	27.6	41.9	0.5	23.5	1.4	
Theander	4.5	26.4	41.6	2.0	24.2	1.3	
soy polysaccharide							
AOAC first action		17.6	8.1	47.4	22.5	1.5	2.9
Theander		12.6	7.5	49.9	25.0	2.1	3.0

[a] Mean of two hydrolysates. [b] Not determined.

Table V. Composition of Dietary Fiber Recovered by Two Methods

	% of dry wt of food			
	neutral sugars	Klason lignin	uronic acids	total
peas				
AOAC first action	16.4[a]	2.5	2.6	21.4
Theander	16.6	5.4	2.1	24.0
food composite				
AOAC first action	6.4	1.2	1.6	9.2
Theander	5.4	0.9	1.4	7.7
apples				
AOAC first action	8.0	3.2	3.6	14.9
Theander	6.5	2.4	3.4	12.2
wheat bran				
AOAC first action	39.9	8.5	1.7	50.1
Theander	39.1	5.4	1.4	45.9
soy polysaccharide				
AOAC first action	65.0	1.0	11.3	77.3
Theander	63.5	0.6	14.8	78.9

[a] Mean, $n = 2$.

experimentation demonstrates that it is not digested during movement through the upper gut.

When the fiber content of the AOAC residue was determined as the sum of the neutral sugar, uronic acid, and Klason lignin contents in a manner similar to the analyses performed in the modified Theander method, another set of fiber values were obtained (Table II). Comparisons of additional analyses performed on the two fiber residues only partially explained the differences in dietary fiber content measured by the two methods. There were no significant differences in neutral sugar composition or distribution of neutral sugars between the two fibers in any of the foods (Table IV). With use of glucose as an indication of cellulose content, both methods detected significant amounts of cellulose in peas, food composite, and apples. The arabinose and xylose contents of wheat bran reflect its high content of arabinoxylans. Data from both methods suggested that about 70% of the neutral sugars in the soy polysaccharide represented an arabinogalactan (Table IV).

The two methods of fiber extraction recovered identical amounts of fiber-derived neutral sugars from three of the five foods that were analyzed: peas, wheat bran, soy polysaccharide (Table V). The AOAC method recovered more neutral sugars from the food composite and apples. Conceivably, fiber polysaccharides could have been extracted in the initial 80% ethanol step in the preparation of the EFR in the Theander method. However, Theander recently examined this possibility and was unable to demonstrate any significant differences (Theander, 1983; Theander and Westerlund, 1986). Alternately, small polysaccharides could be dialyzed away from the soluble fiber fraction, although we were unable to demonstrate any differences in the amount or composition of the soluble fraction of peas concentrated by dialysis in three different sizes of membrane: 12 000–14 000, 6000–8000, 3500 molecular weight cutoff (Marlett and Harned, unpublished observations). Further, comparable recoveries of fiber from wheat bran, potato, carrot, and wheat flour by either dialysis or precipitation in 80% ethanol do not support the possibility that polysaccharide was lost during dialysis in the Theander method or that additional fiber carbohydrate was recovered with ethanol precipitation used in the AOAC

Table VI. Recovery of Fiber Residues Obtained by Two Methods

	% of fiber residue						
	neutral sugars	Klason lignin	uronic acids	residual starch	crude protein	ash	recovery, %
peas							
AOAC first action	49.5	7.7	8.1	9.3	17.1	6.2	97.7 ± 3.6
Theander	36.6	11.9	4.6	8.4	42.9		104.2 ± 5.5
food composite							
AOAC first action	50.2	9.4	12.9	2.8	15.7	6.8	97.8 ± 5.1
Theander	38.4	6.0	9.8	3.0	44.5		101.7 ± 0.5
apples							
AOAC first action	49.6	19.8	21.2	0	7.0	5.2	103.7 ± 1.9
Theander	47.7	17.7	25.1	0	9.6		100.0 ± 0.2
wheat bran							
AOAC first action	61.2	13.0	2.7	0.5	17.0	9.2	103.6 ± 1.9
Theander	62.8	8.6	2.2	0.5	23.4		97.1 ± 0.9
soy polysaccharide							
AOAC first action	72.2	1.1	13.5	0	5.9	4.4	97.1 ± 9.8
Theander	69.6	0.7	16.3	0	13.4		100.0 ± 1.9

procedure (Theander and Westerlund, 1986). In fact, some evidence suggests that some polysaccharides may not be precipitated with 80% ethanol (Larm et al., 1975), a potential problem in the application of a fiber analysis procedure that uses 80% ethanol to precipitate fiber components to a wide variety of foods.

In contrast to the Theander method the AOAC procedure does not contain an initial extraction with 80% ethanol to remove soluble sugars. About 88% of the dry weight of apple and 30% of the food composite, in contrast to 10% of the dry weight of peas, were measured as simple sugars (sucrose, glucose, fructose) by HPLC in the 80% ethanol soluble fraction (Marlett and Chesters, unpublished observations). When high concentrations of simple sugars are present in a sample, measurable amounts may be trapped in the 80% ethanol precipitate. In addition, failure to remove simple sugars makes them available for combining with products of protein degradation during the enzymatic acid hydrolysis steps involving heat. Theander and colleagues reported the production of a brown, water-insoluble polymer by refluxing a slightly acidic aqueous solution of glucose and glycine (Olsson et al., 1978). This material, which contains substantial amounts of nitrogen, was recovered as Klason lignin (Theander, 1983). The recovery of more Klason lignin in the two residues obtained by the gravimetric AOAC procedure that also contain more neutral sugars, i.e. food composite and apples, is consistent with this hypothesis (Table V).

The major cause for the higher total dietary fiber content of peas by the Theander method and of wheat bran by the AOAC procedure was the Klason lignin (Table V). As recently reviewed by Theander (1983), material insoluble in 72% sulfuric acid, i.e. Klason lignin, contains not only true lignin but Maillard and caramelization products, tannins and cutins. In addition, we found that more Klason lignin was recovered when fractions containing nitrogen were acid hydrolyzed vs samples with similar carbohydrate composition but from which the nitrogen had been extracted (Nielson and Marlett, 1983; Marlett and Chesters, 1985). In addition, increasing the concentration of sulfuric acid during the secondary hydrolysis step also produced higher yields of Klason lignin (Marlett and Harned, unpublished observations). Generally, recoveries of the samples with higher Klason lignin contents, the pea fiber fractions obtained by the Theander method and the wheat bran and apple fiber obtained by the AOAC, were greater than 100% (Table VI). This would occur if part of the nitrogen was measured twice, as crude protein and as a portion of the Klason lignin. Our recoveries of the fiber fractions indicate that no significant amounts of the fiber components went undetected.

Registry No. Klason lignin, 8068-04-0; starch, 9005-25-8.

LITERATURE CITED

American Association of Cereal Chemists "Starch-Glucoamylase Method with Subsequent Measurement of Glucose with Glucose Oxidase". In *Approved Methods of the AACC*; AACC: St. Paul, MN, 1976; Method 76-11.

AOAC "Total Solids (Moisture, Indirect Method)". In *Official Methods of Analysis*, 12th ed.; Association of Official Analytical Chemists: Washington, DC, 1980; p 222.

Blake, J. D.; Richards, G. N. "A Critical Re-Examination of Problems Inherent in Compositional Analysis of Hemicelluloses by Gas-Liquid Chromatography". *Carbohydr. Res.* **1970**, *14*, 375–387.

Blumenkrantz, N.; Asboe-Hansen, G. "New Method for Quantitative Determination of Uronic Acids". *Anal. Chem.* **1973**, *54*, 484–489.

James, W. P. T., Theander, O., Eds. *The Analysis of Dietary Fiber in Food*; Marcel Dekker: New York, 1981.

Larm, O.; Theander, O.; Aman, P. "Structural Studies on a Water-Soluble Arabinan Isolated from Rapeseed (*Brassica rapus*)". *Acta Chem. Scand., Ser. B* **1975**, *B29*, 1011–1014.

Marlett, J. A.; Chesters, J. G. "Measuring Dietary Fiber in Human Foods". *J. Food Sci.* **1985**, *50*, 410–414, 423.

Neilson, M. J.; Marlett, J. A. "A Comparison between Detergent and Nondetergent Analyses of Dietary Fiber in Human Foodstuffs, Using High-Performance Liquid Chromatography to Measure Neutral Sugar Composition". *J. Agric. Food Chem.* **1983**, *31*, 1342–1347.

Olsson, K.; Pernemalm, P. A.; Theander, O. "Formation of Aromatic Compounds from Carbohydrates. VII. Reaction of D-Glucose and Glycine in Slightly Acidic, Aqueous Solution". *Acta Chem. Scand., Ser. B* **1978**, *B32*, 249–256.

Prosky, L.; Asp, N. G.; Furda, I.; DeVries, J. W.; Schweizer, T. F.; Harland, B. F. "Determination of Total Dietary Fiber in Foods, Food Products, and total Diets: Interlaboratory Study". *J. Assoc. Off. Anal. Chem.* **1984**, *67*, 1044–1052.

Prosky, L.; Asp, N. G.; Furda, I.; DeVries, J. W.; Schweizer, T. F.; Harland, B. F. "Determination of Total Dietary Fiber in Foods and Food Products: Collaborative Study". *J. Assoc. Off. Anal. Chem.* **1985**, *68*, 677–679.

Shinnick, F. L.; Longacre, M. J.; Ink, S. L.; Marlett, J. A. "Oat Fiber: Composition vs Physiological Function". *J. Nutr.* **1988**, in press.

Slavin, J. L.; Marlett, J. A. "Evaluation of High-Performance Liquid Chromatography (HPLC) for Measurement of Neutral Saccharides in Neutral Detergent Fiber". *J. Agric. Food Chem.* **1983**, *31*, 467–471.

Theander, O. "Advances in the Chemical Characterization and Analytical Determination of Dietary Fibre Components". In *Dietary Fibre*; Birch, G. G., Parker, K. J., Eds.; Applied Science: New York, 1983; Chapter 6, p 77.

Theander, O.; Aman, P. "Analysis of Dietary Fibers and Their Main Constituents". In *The Analysis of Dietary Fiber*; James, W. P. T., Theander, O., Eds.; Marcel Dekker: New York, 1981; Chapter 5, p 51.

Theander, O.; Westerlund, E. A. "Studies on Dietary Fiber. 3. Improved Procedures for Analysis of Dietary Fiber". *J. Agric. Food Chem.* **1986**, *34*, 330–336.

"Total Dietary Fiber in Foods", First Action. *J. Assoc. Off. Anal. Chem.* **1985**, *68*, 399.

Watt, B. K.; Merrill, A. L. *Composition of Foods*; U.S. Department of Agriculture: Washington, DC, 1963.

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