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Pectic Substance Content of Detergent-Extracted Dietary Fibers

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Acid and neutral detergent fibers (ADF and NDF) prepared from apple, pear, tomato, carrot, onion, potato, wheat bran, and orange albedo were analyzed for total pectic substances (PS). Total PS content and the relative proportion of the original total PS recovered as dietary fiber (DF) were higher in ADF than in NDF, suggesting more PS were solubilized and extracted by neutral detergent. About 50 and 38% of total PS in orange albedo were recovered in ADF and NDF, respectively. In fruits, vegetable, and wheat bran, total PS recovered as ADF and NDF ranged from 3.0 to 15.0% and 0.6 to 7.0%, respectively. For potato, the proportion of total PS recovered in extracts and washings during ADF and NDF analysis was 94.8 and 99.8%, respectively. These data emphasize that the detergent methods which are increasingly used to report dietary fiber values in foods underestimate PS, a component of dietary fiber complex with important nutritional and physiological effects. More attention should be directed toward separate analysis of pectin or modifications of existing methods to reflect pectin more accurately.

Ever since the epidemiological demonstrations of the beneficial effect of what is now called dietary fiber (DF) on the etiology of certain "Western" diseases by Burkitt (1973) and Trowell (1972), a great deal of effort has been directed toward development of simple routine methods to assay the dietary fiber content of food. The acid detergent fiber (ADF) and neutral detergent fiber (NDF) methods (Van Soest, 1963; Van Soest and Wine, 1967) are at present considered the most convenient analytical methods for fiber determination and have largely replaced the crude fiber method (AOAC, 1975). Compared to the enzymatic procedure by Hellendoorn et al. (1975) and to the fractionation method of Southgate (1969, 1976), they are simpler, less expensive, more rapid, and practical for routine analysis of large number of samples. The use of these methods is increasing. The ADF method is listed as an official first action method of the Association of Official Analytical Chemists (AOAC, 1975) while a modified NDF is an official method of the American Association of Cereal Chemists (AACC, 1978).

Due to the complex nature of DF in food materials, the development of a method that will encompass all of the components in the DF complex has presented a difficulty and challenge to workers in this subject. For instance, the ADF and NDF methods are designed to measure insoluble components and therefore exclude most of the water-soluble polysaccharides and other polymers.

Pectic substances (PS) or pectins along with cellulose, hemicellulose, and lignin are the major components of the plant cell wall, and collectively they are part of what is now called DF (Southgate, (1979). PS had been reported to have important physiological and nutritional effects such as hypocholesterolemic effect, increased excretion of fecal sterols and lipids, binding of bile salts, binding of polyvalent cations (Story and Kritchevsky, 1969; Kay and Truswell, 1977; Kay et al., 1978; Kelsay, 1978), and increased requirement for vitamin B_{12} (Cullen and Oace, 1978). With the growing interest in its biological effects, it is desirable to determine this DF component more accurately in foods. Due to their relative water solubility, some PS are solubilized during analysis and therefore not included in the total fiber value (Bailey and Ulyatt, 1970; Bailey et al., 1978). However, some insoluble PS may remain with the fiber (Baker et al., 1979). Bailey et al. (1978) determined the solubility of pectins from a number of common plant foods, indicating that a considerable portion of pectin is lost to commonly used analytical procedures. However, PS analyses of actual detergent fibr residues do not appear to have been reported in the literature. This information is especially important with the increased usage of ADF and NDF in reporting fiber values. For these reasons, PS content of selected foods and their detergent-extracted DF were investigated, and results are discussed in this report.

MATERIALS AND METHODS

Sample and Sample Preparation. Food samples used in this study were potato, carrot, tomato, apple, pear, white onion, orange albedo, and wheat bran. Except for wheat bran, all samples were bought from a local grocery store. Wheat bran was AACC grade obtained from Dr. G. Briggs' laboratory (Department of Nutritional Sciences, University of California, Berkeley). Fruits and vegetable samples were washed and dried. Dried outer skins of onions were re-

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moved. Carrots and potatoes were peeled.

Apples, pears, tomatoes, onions, and potatoes were sliced and homogenized in a Waring blender without addition of water. Sodium bisulfite was added to homogenates to a final concentration of 200 ppm to prevent enzymatic browning. Homogenates were freeze-dried. Peeled carrots were sliced and Freeze-dried. Orange albedo sample was prepared from orange peels and freeze-dried. Dry matter content was calculated from weight loss after freeze-drying. Dried samples were ground to pass through a 1.00-mm sieve. Wheat bran sample was ground to pass through a 1.0-mm sieve and moisture determined by using the AACC (1978) method.

Detergent Fiber Methods. ADF was determined according to the Goering and Van Soest (1970) procedure. One gram of freeze-dried sample (weighed to within 0.1 mg) was refluxed with 100 mL of acid detergent solution in a boiling water bath for 1 h. The digest was filtered through a coarse porosity sintered glass crucible, and the residue washed twice with 100 mL of hot distilled water (90–100 °C) followed by acetone until no further color is extracted. The residue was dried at 100 °C overnight and weighed. For determination of losses of PS during analysis, detergent extract and hot water and acetone washings were collected and analyzed.

A modified NDF method described by Mongeau and Brassard (1979) was employed with slight modifications. The method is essentially similar to the AACC (1978) method except that a heat-resistant α-amylase preparation was used. One gram of dry sample (weighed to the nearest 0.1 mg) was refluxed in a boiling water bath with 50 mL of neutral detergent reagent (Goering and Van Soest, 1970), 2 mL of decahydronaphthalene, and 0.5 g of sodium sulfite. After 30 min, 50 mL of neutral detergent reagent was added and the mixture allowed to cool to 50–60 °C before the addition of 3 mL of 2% (w/v) filtered α-amylase aqueous solution (from *Bacillus subtilis*; G. B. Fermentation, Industries, Inc. Des Plaines, IL; 610 units/mg). After 15 min, the mixture was refluxed for another 30 min, in a boiling water bath, and the digest filtered through a tared sintered glass crucible (coarse porosity). The residue was rinsed with hot distilled water (90–100 °C), and the crucible was filled with hot water and 3 mL of α-amylase solution. After 15 min, filtration was resumed and the residue washed with hot distilled water (90–100 °C) followed by acetone until no further color is extracted. The residue was dried overnight at 100 °C and weighed. Neutral detergent extracts and hot water and acetone washings were collected for analysis to trace losses of pectic substances during NDF analyses.

Extraction of Total PS. The method used in the extraction of total PS was essentially that of Dekker and Richards (1972) with slight modifications. A 1-g sample (weighed to the nearest 0.1 mg) was dispersed in 20 mL of 0.25% aqueous solution of ammonium oxalate–oxalic acid and heated to 100 °C for 2 min to inactivate enzymes. After being cooled, the suspension was homogenized for 5 min in an Elvehjem homogenizer run with an electric drill. The homogenate was then refluxed in a boiling water bath for 1 h, cooled, and centrifuged at 12000 rpm for 20 min. The supernatant was collected and the residue subjected to further extraction. The process was repeated until all extractable PS were extracted.

Except for the amount of sample (50–200 mg), volume of oxalate solution (10 mL), and omission of the enzyme inactivation step, extraction of PS from ADF and NDF residues was essentially the same as that described above. In both cases, the optimum number of extractions was determined (see Results and Discussion). All extracts were stored in the refrigerator prior to PS analysis.

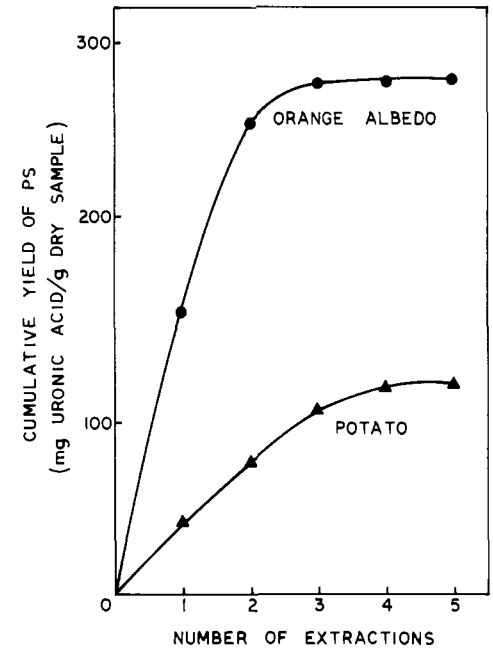

Figure 1. Successive oxalate extractions of pectic substances from potato and orange albedo.

Analysis of Total PS. Total PS in all oxalate extracts and extracts and washings from ADF and NDF analysis was first hydrolyzed with pectinase (Sigma Chemical Co.; EC 3.2.1.15) (Dekker and Richards, 1972). The galacturonic acid produced was determined by a modified carbazole method (Bitter and Muir, 1962) using glucuronolactone standards (4–40 μg/mL), and results are expressed as uronic acid.

Blank determinations contained all of the pectinase hydrolysis and carbazole reagents except for PS extracts or glucuronolactone solution, which was replaced by either oxalate solution (in the case of oxalate extracts) or detergent fiber extracting solutions (in the case of ADF and NDF extracts). The acetone wash was evaporated to dryness, and the residue taken up with water and analyzed for PS against a distilled-water blank.

RESULTS AND DISCUSSION

The completeness of extraction was first ascertained to obtain an accurate estimate of total PS in the original sample and in the detergent fiber residues. The results of successive oxalate extractions of total PS from potato and orange albedo and from their corresponding ADF and NDF residues are presented in Figures 1 and 2. It appears that most of the extractable PS is removed in the first three extractions. There was no apparent difference in the optimum number of extractions between the low PS (potato) and high PS (orange albedo) sample. The total PS values of potato and orange albedo recovered are comparable with reported values (Kertesz, 1951). The recommended sequence of steps for total PS extraction with oxalate from dry food samples is therefore as follows: (1) inactivation of enzymes, (2) homogenization, and (3) heat treatment under reflux at least 3 times. Because of the heat treatment involved in DF analysis, the enzyme inactivation step was omitted in the extraction of total PS from ADF and NDF.

The total PS compositions of apple, pear, onion, tomato, potato, wheat bran, and orange albedo are presented in Table I. As expected, total PS on a dry weight basis was highest in orange albedo and lowest in wheat bran. Al-

Table I. Pectic Substances and Total Dietary Fiber Composition of Some Fruits, Vegetables, and Wheat Bran and Pectic Substance Composition of Their Dietary Fibers

	apple	pear	tomato	carrot[b]	onion	potato[b]	wheat bran	orange albedo
% total pectic substances (PS)								
on dry weight basis	10.90	8.60	12.40	15.40	4.21	12.15	1.29	27.88
on fresh basis	1.81	1.31	0.72	1.59	0.53	2.78	1.15[c]	7.72
CV, %[d]	2.32	6.48	13.74	2.56	13.34	1.53	3.69	0.73
% total dietary fiber (DF)								
acid detergent fiber (ADF)								
on dry weight basis	4.71	9.40	13.91	9.89	5.60	2.67	11.06	19.97
on fresh basis	0.78	1.43	0.80	1.02	0.70	0.61	9.85[c]	5.53
CV, %	1.80	2.71	7.01	0.71	5.30	13.77	1.92	2.91
neutral detergent fiber (NDF)								
on dry weight basis	6.31	11.54	15.06	11.22	7.48	9.90	37.72	36.96
on fresh basis	1.05	1.76	0.87	1.16	0.94	2.26	33.63[c]	10.23
CV, %	6.05	6.62	4.13	3.02	10.21	1.71	3.34	3.21
% PS in DF								
ADF	7.78	3.39	12.82	17.14	4.60	39.20	0.86	69.00
CV, %	10.54	5.42	3.97	5.56	10.76	3.24	14.80	6.35
NDF	2.02	1.59	0.55	1.15	0.45	36.78	0.23	28.60
CV, %	2.28	8.00	12.85	12.30	9.43	1.38	12.30	3.95
% of total PS recovered as DF[a]								
ADF	3.39	3.60	14.40	11.01	6.13	8.61	7.37	49.40
NDF	1.19	2.10	0.65	0.71	0.80	6.16	6.72	37.90

[a] Values were calculated from averages of two determinations of percent total PS and percent PS in DF. [b] Edible portion only, peels excluded. [c] As received basis. [d] CV (%) = coefficient of variation = standard deviation/mean × 100.

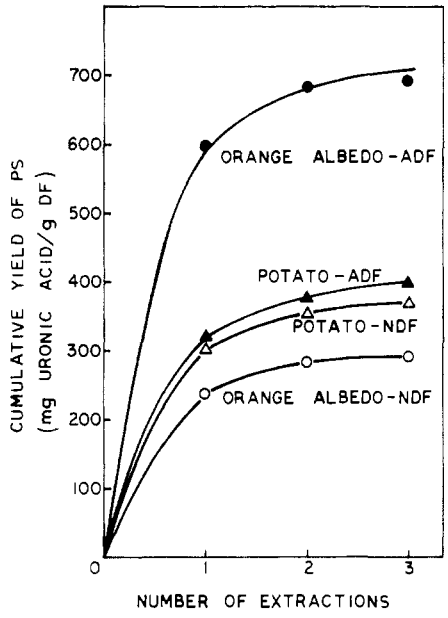

Figure 2. Successive oxalate extractions of pectic substances (PS) from ADF and NDF of potato and orange albedo.

though direct comparison with values in the literature is not valid, because of differences in variety, degree of ripeness, analytical method used, and other factors affecting PS composition, total PS values of fruits and vegetables are within the reported ranges (Kertesz, 1951). In both fruits and vegetables, the total PS was relatively low on a fresh weight basis although it represented quite a substantial proportion of the dry matter. The total PS value represents all polygalacturonides and it includes protopectin and pectinic and pectic acids as well as pectates and pectinates (Doesburg, 1965). It includes both water-soluble and water-insoluble PS.

Table I also shows ADF and NDF values and their total PS content. On a dry weight basis ADF was highest in orange albedo and lowest in potato. On the other hand, NDF was highest in wheat bran and lowest in apple. The relatively high amount of both ADF and NDF in the dried tomato sample could be attributed to the high amount of seeds present which were quite resistant to acid and neutral detergent extraction. In fruits and vegetables, although DF values represented quite a substantial proportion of the dry matter content, ADF and NDF values were very low on a fresh weight basis. NDF values were consistently higher than ADF values, suggesting that more DF components were solubilized by acid detergent. It is interesting to note that the percent total PS of apple, carrot, and potato are higher than either ADF or NDF. According to Goering and Van Soest (1970), extraction of plant tissue with neutral solutions of sodium lauryl sulfate and EDTA is essentially nonhydrolytic and removes soluble carbohydrates, proteins, and lipids as detergent complexes. The residue (NDF) is essentially lignin, cellulose, and hemicellulose. The residue after heat treatment with 1 N H_2SO_4 and 2% cetyltrimethylammonium bromide (i.e., ADF), however, represents only lignin and cellulose. The difference between these two values is generally used to estimate the hemicellulose component of the noncellulosic polysaccharides (Southgate, 1976).

In this study, a rapid α-amylase treatment for analyzing NDF was used. Treatment with heat-resistant α-amylase from *B. subtilis* during digestion and filtration of the NDF residue resulted in complete removal of starches. NDF residues from high-starch samples (potato and wheat bran) were negative to the iodine test. The treatment also facilitated the filtration process which is a major problem in the original NDF procedure when applied to a high-starch sample (Van Soest, 1963). This modification is more rapid than the current AACC (1978) method for NDF analysis where heat-labile hog pancreas α-amylase is used.

Total PS content of DF varied among samples with the highest being 69.0% in orange albedo ADF and the lowest 0.23% in wheat bran NDF (Table I). ADF showed consistently higher total PS than NDF. This plus the high proportion of total PS in the original sample recovered in ADF indicates that more PS was solubilized and extracted during NDF determination. This could be attributed partly to the presence of a significant amount of pectic acid or low-methoxyl pectinic acids in these samples. These substances are insoluble under acid condition (Doesburg, 1965) and therefore remained in acid detergent residue during ADF analysis. However, at higher pH (>6) and in

Table II. Relative Proportion of Total PS in Dry Potato Recovered in Detergent Fiber, Detergent Extracts, and Hot Water and Acetone Washings during ADF and NDF Analysis

	detergent fiber, %	
	ADF	NDF
original total PS sample[a]	100	100
% of total PS recovered in detergent extract	89.6	93.2
% of total PS recovered in hot water wash[a]	5.2	6.6
% of total PS recovered in acetone wash[a]	0.0	0.0
% of total PS recovered in detergent fiber[a]	8.6	6.2

[a] Values are averages of two determinations.

the presence of sequestering agents (EDTA) as in the NDF procedure, these low-methoxyl pectic substances are solubilized and extracted readily (Doesburg, 1965). The presence of EDTA can prevent flocculation of low-methoxyl PS by polyvalent cations such as calcium, thus enhancing solubilization which can result in low total PS recovery in NDF. Evidence in the literature also points to the removal of calcium and the ester group by acid detergent to produce a highly acidic and insoluble polygalacturonic acid as a possible explanation for this effect (Bailey and Ulyatt, 1970).

The proportion of original total PS recovered as DF was highest in orange albedo which had the highest total PS. About 50.0 and 38.0% of the total PS in orange albedo were recovered in ADF and NDF, respetively. The next three samples highest in percent total PS (carrot, tomato, and potato) also showed relatively higher recoveries of PS in ADF but not in NDF compared to the others. This trend of higher PS recoveries in ADF in high-pectin samples needs to be confirmed with analyses of more samples with a wide range of pectin content. Recoveries of PS in fruits, vegetable, and wheat bran were, however, consistently low, ranging from 3.0 to 15.0% and 0.6 to 7.0% for ADF and NDF, respectively. It appeared that the bulk of the total PS was solubilized and extracted during ADF and NDF analysis.

Detergent extracts and hot water and acetone washings were collected and analyzed to account for the losses of total PS during DF determination. Results obtained for potato are summarized in Table II. In both DF methods, the major portion of total PS was recovered in the detergent extracts. Only a small proportion was recovered in the hot water washings and none in the acetone wash. The relative proportion of total PS recovered in the detergent extract and hot water washings was slightly higher in NDF than in ADF, which further indicates that more PS was solubilized in the NDF method. The proportion of total PS in extracts and washings was 94.8 and 99.8% for ADF and NDF of potato, respectively.

Taken together, these data suggest that ADF and NDF methods which are increasingly used for DF analysis underestimate PS as dietary fiber components. The degree of underestimation is slightly higher in NDF than in ADF. Our values for total PS recovery in ADF are lower than those obtained by Bailey and Ulyatt (1970) with pasture grasses and legumes. They showed that the neutral detergent does solubilize practically all of the total PS whereas the acid detergent solubilizes only ~50% of them.

In conclusion, our results emphasize that the detergent methods which are increasingly used to report dietary fiber values underestimate PS, a component of dietary fiber complex with important physiological and nutritional effects. Therefore, more attention should be directed toward separate analysis of pectin as a dietary fiber component or modifications of existing methods to reflect pectin accurately.

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