Recovery of Lignin During Nonstarch Polysaccharide Analysis[1]

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ABSTRACT

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Lignin occurs naturally with nonstarch polysaccharides (NSP) in plants and may possess physiological functions when consumed. Since NSP methodology does not include lignin, the dietary fiber values obtained using this method may be lower than AOAC total dietary fiber values for foods containing lignin. Therefore the residue from NSP analysis of 11 foods was recovered gravimetrically and compared with apparent lignin determined by both Klason and permanganate methods. Whereas the NSP residue value was generally higher than values obtained by these methods, correlation was high ($r = 0.86$, Klason; $r = 0.91$, permanganate). Residue recovery from finely ground AACC wheat bran was significantly lower ($P < 0.05$) from bran that passed a no. 20 or no. 10 screen. Toasting did not affect residue recovery from whole wheat bread. The recovery of lignin during NSP analysis represents savings in time and reagents over separate analysis for lignin.

The measurement of dietary fiber in foods is a complex issue involving not only choice of analytical method, but also the definition of fiber (Marlett, 1990, Cummings and Englyst 1991). While there is little disagreement that cellulose, hemicelluloses, gums, and pectins should be measured as fiber, Cummings and Englyst (1991) oppose the inclusion of digestion-resistant starch, lignin, cell wall proteins, and other nonpolysaccharides in the definition of dietary fiber. The nonstarch polysaccharide (NSP) procedure advocated by Englyst and coworkers typically produces lower dietary fiber values than those obtained by gravimetric methods (Mongeau and Brassard 1986). This difference has been attributed primarily to the omission of lignin. Despite this disadvantage, Englyst's colorimetric procedure offers fairly rapid analysis (Englyst and Hudson 1987). We favor the inclusion of lignin in the dietary fiber value and therefore have modified Englyst's procedure in order to recover lignin as the acid-insoluble residue remaining after polysaccharide hydrolysis. Theander and Westerlund (1986), Southgate (1969), and others have also recovered lignin in a similar fashion, but comparisons with other lignin methods are lacking.

Lignin is an amorphous polyphenol of high molecular weight that is a constituent of plant cell walls (Higuchi 1980). The close proximity of lignin to NSPs in plant cell walls may have led to the initial findings of health benefits from fiber consumption. In vitro studies have found that purified lignin binds various bile acids (Eastwood and Hamilton 1968, Story and Kritchevsky 1976, Vahouny et al 1980, Calvert and Yeates 1982). Lignin has also been shown to bind significant amounts of cholesterol (Balmer and Zilversmit 1974) and bile acids in vivo (Gallagher and Schneeman 1986). Other possible health benefits of lignin consumption include inhibition of colonic carcinogenesis (Newmark 1987) and enhanced absorption of vitamin A (Catignani and Myers 1989).

MATERIALS AND METHODS

Materials

Whole wheat flour (Gold Medal, General Mills, Inc., Minneapolis, MN), and rice bran (Rite-Bran, Uncle Ben's, Houston, TX) were used as is. Fresh broccoli flowerets, and Bosc pears were chopped in a Waring Blendor, freeze-dried, then ground with a mortar and pestle to pass a no. 20 mesh screen. Canned pears (Shop'N'Save Bartlett pear halves in unsweetened pear juice concentrate, Hannaford Bros., Scarborough, ME) were first drained, then processed as for the other produce. Kenmei and All-Bran breakfast cereals (Kellogg's, Battle Creek, MI), Sunchips (regular flavor, Frito-Lay, Inc., Dallas, TX), dulse (*Rhodymenia palmata*, Maine Sea Coast Vegetables, Franklin, ME), and whole wheat bread (Country Kitchen stoneground 100% whole wheat flour with molasses and bran, F.R. LePage Bakery, Inc., Auburn, ME) were ground with a blender to pass a no. 20 screen. The bread was also toasted on "light" and "dark" toast settings, and then each type of toast was ground as above. Hard red wheat bran (AACC, St. Paul, MN) was sifted through a no. 10 screen to obtain a fraction with large particles. Additional bran from the same lot was ground in a blender to pass through no. 20 and 30 screens. Moisture, in triplicate for all materials as is and for freeze-dried broccoli and pears, was determined by loss in weight after drying overnight at 102°C in a forced-air oven.

NSP Procedure

Englyst and Cumming's (1988) method was modified as shown in Figure 1. Porcine pancreatin (Sigma 1675, St. Louis, MO) was used as the source of α-amylase for starch removal. The pancreatin also contained proteolytic and lipolytic enzymes that may have assisted in efficient removal of starch. NSP and uronic acids were determined colorimetrically from the hydrolysate (Englyst and Cummings 1988).

Additional modifications were made in the analysis of All-Bran cereal. This food was selected for additional study because lignin and fiber values have been reported for it by Anderson and Bridges (1988). For one set of triplicate samples, residues in crucibles were washed with 85% ethanol, then with several volumes of acetone instead of water. Anderson and Clydesdale (1981) and Anderson and Bridges (1988) recovered apparent lignin in a similar fashion but used aqueous ethanol rinses followed by washings with ether to remove sugar residues. Another set of samples was not filtered after hydrolysis, but the hydrolysate was removed by aspiration with a dropping pipet. Next, 40 ml of 85% ethanol was added to each tube, stirred for 10 min, then centrifuged at 1,500 × *g* for 10 min. The resulting supernatant was removed by aspiration, and 40 ml of acetone was added. The tubes were handled as for the ethanol addition; then the stir bar was removed. After being placed in a 65°C water bath to evaporate the remaining acetone, the tubes were dried overnight at 102°C. Tubes were weighed after cooling, and lignin was calculated as: [(tube weight + residue weight) − empty tube weight]/(sample weight × % dry weight).

Klason Lignin

The Klason lignin procedure measures Maillard reaction products, condensed polyphenols such as tannins, some cell wall proteins, and cuticular waxes in addition to true lignin (Dreher 1987). Silica is also recovered with these compounds after acid hydrolysis, but the ashing step allows this material to be subtracted from the weight of the crucible contents.

All samples, with the exception of the freeze-dried produce and wheat bran, were air-dried at 102°C before analysis. Rice bran and Sunchips were defatted with 50 ml of acetone in 600-ml tall-form beakers. After 1 hr with stirring, the acetone was drawn off with a Pasteur pipet, and the remaining solvent

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was allowed to evaporate at room temperature overnight. Acid detergent fiber (ADF) residues were prepared from triplicate 1-g samples, hydrolyzed with 72% sulfuric acid for 3 hr, dried at 102°C, weighed, and finally ashed at 425°C for 5 hr to determine Klason lignin (Van Soest 1963). Decahydronapthalene was not used as an antifoaming agent.

Permanganate Lignin

Triplicate ADF residues were prepared as above, then dried overnight and weighed. Lignin was solubilized with buffered potassium permanganate solution according to the method of Van Soest and Wine (1967). In this procedure, lignin is oxidized and removed by filtration, leaving silica, minerals, and cell wall proteins as residue in the crucible. Crucibles containing the permanganate residue were dried overnight, and the difference in weight between the ADF and the permanganate residues was recorded as permanganate lignin.

Statistical Analyses

Lignin values obtained by the three methods were compared using analysis of variance for each food. Duncan's analysis ($P = 0.05$) was applied to means with significant differences. Likewise, values obtained for the various levels of toasting and wheat bran particle size were compared. Mean values for each food by method were correlated using simple regression (Number Cruncher Statistical System, Kaysville, UT).

Weigh sample to 0.1 mg (50-175 mg)
in a 50-ml centrifuge tube
Defat samples containing >5% lipid with acetone
↓
Add stir bar + 2 ml DSMO
↓
Stir 1 hr, 100°C
↓
Add 8 ml 50°C buffer; cool
↓
Add 0.5 ml pancreatin extract
(250 mg Sigma 1675 + 9 ml H_2O)
Add 0.1 ml pullulanase solution
↓
Shake for 16-18 hr at 42.2°C
↓
Add 40 ml ethanol, stand for 1 hr
Centrifuge for 10 min,
remove supernatant by aspiration
↓
Extract twice with 85% ethanol,
then acetone, as above
↓
Add 2 ml 12*M* H_2SO_4;
stir for 1 hr at 35°C
↓
Add 22 ml H_2O; stir 2 hr at 100°C
↓
Remove aliquots for colorimetric determination
of NSP and uronic acids
↓
Filter residue through coarse frit pyrex crucible
Wash with 5 volumes of H_2O; scrape tube for residue
↓
Dry crucible overnight at 102°C; weigh after cooling
↓
Ash crucible 4 hr at 425°C, weigh

$$\% \text{ lignin} = \frac{\text{Weight after drying - Weight after ashing}}{\text{Weight of original sample}} \times 100\%$$

Fig. 1. Nonstarch polysaccharide residue recovery procedure.

RESULTS AND DISCUSSION

NSP residue values were generally highest and permanganate values lowest (Table I). Overall, NSP residue was significantly correlated with both the permanganate (Fig. 2) and Klason (Fig. 3) lignin values obtained for the same foods. Values for NSP residues from whole wheat flour and rice bran were nearly twice as high as the values for permanganate and Klason lignin. Permanganate and NSP residue values agreed for broccoli, but we were unable to obtain consistent Klason lignin values for this material. Dulse was included in the study because lower plants do not produce lignin. The lignin that was apparently recovered from this material may have been other polyphenolic compounds or lignin precursors that were susceptible to permanganate oxidation (Sarkanen and Ludwig 1971).

Englyst and Cummings (1991) oppose the inclusion of lignin

TABLE I
Lignin and Water Contents (%)[a]

Sample	NSP[b] Residue	Permanganate Lignin	Klason Lignin	Moisture
Whole wheat flour	2.0 ± 0.2 a	0.9 ± 0.1 b	1.0 ± 0.0 b	7.6
Rice bran	7.7 ± 0.4 a	4.5 ± 0.4 c	5.3 ± 0.3 b	7.1
Broccoli	2.2 ± 0.1 a	2.1 ± 0.6 a	ND[c]	86.8
Fresh pears	2.2 ± 0.4 a	1.4 ± 0.1 b	1.3 ± 0.1 b	83.0
Canned pears	1.5 ± 0.3 a	1.9 ± 0.3 a	1.8 ± 0.0 a	86.7
Kenmei	1.8 ± 0.3 a	2.1 ± 0.1 a	3.4 ± 0.3 a	3.4
All-Bran	4.9 ± 0.7 a	3.3 ± 0.1 b	2.8 ± 0.0 b	2.7
Sunchips	0.4 ± 0.1 b	0.7 ± 0.2 b	1.8 ± 0.1 a	3.0
Dulse	0.6 ± 0.5 b	1.1 ± 0.3 a	0.6 ± 0.1 b	5.4
Wheat bread	3.1 ± 0.0 b	1.9 ± 0.1 c	3.5 ± 0.2 a	30.7
Light toast	3.4 ± 0.5 a	1.0 ± 0.2 b	3.1 ± 0.4 a	17.8
Dark toast	3.7 ± 0.8 a	1.3 ± 0.2 b	3.1 ± 0.3 a	14.2
Wheat bran				
No. 10[d]	7.6 ± 0.5 a	5.1 ± 0.2 b	6.7 ± 0.7 a	7.3
No. 20[d]	6.9 ± 0.6 a	4.3 ± 0.1 b	4.1 ± 0.6 b	6.7
No. 30[d]	5.6 ± 0.5 b	4.0 ± 0.0 c	7.0 ± 0.7 a	6.6

[a] Means ± SD (dry weight basis) followed by different letters within rows are significantly different ($P = 0.05$, Duncan's test). The letter "a" within a row denotes the higher value or indicates that all lignin values for a food are not different. Differences due to toasting and grinding are presented in the text.
[b] Nonstarch polysaccharides
[c] Not determined.
[d] Ground to pass a U.S. no. 10, 20, or 30 sieve.

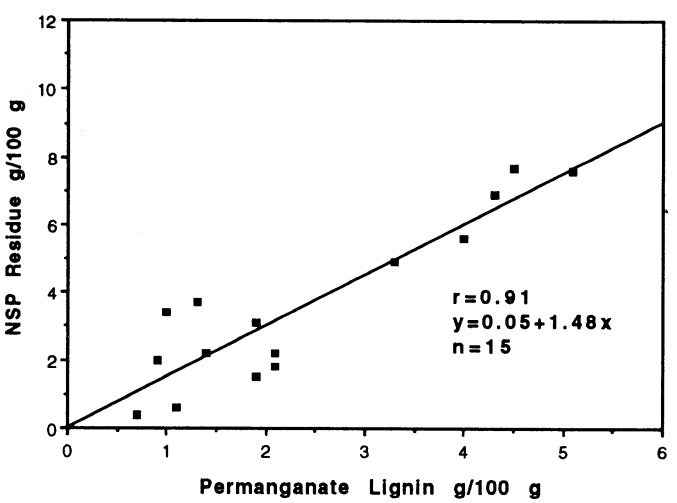

Fig. 2. Correlation of mean nonstarch polysaccharide (NSP) residues with permanganate lignin values for 15 foods on a dry weight basis.

with fiber values in part because Maillard reaction and other browning compounds may be measured by Klason lignin. NSP residue and Klason lignin values for bread were not influenced significantly by toasting, which should have produced acid-insoluble compounds. Nevertheless, permanganate lignin values for the bread and toast were 1–2% less than the other lignin values obtained for those foods.

The size of food particles affects dietary fiber recovery in that enzymes and acid used to remove polysaccharides may be prevented from reaching the center of the particles. This undigested material is then detected as fiber or lignin. We attempted to measure the ability of the NSP procedure to overcome this problem by grinding wheat bran and measuring the lignin from the different-sized fractions. NSP residue from the largest bran was significantly higher ($P \leq 0.01$) than that from bran that passed a no. 30 screen. However, permanganate lignin values followed a similar trend, suggesting that the true lignin content was different between samples, perhaps due to inherent differences in composition among bran particles of different sizes or to errors in sampling or grinding. Since starch was not measured in these samples, we do not know whether the smaller particle size fractions

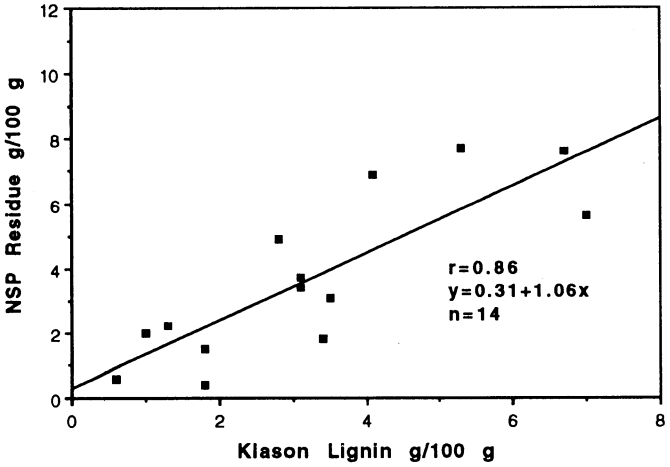

Fig. 3. Correlation of mean nonstarch polysaccharide (NSP) residues with Klason lignin values for 14 foods on a dry weight basis.

TABLE II
Nonstarch Polysaccharide (NSP) and Uronic Acids[a]

Sample	Total NSP	Uronic Acids	Total Fiber[b]	Total AOAC Dietary Fiber[c]
Whole wheat flour	11.21 ± 0.32	0.59 ± 0.04	13.78 ± 0.18	11.8[d]
Rice bran	25.26 ± 0.58	2.07 ± 0.32	35.07 ± 0.43	31.7[d]
Broccoli	19.61 ± 2.40	5.16 ± 0.37	26.95 ± 2.88	31.2[e]
Fresh pears	11.14 ± 0.45	4.68 ± 0.19	17.98 ± 0.16	...[f]
Canned pears	13.60 ± 1.48	6.02 ± 0.53	21.10 ± 1.26	...
Kenmei	7.15 ± 0.32	0.17 ± 0.01	9.14 ± 0.64	3.5[d]
All-Bran	35.19 ± 1.96	0.63 ± 0.4	40.71 ± 2.66	49.3[d]
Sunchips	6.40 ± 0.15	0.34 ± 0.04	7.12 ± 0.27	<7.0[d]
Dulse	30.08 ± 0.52	0.73 ± 0.16	31.38 ± 0.57	...
Wheat bread	13.52 ± 0.35	0.48 ± 0.15	17.11 ± 0.46	...
Light toast	13.20 ± 2.21	0.34 ± 0.06	16.68 ± 0.21	...
Dark toast	13.21 ± 2.59	0.21 ± 0.19	16.71 ± 3.10	...
Wheat bran				
No. 10[g]	32.26 ± 0.50	2.10 ± 0.12	41.91 ± 0.28	42.7[d]
No. 20[g]	30.07 ± 0.30	2.39 ± 0.08	39.39 ± 0.87	...
No. 30[g]	31.10 ± 0.47	2.42 ± 0.15	39.05 ± 0.90	...

[a] Means ± SD (dry weight basis).
[b] Sum of NSP, uronic acids, and NSP residue per sample.
[c] As determined by method 985.29 (AOAC 1990).
[d] Data obtained from manufacturer.
[e] Mongeau and Brassard (1986).
[f] Total dietary fiber not available.
[g] Ground to pass a U.S. no. 10, 20, or 30 sieve.

contained more endosperm than did the bran that passed a no. 10 screen. Sieving could have removed starch-containing endosperm particles from the larger bran fraction, thereby concentrating more lignin and NSP in the no. 10 screen bran.

The inclusion of NSP residue with NSP and uronic acids gave a total dietary fiber value that is compared in Table II with published AOAC total dietary fiber (TDF) values for several foods. Some differences between the TDF values for some foods may be explained by natural variation among samples. Correlation between the two TDF values for the seven foods listed was high ($r = 0.97$, $P \leq 0.001$, $y = 4.44 + 0.81x$). Although TDF also measures resistant starch, these results indicate that the inclusion of the residue from NSP analysis as apparent lignin brings NSP values much closer to TDF values for foods containing lignin.

Modifications of the procedure for All-Bran offer some improvements in reducing NSP residue values to levels closer to those of permanganate lignin. NSP residue recovery after washing with 85% ethanol and acetone was 3.2 g per 100 g, dry weight, which was not significantly different from the permanganate and Klason lignin values (Table I). This value was also in agreement with that reported by Anderson and Bridges (1986). We originally washed the NSP residue with water to reduce the use of organic solvents in the laboratory and to minimize losses of lignin. Sarkanen and Ludwig (1971) reported that thermal processing increased the solubility of lignins in nonpolar solvents, and we suspected that the 2-hr NSP hydrolysis at 100° C could produce such changes in the lignin. Retention of NSP residue in the centrifuge tubes used during NSP analysis proved to be less successful, since a significantly higher ($P \leq 0.05$) amount, 6.4 g/100 g, was found. The standard deviation for this method was also high (1.1 g/100 g).

In summary, the recovery and measurement of the residue from nonstarch polysaccharide analysis provides a value similar to lignin values obtained from the acid detergent residue of foods. The value obtained by adding this NSP residue to the NSP and uronic acid values approximates the total dietary fiber values obtained using the AOAC procedure. The use of 80–85% aqueous ethanol rather than water for the final washings of the NSP residue in coarse-fritted crucibles followed by drying with acetone may remove additional carbohydrates without loss of lignin.

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LITERATURE CITED

ANDERSON, J. W., and BRIDGES, S. R. 1988. Dietary fiber content of selected foods. Am. J. Clin. Nutr. 47:440.

ANDERSON, N. E., and CLYDESDALE, F. M. 1980. An analysis of the dietary fiber content of a standard wheat bran. J. Food Sci. 45:336.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1990. Official Methods of Analysis, 15th ed. The Association: Washington, DC.

BALMER, J., and ZILVERSMIT, D. B. 1974. Effects of dietary roughage on cholesterol absorption, cholesterol turnover and steroid excretion in the rat. J. Nutr. 104:1319.

CALVERT, G. D., and YEATES, R. A. 1982. Adsorption of bile salts by soya-bean flour, wheat bran, lucerne (*Medicago sativa*), sawdust and lignin; the effect of saponins and other plant constituents. Br. J. Nutr. 47:45.

CATIGNANI, G. L., and MYERS, K. H. 1989. Increase in the bioavailability of vitamin A in the rat by dietary lignin. Pages 365-367 in: Nutrient Availability: Chemical and Biological Aspects. D. A. T. Southgate, I. T. Johnson, and G. R. Fenwick, eds. Royal Society of Chemistry, Cambridge, England.

CUMMINGS, J. H., and ENGLYST, H. N. 1991. What is dietary fibre? Trends Food Sci. Technol. 2:99.

DREHER, M. L. 1987. Handbook of Dietary Fiber. Marcel Dekker, Inc.: New York.

EASTWOOD, M. A., and HAMILTON, D. 1968. Studies on the adsorption of bile salts to non-absorbed components of the diet. Biochim. Biophys. Acta 152:165.

ENGLYST, H. N., and CUMMINGS, J. H. 1988. Improved method for measurement of dietary fiber as non-starch polysaccharides in plant foods. J. Assoc. Off. Anal. Chem. 71:808.
ENGLYST, H. N., and HUDSON, G. J. 1987. Colorimetric method for routine measurement of dietary fibre as non-starch polysaccharides. A comparison with gas-liquid chromatography. Food Chem. 24:63.
ENGLYST, H. N., CUMMINGS, J. H., and WOOD, R. 1987. Determination of dietary fibre in cereals and cereal products—Collaborative trials. Part III: Study of further simplified procedures. J. Assoc. Publ. Anal. 25:73.
GALLAGHER, D., and SCHNEEMAN, B. O. 1986. Intestinal interaction of bile acids, phospholipids, dietary fibers, and cholestyramine. Am. J. Physiol. 250(13):G420.
HIGUCHI, T. 1980. Lignin structure and morphological distribution in plant cell walls. Pages 1-19 in: Lignin Degradation: Microbiology, Chemistry, and Potential Applications, Vol. I. T. X. Kirk, T. Higuchi, and H.-M. Chang, eds. CRC Press: Boca Raton, FL.
KRIS, E. S., and MARLETT, J. A. 1990. Measuring lignin and neutral sugars in neutral detergent soluble and insoluble fractions of human and rat feces. J. Agric. Food Chem. 38:1218.
MARLETT, J. A., and CHESTERS, J. G. 1985. Measuring dietary fiber in human foods. J. Food Sci. 50:410.
MARLETT, J. A., CHESTERS, J. G., LONGACRE, M. J., and BOGDANSKE, J. J. 1989. Recovery of soluble fiber is dependent on the method of analysis. Am. J. Clin. Nutr. 50:479.
MONGEAU, R., and BRASSARD, R. 1986. A rapid method for the determination of soluble and insoluble dietary fiber: Comparison with AOAC total dietary fiber procedure and Englyst's method. J. Food Sci. 51:1333.
NEWMARK, H. L. 1987. Plant phenolics and inhibitors of mutational and precarcinogenic events. Can. J. Physiol. Pharmacol. 65:461.
SARKANEN, K. V., and LUDWIG, C. H. 1971. Lignins. Wiley-Interscience: New York.
SOUTHGATE, D. A. T. 1969. Determination of carbohydrate in foods. II. Unavailable carbohydrates. J. Sci. Food Agric. 20:331.
STORY, J. A., and KRITCHEVSKY, D. 1976. Comparison of the binding of various bile acids and bile salts in vitro by several types of fiber. J. Nutr. 106:1292.
THEANDER, O., and WESTERLUND, E. A. 1986. Studies on dietary fiber. 3. Improved procedures for analysis of dietary fiber. J. Agric. Food Chem. 34:330.
VAHOUNY, G. V., TOMBES, R., CASSIDY, M. M., KRITCHEVSKY, D., and GALLO, L. L. 1980. Dietary fibers: V. Binding of bile salts, phospholipids and cholesterol from mixed micelles by bile acid sequestrants and dietary fibers. Lipids 15:1012.
VAN SOEST, P. J. 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J. Assoc. Off. Anal. Chem. 46:829.
VAN SOEST, P. J., and WINE, R. H. 1967. Methods for determination of lignin-cellulose and silica. J. Anim. Sci. 26:940.

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