

DIETARY FIBER METHODOLOGIES: STATUS AND CONTROVERSIAL ISSUES

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Before 1986, the United States did not have an official method for the determination of total dietary fiber (TDF). Even though the Association of Analytical Chemists (AOAC) had a *Crude Fiber and an Acid Detergent Fiber methods*; the American Association of Cereal Chemists (AACC) had the *Neutral Detergent Fiber method*, none of these methods provided complete data on dietary fiber content of foods.

An enzymatic-gravimetric method for TDF was officially adopted by AOAC after two international collaborative studies which were conducted between 1982 and 1984 (1,2). This method is based on several earlier methods which employ various enzymes to remove starch and protein followed by isolation of the remaining residue gravimetrically with or without alcohol precipitation. Meanwhile, an enzymatic-chemical method has been developed by Southgate (3) in the late 1960's and recently modified by Englyst (4). It is also known as a fractionation procedure, in which the starch free residue is hydrolyzed in acid and the resulting monosaccharides are measured either calorimetrically or chromatographically.

Several years ago, Hollman and Katan (5,6) of the Netherlands organized a collaborative study - Eurofoods Interlaboratory Trial 1985 - "to determine the influence of differences in analytical and other procedures in laboratories that contribute to food tables, on the nutrient values in these tables." Twenty laboratories took part in this study; each used its preferred method to analyze six foods for any of the following food components: protein, fat, available carbohydrates, dietary fiber (DF) and ash. Fourteen of the collaborators reported DF values. The methods they used are outlined in Table 1, and the results for three of the foods are given in Table 2-4. From these data, one would conclude that TDF values are method dependent. However, those enzymatic-gravimetric methods, which only correct for residual ash and not residual protein, consistently give higher values while those without alcohol precipitation tend to give lower values similar to the enzymatic-chemical method and the neutral detergent fiber method.

Despite the fact that we now have an AOAC-approved method for total dietary fiber determination, there are still problems inherent in this enzymatic-gravimetric procedure. For example, the analytical variability is quite high within a number of laboratories, partly due to the use of phosphate buffer, and the method requires three pH-adjustment steps, which makes it rather tedious. Recently, a modified and slightly simplified procedure has been proposed by Lee (7) with the use of MES-TRIS buffer and a collaborative study is now underway for TDF, soluble and insoluble dietary fiber analyses.

Another simplified enzymatic-gravimetric method had been developed by Li (8). It is based on the same principles as the AOAC method, but uses a different approach; starch is removed by the use of a single enzyme (amyloglucosidase) and no attempt is made to remove any of the protein in the original sample, thus whatever protein remains in the residue is corrected based on a Kjeldahl nitrogen determination. Figure 1 is a schematic diagram depicting the three versions of the enzymatic-gravimetric procedure.

Currently, there are other published methods which may be considered either as enzymatic-gravimetric or enzymatic-chemical method. Brief descriptions of some of the methods are given below.

Enzymatic-Gravimetric Methods

Mongeau (9) of Canada has developed a rapid gravimetric method to determine soluble dietary fiber (SDF) in food samples which are autoclaved, treated with a heat stable amylase, amyloglucosidase, and protease to remove

protein and starch. Soluble fiber is isolated after filtration and precipitated by the addition of absolute ethanol. Insoluble dietary fiber (IDF) is obtained using the Neutral Detergent Fiber procedure. The sum of SDF and IDF is taken to be the value for total dietary fiber. Only ash and no crude protein determination is required for either of the procedures.

Jeraci and co-workers have developed a new method known as the urea enzymatic dialysis procedure. "It utilizes 8M urea to hydrate and extract starch and other water-soluble polysaccharides, a heat-stable amylase and dialysis to remove starch, and protease digestion of plant proteins..... This method quantitatively recovered, with less variation, more of the purified and semipurified dietary fiber products." (10) A collaborative study was conducted in 1989 and the results are being evaluated statistically.

Enzymatic-Chemical Methods

Englyst (11) modified the Southgate procedure by the use of dimethyl-sulfoxide to solubilize starch (including resistant starch) before enzyme hydrolysis. The non-starch polysaccharides isolated after addition of ethanol are hydrolyzed in sulfuric acid to the monosaccharides, which are quantified as their alditol acetates by gas-liquid chromatography. The hydrolyzate is also reacted with dimethylphenol and analyzed calorimetrically for uronic acids. Lignin is, intentionally, not included in this method. By Englyst's definition, dietary fiber is synonymous with non-starch polysaccharides.

Theander has proposed three related methods, A-C, for analysis and chemical characterization of dietary fiber, which by his definition is the sum of non-starch polysaccharides and Klason lignin. "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." (12)

Comparison of the Methods

A mixed diet was prepared at the Beltsville Human Nutrition Research Center and distributed by the National Institute of Standards and Technology (formally NBS) as Reference Material 8431. As part of its characterization, the mixed diet was analyzed for its TDF content by several laboratories using various methods. Table 5 shows a wide range of values, of which those from Crude Fiber and Neutral Detergent Fiber methods obviously represent underestimations of the dietary fiber content of this diet material. The difference between Englyst's value and those of Theander or the enzymatic-gravimetric values is due mainly to the exclusion of lignin.

The Human Nutrition Information Service of USDA conducted a study in 1988-89 in which 25 foods in blind duplicates were sent to six laboratories for dietary fiber analysis. Each laboratory used the AOAC/TDF method and one of the three other methods mentioned above; namely, Englyst, Mongeau and Li's Simplified Method. Among the foods, there were several that were very difficult to analyze for dietary fiber content, e.g. pie crust, peanuts, popcorn and raised donuts. However, preliminary evaluation of the AOAC/TDF data from four laboratories for all 25 foods provided a mean value ranging from 12.73 to 13.62 g/100g dry weight, with a coefficient of variation of 14.8. Total dietary fiber values for 8 of the 25 foods as determined by the AOAC method are shown in Table 6. Data on the soluble and insoluble fractions of dietary fiber in these same foods from four different methods will be presented later.

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Except for Mongeau's method, all the others were developed originally for the determination of TDF with alternative steps for the fractionation of soluble and insoluble dietary fiber components. Englyst method provides an extraction step, which removes the soluble fiber fraction with buffer, thus the remaining fraction is taken to be the insoluble dietary fiber. The difference between TDF and IDF will be SDF. The latest versions of AOAC (13) and Simplified (14) procedures separate the IDF and SDF by filtration before the addition of 95% ethanol to precipitate the soluble fiber components from the filtrate. The two fractions are isolated gravimetrically, then corrected for crude protein and ash contaminations. Values for soluble and insoluble dietary fiber content for eight selected foods from the HNIS study are presented in Table 7. The absolute and relative amounts of SDF and IDF in a given food vary tremendously between methods and sometimes even within a method. At present, we have some understanding of the differences between methods for TDF, e.g. the exclusion of lignin leads to lower TDF values with the Englyst method for foods other than fruits and vegetables while the incomplete removal of protein will give higher TDF values with the Mongeau method for soy products. However, the differences for SDF and IDF are more random and difficult to predict. We have observed that among other things, the actual porosity of crucibles and the particle-size of Celite (filtering aid) may affect the overall filtration time, hence the proportion of soluble to insoluble fractions as they are being separated during the first filtration step of any enzymatic-gravimetric procedure.

In general, enzymatic-gravimetric methods are more suited for routine analyses, but they do not provide detailed information on fiber components. On the other hand, enzymatic-chemical methods are more difficult, require highly skilled analysts and more expensive instruments; such as gas-liquid or high-performance liquid chromatographs in order to obtain individual sugar composition of the non-starch polysaccharides. Furthermore, the determination of lignin and uronic acid content of most fiber containing foods is still imprecise and needs much improvement.

The operational definition of dietary fiber is still controversial; however, many analysts agree that lignin and resistant starch should be considered as part of dietary fiber. Even though we have no clear knowledge of the actual physiological roles of these food components, they are known to resist digestion in the mammalian upper gastrointestinal tract, a characteristic originally attributed to dietary fiber.

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SIMPLIFIED

AQAC

Sample (0.5 g)
25mL deionized H₂O

autoclave at 121 °C, 1 h
cool to 60° C

0.15 mL amyloglucosidase, 0.85 mL H₂O
1.0 mL acetate buffer (pH 4.8)

incubate at 55 °C, 2 h

Sample (1 g)
50 mL phosphate buffer (pH 6.0)
{40 mL MES-TRIS buffer }
0.2 mL heat stable α -amylase

incubate at 100 °C, 30 min
cool, adjust pH to 7.5
{no pH adjustment}

0.1 mL of 50 mg protease/mL phosphate
buffer

incubate at 60 °C, 30 min
cool, adjust pH to 4.5

0.3 mL amyloglucosidase

incubate at 60 °C, 30 min

4 volumes of 95% ethanol

leave at room temperature 1 h
filter through crucible
rinse with ethanol and acetone
dry in oven at 105 °C overnight
weigh crucible + content

TOTAL DIETARY FIBER
containing protein & ash

protein determination
(Kjeldahl)

ash determination
(525 °C for 5 h)

Figure 1 Total Dietary Fiber Determination

Table 1

Dietary Fiber Methods - Eurofoods Interlaboratory Trial 1985

ENZYMATIC-GRAVIMETRIC METHODS

L. Prosky et al. J. Assoc. Off. Anal. Chem. 67, 1044 (1984)
gelatinize 15 min, 100 °C with Termamyl
digest 30 min, 60 °C with protease
30 min, 60 °C with amyloglucosidase
precipitate with ethanol, filter, dry and weigh
(correct for ash and protein)

N.-G. Asp et al. J. Agric. Food Chem. 31, 476 (1983)
gelatinize 30 min, 100 °C with Termamyl
digest 60 min, 40 °C with pepsin
60 min, 40 °C with pancreatine
(correct for ash and protein)

T. F. Schweizer et al. J. Sci. Food Agric. 30, 613 (1979)
extract with boiling 80% ethanol
gelatinize 60 min, 120 °C in water
digest 20 hrs, 37 °C with pepsin
18 hrs, 37 °C with pancreatine and amyloglucosidase
(correct for ash only)

E. Hellendoorn, J. Sci. Food Agric. 26, 1461 (1975)
suspend sample in water
digest 18 hrs, 40 °C with pepsin
1 hr, 40 °C with pancreatine
(no ethanol precipitation and no correction for ash or protein)

FRACTIONATION METHODS

H. Englyst et al. Analyst 107, 307 (1982)
gelatinize 1 hr, 100 °C in water
digest 16 hrs, 42 °C with α -amylase, pullulanase
precipitate with 80% ethanol
hydrolyze residue with 12M H_2SO_4 , 1hr, 35 °C
1M H_2SO_4 , 2 hrs, 100 °C
analyze sugars by GLC, uronic acid colorimetrically

Same as above, lignin is determined gravimetrically in residue
after acid hydrolysis

NEUTRAL DETERGENT METHOD

AACC approved method 32-20 (1978)
boil 1 hr in neutral detergent solution
digest overnight, 37 °C with α -amylase
filter, dry and weigh

Table 2

BISCUITS

Total Dietary Fiber
g/100 g dry wt

Lab No.	Results		Mean	Method
1	2.459	2.609	2.534	AOAC
2	2.700	2.300	2.500	AOAC
12	3.990	3.480	3.735	AOAC
14	2.050	1.840	1.945	AOAC
15	2.500	2.500	2.500	AOAC
4	3.070	2.700	2.885	Asp
5	2.630	2.320	2.475	Asp
16	6.270	6.480	6.375	Asp(ash only)
7	10.970	10.880	10.925	Schweizer(ash Only)
8	1.030	0.410	0.720	Hellendoorn
11	2.300	2.300	2.300	Englyst
19	1.940	2.110	2.025	Englyst
3	1.940	1.940	1.940	Englyst(+ lignin)
6	0.980	0.550	0.765	NDF

Table 3

WHOLE WHEAT MEAL

Total Dietary Fiber
g/100 g dry wt

Lab No.	Results	Mean	Method	
1	12.983	14.134	13.559	AOAC
2	13.200	12.700	12.950	AOAC
12	13.860	13.860	13.860	AOAC
14	15.810	15.120	15.465	AOAC
15	14.400	15.100	14.750	AOAC
4	13.250	12.590	12.920	Asp
5	11.390	12.850	12.120	Asp
16	21.280	19.030	19.655	Asp(ash only)
7	20.240	19.440	19.840	Schweizer(ash Only)
8	10.470	10.130	10.300	Hellendoorn
11	9.900	10.100	10.000	Englyst
19	8.630	8.850	8.740	Englyst
3	8.420	10.580	9.500	Englyst(+ lignin)
6	9.650	10.080	9.865	NDF

Table 4

FRENCH BEANS

Total Dietary Fiber
g/100 g dry wt

Lab No.	Results	Mean	Method	
1	31.938	31.346	31.642	AOAC
2	30.600	31.000	30.800	AOAC
12	25.160	26.220	25.690	AOAC
14	32.500	31.550	32.025	AOAC
15	33.400	31.100	32.250	AOAC
4	30.640	31.160	30.900	Asp
5	31.370	31.050	31.210	Asp
16	35.210	36.280	35.745	Asp(ash only)
7	33.630	36.940	35.285	Schweizer(ash Only)
8	19.680	17.230	18.455	Hellendoorn
11	22.200	22.300	22.250	Englyst
19	25.530	21.920	23.725	Englyst
3	24.810	17.150	20.980	Englyst(+ lignin)
6	15.630	15.530	15.580	NDF

Table 5

REFERENCE MATERIAL 8431 - Mixed Diet

<u>Method</u>	<u>Total Dietary Fiber</u> g/100g dry wt
Crude Fiber	1.4
Neutral Detergent Fiber	1.8
Englyst	3.6
Theander	5.1
AOAC	5.3
Simplified	5.6

Table 6

HNIS Collaborative Study - AOAC

<u>Sample</u>	<u>Total Dietary Fiber</u> g/100g dry wt \pm SD			
	Lab 1	Lab 2	Lab 3	Lab 4
Cooked potatoes	7.45 \pm 0.31	6.84 \pm 0.10	8.30 \pm 0.14	8.02 \pm 0.41
Peanuts	10.81 \pm 2.21	11.35 \pm 1.45	7.82 \pm 0.59	11.01 \pm 2.66
Apple	13.04 \pm 0.32	11.50 \pm 0.22	12.43 \pm 0.41	12.05 \pm 0.58
Soy flour	16.31 \pm 0.63	14.98 \pm 0.68	13.95 \pm 0.36	15.67 \pm 1.11
Lo-cal white bread	17.20 \pm 0.68	16.11 \pm 0.27	16.17 \pm 0.27	16.83 \pm 0.33
Cabbage	22.58 \pm 0.24	21.40 \pm 0.46	21.76 \pm 0.20	23.18 \pm 1.21
Carrots	25.38 \pm 0.75	23.48 \pm 0.89	23.76 \pm 0.54	24.64 \pm 0.61
Cocoa	30.16 \pm 0.37	27.98 \pm 0.43	28.59 \pm 0.50	29.09 \pm 0.36

Table 7

SOLUBLE AND INSOLUBLE DIETARY FIBER CONTENTS (g/100g dry wt) - By Four Methods

Sample	Simplified		AOAC-1		AOAC-2		Mongeau		Englyst-1		Englyst-2	
	Sol	Ins (S+I)	Sol	Ins (S+I)	Sol	Ins (S+I)	Sol	Ins (S+I)	Sol	Ins (S+I)	Sol	Ins (S+I)
Cooked potatoes	2.52 (5.67)	3.15	2.00 (6.05)	4.05	3.02 (7.85)	4.83	3.35 (6.35)	3.00	3.80 (5.78)	1.98	3.20 (5.45)	2.25
Peanuts	2.58 (10.77)	8.19	0.06 (13.74)	13.68	0.61 (9.57)	8.96	4.07 (8.12)	4.05	1.10 (5.96)	4.86	1.25 (6.27)	5.02
Apple	4.24 (10.98)	6.74	2.84 (12.14)	9.30	4.69 (12.79)	8.10	4.24 (10.93)	6.69	5.02 (10.64)	5.62	5.30 (11.05)	5.75
Soy flour	6.60 (14.86)	8.26	0.56 (15.28)	14.72	2.74 (19.22)	16.48	12.61 (17.94)	5.33	1.98 (13.36)	11.38	2.60 (13.15)	10.55
Lo-cal white bread	4.00 (16.12)	12.12	1.98 (15.74)	13.76	2.88 (15.98)	13.10	4.69 (15.06)	10.37	3.25 (13.22)	9.97	3.65 (13.55)	9.90
Cabbage	6.84 (20.94)	14.10	4.08 (22.40)	18.32	6.19 (24.24)	18.05	8.07 (20.02)	11.95	9.80 (19.80)	10.00	10.70 (22.55)	11.85
Carrots	9.01 (22.56)	13.55	7.45 (24.65)	17.20	8.35 (25.51)	17.16	11.04 (20.24)	9.20	12.60 (20.90)	8.30	13.00 (21.65)	8.65
Cocoa	4.52 (27.87)	23.35	4.15 (31.27)	27.12	5.70 (30.60)	24.90	8.28 (30.96)	22.68	4.06 (12.04)	7.98	3.30 (12.15)	8.85