Minerals and Phytate in the Analysis of Dietary Fiber from Cereals. II

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ABSTRACT

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Dietary fiber and especially soluble fiber components in a wheat bread dough with a high content of fiber were associated with considerable amounts of ash when assayed with an enzymatic gravimetric method, using ethanol precipitation to recover soluble fiber. Only a small amount of minerals occurred in the insoluble fiber fraction, except for iron, 40% of which was associated with this fraction. Only a fraction of the minerals and phytate associated with the alcohol-precipitated soluble fiber could be removed by dialyzing the redissolved fiber. Soluble fiber dialyzed without previous alcohol precipitation contained much smaller amounts of minerals, but nearly 30% of the iron was bound to the soluble fraction also when prepared without ethanol precipitation. This study shows that the isolation procedure of fiber components heavily modifies their mineral-binding capacity. However, the different cations investigated (Ca, Mg, Fe, and Zn) were not uniformly affected by the isolation procedures. Great care is needed when extrapolating from in vitro studies with isolated fiber components to the native dietary fiber or to its effect in vivo.

Previous studies (Schweizer and Würsch 1979, Frølich and Asp 1980) demonstrated that a considerable amount of ash was associated with dietary fiber assayed with enzymatic, gravimetric methods. This ash was found almost exclusively in the soluble-fiber fraction, but the nature of this association has not yet been elucidated.

Some observations about the influence of buffer choice, ionic strength, and fiber-isolation method on fiber-associated minerals were reported in part I of this study (Schweizer et al 1984), in which bran was the fiber source. For this second part of the study, a bread dough was chosen as the fiber source, and more minerals were included. In addition to studying methodology, the purpose of the study was to contribute to the knowledge of in-vitro mineral binding to cereal fiber.

MATERIALS AND METHODS

Samples

Four bread doughs were prepared from whole grain wheat flour with an addition of bran. The dough recipe was as follows: water, 700 ml; whole grain wheat flour; 700 g; bran, 300 g; salt, 17.5 g; and yeast, 35 g. Samples were taken for analysis directly after the ingredients were mixed.

Sample Preparation

The samples did not contain more than 2–3% lipids and were therefore not defatted (Schweizer and Würsch 1979, Asp et al 1983). The samples were immediately dried at 105° C for 2 hr and ground in a Cyclotec sample mill (Tecator AB, Höganäs, Sweden) to a particle size of <0.45 mm. Ash content was determined by ashing at 500° C to constant weight.

Mineral Determination

After ashing, the samples were dissolved in a 1:1 solution of 2% (v/v) HCl and HNO_3. The individual minerals were then determined by plasma spectrophotometry by emission, using a Direct Reading Spectrometer (Simultaneous Instrument, no. 975 1 CAP, Jarrell-Ash, Boston).

Dietary Fiber

The method used to determine dietary fiber content was based on Hellendoorn's enzymatic, gravimetric method (Hellendoorn et al 1975) as described by Asp et al (1983).[4] To remove starch completely, an extremely heat-stable α-amylase (Termamyl 60L, Novo, Copenhagen, Denmark) was used in an initial gelatinization step at 100° C for 15 min. Further enzyme digestions were performed with pepsin (Merck no. 7190) at pH 1.5 for 1 hr, and with pancreatin (Sigma no. P-1750) at pH 6.8 for 1 hr, both at 40° C. The mineral content (mg) of the enzyme preparations used for a 1-g sample (dry matter) of bread dough was as follows: Termamyl (100 μl; Ca 0.46, Mg 0.06, P 0.20, Fe 0.001, Zn 0.001; pepsin (100 mg): Ca 0.05, Mg 0.14, P 0.58, Fe 0.003, Zn 0.002; pancreatin (100 mg); Ca 1.50, Mg 0.06, P 1.00, Fe 0.007, and Zn 0.014.

The final phosphate buffer concentration used in this study before ethanol precipitation was 50 m*M*. In view of the present results, the phosphate buffer concentration was decreased to 25 m*M* in the final version of the method as described by Asp et al (1983).

The fiber components were recovered either by filtration or by centrifugation. In filtration, insoluble-fiber components were recovered by filtration and soluble components by precipitation with four volumes of 95% ethanol (final concentration, 78%; v/v), followed by filtration. The filtrations were done with Tecator's Fibertec system (Tecator AB, Höganäs, Sweden), using 0.5 g of Celite 545 as a filter aid. In centrifugation, insoluble-fiber components were recovered by centrifugation for 30 min at 1,600 × *g* in a Beckman centrifuge J 2-21 at 4° C. The supernatants and washings (100 ml) were precipitated with four volumes of ethanol, and the soluble-fiber components were recovered by centrifugation in the same centrifuge for 30 min at 3,000 × *g* at 4° C.

The dietary fiber values reported in the tables are means of duplicate gravimetric analyses. All values are given in percent, corrected for protein (Kjeldahl N × 6.25) and ash associated with the fiber.

Phytate

Phytate was determined with a modification of Holt's method (Holt 1955). This method is based on complex formation of phytic acid and Fe (III)-ions at pH 1–2. An excess of Fe (III) present in the solution will react with thiocyanate ions to form a characteristic pink complex, $Fe(SCN)_3$. The optical density at 465 nm in an amyl-alcohol layer is measured, and an inverse linear relation is found for phytate concentrations ranging from 40 to 200 nmol/L.

Dialysis

The alcohol-precipitated fiber fractions were redissolved in 2 ml of distilled water. The solutions were dialyzed against 4 L of double-distilled water for 48 hr at 4° C in Spectrapor sacks (Spectrum Medical Industries, Inc., Los Angeles, CA) with an exclusion limit of 6,000–8,000 daltons. The contents of the sack were then freeze-dried.

As an alternative to the method described above, the soluble

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[4] N. G. Asp., C.-G. Johansson, H. Hallmer., and M. Siljestrøm. 1981. A rapid enzymatic method for assay of insoluble and soluble dietary fiber. Presented at AOAC Spring Workshop, Ottawa.

fiber was recovered by dialyzing the supernatant obtained after centrifuging the enzyme digest, using the same procedure as above. In this experiment with direct dialyzing, the Termamyl was excluded.

RESULTS AND DISCUSSION

Table I gives dietary fiber content of the bread dough, with both the filtration and the centrifugation methods. The total dietary fiber values (corrected for ash and undigestible protein) were the same with the two procedures. However, the soluble fraction was significantly larger with the centrifugation method. The amount of ash associated with soluble fiber was the same with both methods, whereas slightly more ash was found in the insoluble fiber when the filtration method was used. The reason for recovering the fiber components with centrifugation, which is a more time-consuming method, was that the filtration aid, Celite, contains minerals that disturb the assay of individual minerals.

Table II gives the composition of the bread dough with regard to dietary fiber, phytate, ash, and selected minerals. About 70% of the phosphorus in the bread dough is present as phytate.

Individual mineral determinations (Tables III and IV) show similar distribution between insoluble and soluble fiber as total ash (Table I). Only 1.6–13.2% of the total minerals were associated with insoluble fiber. The only exception was iron, 40% of which was associated with this fraction. For some minerals (Ca and Zn), the amount recovered in the soluble-fiber fraction exceeded that in the dough sample analyzed. This can be explained by the mineral content of the enzyme preparations given in Materials and Methods. Termamyl and pancreatin contained large amounts of calcium, and pancreatin also contained a significant amount of zinc.

TABLE I
Content of Dietary Fiber in the Bread Dough Determined with Centrifugation/Filtration[a]

	Centrifugation Method	Filtration Method
Dietary fiber (corrected for residual protein and ash)		
Insoluble fiber	20.7 ± 0.9	21.3 ± 1.8
Soluble fiber	3.7 ± 0.8	2.7 ± 0.3
Total fiber	24.4 ± 1.1	24.0 ± 2.1
Ash associated with dietary fiber		
Insoluble fiber	0.2	0.4 ± 0.2
Soluble fiber	2.4 ± 0.3	2.5 ± 0.5

[a] All figures in percent of dry matter of the dough. Means ± SD of duplicate determinations for each of four doughs. Statistical evaluation was made with Student's *t*-test.

TABLE II
Composition of Two Samples of Wheat Bread Dough (db)

Components	Percent	Mean Range (mg/g)
Dietary fiber[a]	24.4 ± 1.1	
Phytate	1.7 (2.4)[b]	
Total ash	3.0	
Ca	12	0.59; 0.59
Mg	13	2.61; 2.55–2.68
P	14	6.80; 6.70–6.90
Fe	15	0.083; 0.082–0.084
Zn	16	0.053; 0.053–0.054

[a] Corrected for residual protein ash.
[b] Values in parentheses are calculated from P in ash, assuming that all P was from phytate.

TABLE III
Composition of Components of Insoluble Fiber in Four Samples of Bread Dough (db)

Components	Percent	Mean (mg/g)[b]	Range (mg/g)	
Residue weight	23.5 ± 0.9			
Ash	0.5 (16)[a]			
Phytate	Not detectable			
Insoluble fiber[a]	20.6 ± 0.9			
Ca		0.077	0.075–0.090	(13.1)
Mg		0.044	0.035–0.055	(1.6)
P		0.47	0.27–0.69	(6.8)
Fe		0.031	0.027–0.034	(37.3)
Zn		0.007	0.006–0.009	(13.2)

[a] Corrected for residual protein ash.
[b] Values in parentheses are percentage of total content in the dough.

TABLE IV
Composition of Soluble Fiber in Four Samples of Bread Dough (db)

Components	Percent	Mean (mg/g)	Range (mg/g)	
Residue weight	6.5 ± 0.8			
Ash	2.4 ± 0.3 (80)[a]			
Phytate	0.8 (46.5) [2.2][b]			
Soluble fiber[c]	3.7 ± 0.8			
Minerals associated with soluble fiber recovered by ethanol precipitation				
Ca		1.60	1.25–1.95	(270)
Mg		1.70	1.65–1.75	(65)
P		6.10	5.30–6.90	(90)
Fe		0.063	0.058–0.071	(76)
Zn		0.091	0.071–0.117	(170)
Dialysis of redissolved ethanol-precipitated fiber				
Ca		1.40	1.00–1.65	(230)
Mg		1.35	1.30–1.40	(52)
P		3.40	2.90–3.80	(50)
Fe		0.048	0.043–0.054	(59)
Zn		0.074	0.065–0.086	(140)
Dialysis of supernatant without ethanol and Termamyl				
Ca			0.13	(22)
Mg			0.037	(1.4)
P			0.840	(12)
Fe			0.023	(28)
Zn			0.006	(11)

[a] Values of parentheses are percentage of total content in the bread dough.
[b] Values in parentheses are calculated from P in ash, assuming that all P was from phytate.
[c] Corrected for protein and ash.

High amounts of phosphorus were also associated with the fiber, which could be partly due to coprecipitated phosphorus from the 50 m*M* phosphate buffer. Similar findings were reported for bran in part I of this work (Schweizer et al 1983), although the amount of phosphorus was somewhat lower, because of the lower phosphate concentration (17 m*M*) used in that study.

Nearly half (47%) of the content of phytate in the original bread dough was recovered in the soluble-fiber fraction, whereas no phytate could be detected in the insoluble-fiber fraction. The supernatant after precipitation and filtration of the soluble fiber contained 35% of the original phytate. The last 15% could possibly have been broken down during the enzyme incubation steps in the fiber-assay procedure.

In an attempt to measure whether the minerals were really bound to the fiber or just coprecipitated through the ethanol, two experiments were set up. In one experiment, the precipitated soluble fibers were redissolved and dialyzed against water. The redissolution was performed both in distilled water and in hydrochloric acid (pH 4.5), with the same result. Surprisingly, only about 20% of the minerals were dialyzable, except for phosphorus, 40% of which disappeared. In the other experiment, the supernatant was dialyzed directly after the centrifugation of the enzyme digest. Most of the minerals could then be dialyzed (Table IV). In this experiment, Termamyl was omitted in the digestion to avoid unnecessary mineral addition. This gives approximately 1% residual starch in the fiber residue (Asp et al 1983). By replacing precipitation by dialysis, the total ash associated with the soluble-fiber fraction decreased to about one-fourth. All of the magnesium and nearly all of the zinc and phosphorus disappeared through dialysis. In contrast, 28% of the iron in the bread dough remained bound to the soluble fiber after this procedure. These results are in agreement with the findings with bran, where most of the minerals could be dialyzed away from the fiber (Schweizer et al 1983). Therefore, it seems that the soluble fiber or the phytate is altered through ethanol precipitation in such a way that minerals are not released upon redissolution. This clearly shows that the procedure used for isolation of dietary fiber can heavily modify its ion-binding properties. Therefore, great care is needed when extrapolating from in-vitro studies with fiber fractions treated with solvents or detergents to the original fiber sources (Fernandez and Phillips 1982). This has also been pointed out with respect to the bile-acid binding of fibers (Story et al 1982).

CONCLUSION

This study shows that the mineral binding of dietary fiber fractions in-vitro is modified by solvent precipitation. Studies done on isolated fiber fractions do not directly reflect native dietary fiber, and such studies cannot be extrapolated to the effect of dietary fiber in human beings.

The majority of the ash and nearly half of the phytate were associated with the soluble-fiber fraction after isolation of the latter by solvent precipitation. The insoluble-fiber fractions did not contain measurable amounts of phytic acid and contained only minor amounts of minerals. As an exception to this, 37% of the iron in the dough remained associated with this fraction. Iron associated with the soluble fraction also seems to behave differently than other minerals. When the soluble fiber fraction was dialyzed without being alcohol precipitated and redissolved, 28% of the iron remained bound to this fraction, whereas around 90% of other minerals were removed.

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