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

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ABSTRACT

Considerable amounts of ash were found in dietary fiber in foods and especially in soluble-fiber components from cereal products in assays with an enzymatic gravimetric method. With wheat bran as a source of fiber, several digestion and isolation procedures were modified to identify the component minerals and the nature of their association with insoluble- and soluble-fiber components. When buffers with low ionic strength were used, citrate was substituted for phosphate, and soluble fibers were isolated with dialysis instead of precipitated with ethanol, as much as 90% of the ash in the fiber fractions was reduced. On the other hand, more residual protein remained in the fiber fractions. From the assay of the quantitatively important minerals and of phytic acid in all fiber fractions and from separate solubility tests, coprecipitation was determined to be partly responsible for the ash in the soluble-fiber fractions. These results should be helpful in improving current analytical methods for determining dietary fiber.

Several approaches for analyzing dietary fiber in food are currently used, each having its own advantages and limitations (Southgate et al 1978, Schweizer 1980). When assaying the insoluble and soluble fibers by gravimetric methods after enzymatic digestion of protein and starch (Schweizer and Würsch 1979, 1981, Asp and Johansson 1981, Asp et al 1983), we found considerable amounts of ash, especially in the soluble-fiber fraction from cereals (Schweizer and Würsch 1979, Frølich and Asp 1981). Since no satisfactory explanation has yet been provided for this observation, studying the origin of this ash and the nature of its association to insoluble- and soluble-fiber fractions promised to be interesting.

The gravimetric method of Schweizer and Würsch (1979) for the assay of dietary fiber was used to perform blank determinations and digestions of wheat bran. Also, several modifications (type and ionic strength of buffer, and fiber-isolation method) were introduced to show to what extent the ash in the soluble-fiber fraction was caused by coprecipitation by the ethanol used for isolating soluble fibers, by the phytate associated with the soluble fibers, or by the ability of minerals to associate to the polysaccharides. Wheat bran was chosen as a fiber-rich material, because it is also rich in minerals and phytate. These three dietary components could be followed easily throughout the analytical procedures.

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MATERIALS AND METHODS

Reagents and Samples

Analytical reagents of the purest grade available were used for all analyses. Other, specific products used are also mentioned.

Wheat bran was obtained from a local mill and ground to a particle size of less than 0.6 mm. The composition of the bran is given in Table I.

Solubility Tests

The following stock solutions were prepared:

1. 85 and 100 m*M* phosphate buffers, pH 6.0, by dissolving adequate amounts of $Na_2HPO_4 \cdot 2H_2O$ and $NaH_2PO_4 \cdot H_2O$ in distilled water;
2. 85 and 100 m*M* phosphate buffers, pH 4.5, by adjusting the pH of solution 1 to 4.5 with diluted HCl before adjusting the volume;
3. 85 and 100 m*M* citrate buffers, pH 6.0, by dissolving citric acid in distilled water and adding 25% ammonia solution until reaching pH 6.0 before adjusting the volume;
4. 85 and 100 m*M* citrate buffers, pH 4.5, by adjusting the pH of solution 3 to 4.5 with 5*N* HCl before adjusting the volume;
5. 333 m*M* sodium chloride;
6. 2.37 m*M* $Ca(NO_3)_2 \cdot 4H_2O$;
7. 17.9 m*M* $MgCl_2 \cdot 6H_2O$; and
8. 5.38 m*M* sodium phytate (Sigma Chemicals, St. Louis; % Na: 29.8 found, 29.9 calc; % P: 20.0 found, 20.1 calc).

The concentrations of the three latter salts were chosen to simulate, in the subsequent solubility tests, the enzymatic digestion of 2.5 g of wheat bran in a final volume of 150 ml, assuming the following amounts in wheat bran (mg/100 g): Ca, 114; Mg, 522; and

phytic acid, 4,260. These are close to those found in food tables (Southgate and Paul 1978) and in the wheat bran used in this study.

The solubility tests were then performed by mixing the required amounts of the different solutions in a test tube to obtain the final concentrations given in Tables II–IV. Four volumes of ethanol were then added under stirring to the resulting aqueous solutions, and the tube contents were checked for turbidities and precipitates at room temperature after 10 min and after one night.

The results of Table IV were obtained by mixing 1 ml of one of the buffers 1–4, 0.5 ml of NaCl (5), 1 ml of the salts 6–8, and 0.5–2.5 ml of distilled water, followed by addition of 20 ml of ethanol in steps of 5 ml.

Dietary Fiber

The contents of insoluble and soluble fibers were determined as described by Schweizer and Würsch (1979, 1981), with some modifications. When citrate-buffer was used, the pH adjustment between the pepsin step and the pancreatin/glucoamylase step was made with ammonia solution instead of NaOH. In some experiments, the pH was adjusted from 6.0 to 4.5 with HCl before the soluble fibers were isolated. As an alternative to solvent precipitation of the soluble fibers, these were recovered by exhaustively dialyzing the digestion filtrate against distilled water in Visking sacks, which, according to the manufacturer (Serva, Heidelberg, W. Germany), had an exclusion limit of 10,000 daltons, followed by freeze-drying of the retentate. The insoluble and soluble fibers were analyzed separately for residual nitrogen by the Kjeldahl method (conversion factor to protein = 6.25) and for ash.

The values reported in the tables are the means of two to four duplicate gravimetric analyses. The relative standard deviations were less than 3 and 5% for insoluble and soluble fibers, respectively, confirming the previously established reliability (Schweizer and Würsch 1979).

TABLE I
Composition of Wheat Bran (dry basis)

Total Composition	Percentage
Protein (N × 6.25)	16.9
Lipids	6.2
Starch	18.7
Sugars	4.2
Dietary fiber[a]	43.4
Ash	6.2
Phytic acid[b]	3.7 [4.4]
Ash components (mg/100 g)	
Na	7.4
K	1,500
Ca	122
Mg	470
P	1,250
Si	9.3

[a] Corrected for residual protein and ash.
[b] Values in brackets are calculated from P in ash, assuming that all P was from phytic acid.

TABLE II
Solubility of Buffers in 80% Ethanol (v/v) at Different pH[a,b]

Concentration (m*M*)	Phosphate pH 4.5	Phosphate pH 6.0	Citrate pH 4.5	Citrate pH 6.0
100	++	++	–	–
50	+	++	–	–
25	–	+	–	–
17	–	–	–	–
12.5	–	–	–	–

[a] Qualitative observations. – = entirely soluble, + = slightly cloudy, ++ = very cloudy.
[b] Concentrations refer to the aqueous solutions, ie, before addition of four volumes of ethanol.

Minerals and Phytate

The samples were ashed to constant weight at 550°C and the resulting ashes dissolved in 2*N* hydrochloric acid. The individual cations were analyzed by flame atomic-absorption spectrophotometry. Silicon dioxide, although not soluble, was kept in homogenous suspension and could be determined by the same technique. The sum of the cations after conversion to their oxides accounted for 69–102% of the total ash. The complete recovery of phosphorus from the wheat bran and from sodium phytate was assessed in separate experiments by adding an excess magnesium oxide before ashing, as described in official AOAC methods 14.015 and 14.016 (Horwitz 1980).

The content of phytic acid in the bran and in the fiber fractions was determined by the method of Makower (1970) as modified by de Rham and Jost (1979).

RESULTS AND DISCUSSION

Solubility in Aqueous Ethanol of Buffer Salts, Minerals, and Phytic Acid

The concentrations of the phosphate buffers just before precipitating the soluble fibers in the gravimetric methods reported in the literature range from 16.7 m*M* at pH 6.0 (Schweizer and Würsch 1979) to 50 m*M* at pH 4.5.[4] Therefore, the solubility of phosphate buffer salts around these concentrations was determined and compared to an ammonium–citrate buffer. The latter was

[4] N.-G. Asp, C.-G. Johansson, H. Hallmer, and M. Siljeström. 1983. A rapid enzymatic method for assay of insoluble and soluble dietary fiber. Presented at AOAC Spring Workshop, Ottawa.

TABLE III
Solubility of Buffer–NaCl Mixtures in 80% Ethanol (v/v)[a,b]

Concentration of NaCl (m*M*)	Phosphate pH 4.5	Phosphate pH 6.0	Citrate pH 4.5	Citrate pH 6.0
		17 m*M*		
167	–	++	–	++
83	–	+	–	+
42	–	–	–	–
21	–	–	–	–
		25 m*M*		
167	–	++	–	++
83	–	++	–	+
42	–	+	–	–
21	–	+	–	–

[a] Qualitative observations. – = entirely soluble, + = slightly cloudy, ++ = very cloudy.
[b] Concentrations refer to the aqueous solutions, ie, before addition of four volumes of ethanol.

TABLE IV
Solubility in 80% Ethanol (v/v) of Minerals and Phytic Acid in 17 m*M* Buffer Containing 33 m*M* NaCl[a,b]

Addition	Phosphate pH 4.5	Phosphate pH 6.0	Citrate pH 4.5	Citrate pH 6.0
None	–	–	–	–
(A) Ca^{2+} (19 μg/ml)	–	+	+[c]	–
(B) Mg^{2+} (87 μg/ml)	+	++	–	–
(C) Phytic acid (710 μg/ml)	+[d]	++[d]	++[d]	+[e]
A + C	++[d]	++[f]	++[d]	++[d]
B + C	+[f]	+[f]	++[d]	++[d]
A + B + C	++[f]	++[f]	++[d]	++[f]

[a] Qualitative observations. – = entirely soluble, + = slightly cloudy, ++ = very cloudy.
[b] Concentrations refer to the aqueous solutions, ie, before addition of four volumes of ethanol.
[c] After prolonged standing only.
[d] Beginning already at 67% EtOH.
[e] Beginning already at 75% EtOH.
[f] Beginning already at 50% EtOH.

chosen in view of the subsequent digestion experiments because it does not yield ash by itself.

Table II shows that the citrate buffer is soluble in 80% ethanol in a wider concentration range than phosphate. Addition of sodium chloride affects the solubility of the citrate more than that of the phosphate (Table III). This table also shows that much higher concentrations of sodium chloride can be tolerated at pH 4.5 than at pH 6. However, the sodium chloride produced in the procedure of Schweizer and Würsch (1979, 1981) through acidification in the pepsin step and subsequent neutralization does not generally exceed a final concentration of 30 m*M*. Therefore, no phosphate precipitation is expected, even if the sample analyzed contains up to 15% of sodium chloride. In the recently published procedure of Asp et al (1983), the final concentration of phosphate buffer was reduced to 25 m*M* at pH 4.5. Under these conditions, no precipitation is expected, despite the higher concentrations of sodium chloride obtained in this method.

Finally, Table IV shows the solubility of calcium, magnesium, and phytate in 80% ethanol. The conditions of this experiment were chosen to simulate the digestion of 2.5 g of wheat bran. The results indicate that, in contrast to the phosphates of calcium and magnesium, phytate can be expected to precipitate partially under all circumstances. Table IV also shows that the simultaneous presence of phytate, Ca, and Mg favors precipitation, since it occurs at lower concentrations of alcohol. On the other hand, these qualitative experiments cannot adequately simulate the more complex interactions between the minerals, phytic acid, and the polyionic fiber or protein components encountered in real digestion.

TABLE V
Composition of Insoluble Fibers from Wheat Bran (dry basis) Digested in Two Different Buffers[a]

Composition of Insoluble Fibers	Phosphate	Citrate
Residue weight, %	42.9	43.0
Protein, % (N × 6.25)	3.9 (23.1)	4.9 (29.0)
Ash, %	0.8 (13.4)	0.2 (3.4)
Corrected residue weight, %	38.2	37.9
Phytic acid, %	0.19 (5.1) [0.34][b]	0.03 (0.8) [0.09][b]
Ash components (mg/100 g)		
Na	122 (1,650)	11 (149)
K	28 (1.9)	36 (2.4)
Ca	33 (27.2)	7 (5.7)
Mg	46 (9.8)	12 (2.6)
P	97 (7.8)	28 (2.2)
Si	10 (107)	11 (118)

[a] Values in parentheses are the percentage of the content in the original bran.
[b] Values in brackets are calculated from P in ash, assuming that all P was from phytic acid.

Determination in Wheat Bran of Dietary Fiber and Fiber-associated Ash

The content and composition of the insoluble fibers recovered from wheat bran by the method of Schweizer and Würsch (1979) are given in Table V. Residue weights and corrected residue weights were not greatly influenced by the choice of buffer, since the slightly lower residual protein content obtained with the phosphate buffer was compensated by the higher ash content. The average residual protein (26%) closely corresponds to the indigestible nitrogen fraction of wheat bran fed to rats (Saunders and Betschart 1980).

In both cases, silica was entirely recovered in the insoluble fibers. The ash, four times higher, obtained with the phosphate buffer was mainly due to sodium from the buffer that adhered to the fiber despite washing. Phosphorus, magnesium, and calcium were also higher, the latter accounting for 27% of the original calcium content of the bran. However, none of the minerals investigated remained adsorbed to the insoluble fibers to any important extent when citrate buffer was used. Only negligible amounts of phytate were recovered in the insoluble fibers with both buffers.

Table VI shows the corresponding data for soluble fibers. Residue weights and corrected residue weights were comparable for both buffers and all isolation methods, with the exception of the low amount of fibers precipitated from phosphate buffer at pH 4.5 with ethanol, for which we have no explanation. Replacing precipitation by dialysis in phosphate reduced the ash content of the fraction of the fiber from 40 to 10%, whereas the protein content increased from about 11 to 28% at both pH's studied. This confirms our previous findings (Schweizer and Würsch 1979). Phytic acid accounted for roughly 20% of the ash and for 50% of the phosphorus in the soluble fibers precipitated from phosphate buffer. Thus, phosphate from the buffer was coprecipitated, probably as magnesium phosphate (see Table IV). In all three alternative isolation procedures, phytic acid accounted for at least 80% of the phosphorus.

The results for the soluble fibers isolated from citrate buffer are also given in Table VI. As already shown for the insoluble fibers, the use of citrate leads to less ash in both isolation procedures. In contrast to the phosphate buffer, increasing the pH from 4.5 to 6.0 diminished calcium, magnesium, and phosphorus. This is in agreement with the qualitative data of Table IV.

Dialysis removed much of the ash in the soluble-fiber fractions, roughly 70 and 80% in phosphate and citrate, respectively. However, none of the minerals could be completely dialyzed, which suggested that coprecipitation might not be entirely responsible for the fiber-associated ash, and that some binding might nevertheless exist (Frølich and Asp 1981). This view is supported by the fact that separately prepared mixtures of phytate with magnesium or calcium are well dialyzed in comparable conditions.

Nevertheless, the apparent relationship between soluble fiber and the ash associated with it can now largely be explained by coprecipitation in 80% ethanol of phytate and related minerals. Thus, brans from different cereals (wheat, barley, rye, and corn)

TABLE VI
Composition of Soluble Fibers from Wheat Bran (dry basis), Recovered Under Different Conditions

Composition of Soluble Fibers	Percent Content in Bran	Phosphate Buffer				Citrate Buffer			
		80% Ethanol		Dialysis		80% Ethanol		Dialysis	
		pH 4.5	pH 6.0	pH 4.5	pH 6.0	pH 4.5	pH 6.0	pH 4.5	pH 6.0
Residue weight, %		7.3	9.6	9.7	10.1	9.9	8.8	9.3	8.3
Protein, % (N × 6.25)	5.3–16.6	0.9	1.0	2.8	2.8	1.8	2.3	2.3	2.6
Ash, %	4.8–61.3	2.9	3.8	1.0	1.0	2.3	1.4	0.5	0.3
Corrected residue weight, %	···	3.5	4.8	5.9	6.3	5.8	5.1	6.5	5.4
Phytic acid, %[a]	13.5–37.8	0.9 [1.8]	1.0 [1.9]	0.8 [1.0]	0.9 [1.1]	1.4 [1.4]	1.4 [1.3]	0.5 [0.6]	0.5 [0.4]
Ash components (mg/100 g)									
Na	122–6,311	196	467	153	105	16	20	13	9
K	1.2–13	140	193	29	22	139	110	18	35
Ca	7.4–107	95	99	12	28	131	53	18	9
Mg	4.7–89	319	417	54	85	179	59	52	22
P	8.6–42	510	526	290	322	397	373	172	107

[a] Values in brackets are calculated from P in ash, assuming that all P was from phytic acid.

TABLE VII
Content of Soluble Fiber, Phytic Acid, and Soluble Fiber-associated Ash of Different Cereal Brans

Product	Soluble Fiber, %[a]	Ash, %	Phytic Acid, %
Wheat bran 1[b]	4.8	3.8	3.7
Wheat bran 2[c]	2.6	4.0	4.5
Rye bran[c]	2.6	2.5	2.5
Barley hulls[c]	1.1	1.4	0.6
Corn bran[c]	2.1	0.7	0.1

[a]Corrected for residual protein and ash.
[b]Present study.
[c]Unpublished data (Schweizer 1980).

with widely different phytic acid contents show a close relationship ($r = 0.99$) between their phytate content and their fiber-associated ash (Table VII). The relationship between soluble fiber and fiber-associated ash ($r = 0.69$) is clearly less pronounced.

CONCLUSION

Our findings show that the majority of Ca and Mg is released from wheat bran by the enzymatic treatment, which is similar to the one in the upper gastrointestinal tract. This release can be assumed to be largely due to the acidic pepsin-digestion step (Thompson and Weber 1979). Upon neutralization and digestion with pancreatin, there is little binding of the minerals to the insoluble fibers, especially when the chelating citrate buffer is used. The apparent association of the minerals with the soluble fibers is largely due to their coprecipitation in aqueous ethanol, since the majority of the minerals is removed from the digest when this isolation procedure is replaced by dialysis.

The results of this study provide some new information regarding current analytical practice. Because the majority of ash and protein in the soluble fibers originate from coprecipitation with 80% ethanol, it might be adequate to correct the soluble fiber fraction for residual protein and ash. The pure gravimetric value, including the indigestible protein, can be used for the insoluble fibers (Schweizer and Würsch 1981). If, however, total dietary fiber is determined gravimetrically, without separating insoluble and soluble fibers, the convention of correcting for residual ash and protein could be adopted. The buffer strength used should be kept at a low level, however, to minimize coprecipitation and the corrections for residual ash. The alternative use of citrate-buffer or dialysis merits further attention, but the substantial increase of residual protein in the soluble fibers calls for more efficient proteolytic steps, before such an alternative can be recommended.

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