Natural Levels of Nutrients in Commercially Milled Wheat Flours. III. Mineral Analysis[1]

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ABSTRACT

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The mineral components (Fe, Mg, Ca, Zn) of 63 samples of wheat flour (baker's bread, family, hearth, cake, and cookie-cracker types) from mills throughout the United States and Canada were determined by different laboratories. Significant differences were found between laboratories for each of the analyzed elements, mainly because of differences in the extraction procedures. The level of Ca in flour depended mainly on the Ca content of the wheat and was little affected by milling variables. Levels of Fe, Mg, and Zn in flour followed a different pattern from that of Ca and depended more on milling variables. These elements were highly correlated with flour ash and protein content and showed significant differences between flour types. The differences could be explained by Ca having a distribution within the wheat kernel different from that of Fe, Mg, and Zn.

Wheat has traditionally been an important source of mineral nutrients in the human diet, but this contribution is reduced by milling. Nutrients lost in milling, especially those for which there is evidence of nutritional deficiency, are often restored. Wheat flour is usually supplemented with iron in countries that have cereal enrichment programs. In the United States, calcium can be added to flour and bread optionally, but Great Britain requires calcium enrichment. Calcium addition is optional in Canada, except in Newfoundland, where it is required.

The National Academy of Sciences' Proposed Fortification Policy for Cereal-Grain Products (NAS/NRC 1974) included zinc and magnesium in the list of nutrients that should be added. Copper, manganese, selenium, and chromium were considered but not recommended for addition, either because a deficiency in the mineral has yet to be demonstrated or because toxicity problems are possible.

As discussed by Lorenz et al (1977) and Keagy et al (1980), a first step in evaluating the feasibility of the NAS proposal is to determine the naturally occurring level of each of the proposed nutrients in flour. This is required in order to know how much of each nutrient to add to meet either the NAS or the Canadian suggested amounts.

Previous studies on the mineral composition of wheat flour do not provide the necessary data for assessing supplementation levels. A number of reports concern mineral content of various flour fractions and the effect of extraction rate (Cirilli 1971; Garcia et al 1972a, 1972b; O'Dell et al 1972; Tunger and Feller 1971; Zook et al 1970), and other studies relate mineral content to agronomic growing conditions (Ghanbari and Mameesh 1971, Koivistoinen et al 1974).

We report the mineral composition (Fe, Mg, Ca, Zn) of 63 flour samples representing the commercial wheat flour production (baker's bread, family, hearth, cake, and cookie-cracker) in the

United States and Canada for the 1975 crop year.

MATERIALS AND METHODS

Sample Description

The description and proximate analysis of these 63 flour samples has been presented (Kulp et al 1980).

Methods of Analysis

Mineral data on the flour samples were contributed by laboratories representing governmental, industrial, and research and educational organizations. All laboratories were experienced in mineral analysis of cereals, and each used its own in-house analysis in this study. No attempt was made to reduce analytical error by standardizing a single procedure; this was not intended to be a collaborative study of analytical methodology, but rather a survey of the mineral composition of commercially milled wheat flour as determined by well-established laboratories regularly engaged in this type of assay. The methods used by the various laboratories were as follows:

Laboratory D. This laboratory used the AOAC α,α'-dipyridyl colorimetric method for iron 14.011-14.013 (AOAC 1975) and contributed Fe assays only.

Laboratory G. A dry ashing procedure based on AACC method 40-41 (AACC 1962) was used. Samples of flour (2 g) were ashed at 600°C for 6 hr. The ash was cooled, dissolved in 10 ml of 50% HCl, and boiled gently for 15 min in a partially covered ashing crucible. The resulting solution was diluted to appropriate volumes with deionized distilled water to bring element compositions within standard curve ranges. An Instrumentation Laboratory model 251 atomic absorption spectrophotometer was used. This laboratory contributed Fe, Mg, Ca, and Zn data in duplicate and, in some cases, triplicate.

Laboratory H. A pressure digestion technique (Lorenz et al 1977) was used to extract the minerals. This procedure involved initial treatment of 0.5 g of flour, weighed into plastic screw-capped test tubes, with 2 ml of concentrated HNO_3 for a 12-hr predigestion period. Subsequently, 2 ml of concentrated $HClO_4$ was added, after which the tubes were tightly capped and immersed in a 70°C water bath for 3 hr in a closed hood. After this digestion, the samples were

TABLE I
Means and Standard Deviations of Mineral Data[a]

Laboratory	Flour Type[b]	Fe (mg/100 g)	Mg (mg/100 g)	Ca (mg/100 g)	Zn (mg/100 g)
G	All	0.99 ± 0.36	22.8 ± 7.9	14.2 ± 2.6	0.74 ± 0.21
	Hard	1.10 ± 0.33	26.1 ± 6.5	14.3 ± 2.8	0.80 ± 0.18
	Soft	0.75 ± 0.29	15.8 ± 6.0	14.0 ± 2.2	0.60 ± 0.22
	B	1.10 ± 0.30	26.4 ± 6.0	14.9 ± 2.7	0.78 ± 0.11
	F	0.83 ± 0.21	21.3 ± 8.6	14.1 ± 2.8	0.68 ± 0.21
	H	1.51 ± 0.28	30.8 ± 4.3	12.2 ± 1.9	1.01 ± 0.20
	C	0.60 ± 0.18	12.6 ± 4.5	13.6 ± 2.8	0.44 ± 0.10
	C/C	0.95 ± 0.27	19.7 ± 4.1	14.5 ± 1.8	0.76 ± 0.17
H	All	1.88 ± 0.37	26.0 ± 7.8	15.6 ± 2.9	1.23 ± 0.33
	Hard	1.94 ± 0.35	28.8 ± 6.7	15.6 ± 3.1	1.28 ± 0.34
	Soft	1.77 ± 0.41	20.2 ± 6.9	15.5 ± 2.5	1.13 ± 0.26
	B	1.93 ± 0.40	29.4 ± 5.9	16.5 ± 3.1	1.29 ± 0.28
	F	1.91 ± 0.31	23.1 ± 6.5	14.7 ± 2.7	1.04 ± 0.18
	H	1.98 ± 0.28	34.5 ± 7.9	13.9 ± 2.8	1.66 ± 0.45
	C	1.67 ± 0.34	16.9 ± 5.3	15.8 ± 3.5	1.15 ± 0.27
	C/C	1.84 ± 0.48	24.0 ± 5.7	15.5 ± 1.6	1.14 ± 0.27
I	All	1.29 ± 0.42	28.6 ± 8.5	17.7 ± 3.0	0.83 ± 0.26
	Hard	1.40 ± 0.42	32.2 ± 7.0	17.7 ± 3.2	0.88 ± 0.20
	Soft	1.06 ± 0.35	20.8 ± 6.1	17.5 ± 2.7	0.72 ± 0.36
	B	1.39 ± 0.33	32.0 ± 5.5	18.3 ± 3.3	0.88 ± 0.12
	F	1.07 ± 0.33	25.7 ± 7.1	16.4 ± 3.2	0.70 ± 0.18
	H	1.93 ± 0.35	41.3 ± 5.5	17.5 ± 3.2	1.11 ± 0.23
	C	0.94 ± 0.33	16.8 ± 3.9	17.5 ± 3.2	0.51 ± 0.11
	C/C	1.24 ± 0.24	25.5 ± 3.9	18.2 ± 1.8	0.91 ± 0.39
J	All	...	...	...	0.92 ± 0.24
	Hard	...	...	...	0.99 ± 0.23
	Soft	...	...	...	0.75 ± 0.20
	B	...	...	...	0.97 ± 0.16
	F	...	...	...	0.83 ± 0.08
	H	...	...	...	1.27 ± 0.30
	C	...	...	...	0.63 3 0.20
	C/C	...	...	...	0.88 ± 0.11
D	All	1.31 ± 0.43	...	...	...
	Hard	1.39 ± 0.42	...	...	...
	Soft	1.16 ± 0.43	...	...	...
	B	1.40 ± 0.53	...	...	...
	F	1.22 ± 0.38	...	...	...
	H	1.73 ± 0.21	...	...	...
	C	0.97 ± 0.25	...	...	...
	C/C	1.36 ± 0.46	...	...	...

[a] Moisture-free basis.
[b] Flour type codes: all = all samples, hard = hard wheat flours, soft = soft wheat flours, B = baker's bread flours, F = family/all-purpose flours, H = hearth bread flours, C = cake flours, C/C = cookie-cracker flours.

cooled and diluted with glass-distilled water to appropriate volumes. A Perkin-Elmer model 303 atomic absorption spectrophotometer was used. This laboratory provided Fe, Mg, Ca, and Zn data in triplicate.

Laboratory I. This laboratory used two different methods. Approximately half of the flour samples were digested in concentrated HNO_3 in a Technicon model BD-40 block digestor. The samples were cooled and diluted to appropriate volumes with distilled water. Triplicate readings were then taken on a Varian No. 5 flame atomic absorption unit. The remaining flour samples were assayed by means of AACC method 40-70 (AACC 1962) and an Instrumentation Laboratory model 151 atomic spectrophotometer. Fe, Mg, Ca, and Zn data were contributed.

Laboratory J. The flour samples were wet ashed in triplicate with H_2SO_4-H_2O_2. The solutions were filtered and diluted to appropriate volumes for assay on a Perkin-Elmer 503 atomic absorption spectrophotometer. The procedure was calibrated with National Bureau of Standards reference materials as previously described (Wolf 1977). This laboratory contributed Zn data.

Additional laboratories were asked to provide Fe assays so that the experimental Fe assay error could be better assessed and related to the various analytical procedures used to determine this mineral. Special consideration was given to the Fe content because many problems are associated with the enrichment of flour and bread with this nutrient (Ranum and Loewe 1978), despite its inclusion in the enrichment standards from their inception. One problem is the large analytical error for Fe in relation to the narrow range in flour enrichment permitted by the standards.

Analysis of Data

Mineral data from each laboratory were averaged (when multiple assays on a sample were provided) and corrected to a moisture-free basis. The data were analyzed using an analysis of variance. Means, standard deviations, and correlation coefficients were calculated for each laboratory on wheat and flour protein, ash, and mineral levels for all flours, for hard and soft wheat flours, and for each of the five flour types.

RESULTS AND DISCUSSION

Table I shows the average mineral composition for the various flour types and wheat classes, as determined by each laboratory. Analysis of variance showed significant ($P = 0.05$) differences between flour types and between laboratories.

Effect of Flour Type

Flour types varied in their mean Fe, Mg, and Zn contents according to the mean flour ash content of each type, as illustrated in Fig. 1. As ash content increased, so did the levels of these minerals. However, Ca did not vary with ash content. This was also shown in the high correlation between Fe, Mg, and Zn (but not Ca) levels and flour ash content for individual samples (Table II). Unlike Fe and Zn, the Mg level of the different flour types appeared to be influenced by wheat type as well as by ash. Figure 1 shows that the Mg levels in cake and cookie-cracker flours are lower than would be predicted from the relationship between Mg and ash levels in the other three flour types, which were primarily from hard wheats.

Comparison of Data from Different Laboratories

Figure 1 illustrates the significant differences between laboratories, and Table III provides the correlation coefficients between laboratories.

For Fe, Laboratories D and I show good agreement that was confirmed by their correlation coefficients. Laboratory G was consistently low but parallel to D and I, and H was high. A comparison of the pressure digestion technique with an accepted wet ashing procedure for digestion of wheat flours before atomic absorption analysis showed higher Ca, Mg, and Fe values with the pressure digestion technique (Lorenz et al 1977). The relatively high values for Fe reported by Laboratory H are therefore not surprising.

For Zn, Laboratories G, I, and J were all essentially parallel; G

was the lowest. Laboratory H was again significantly higher.

For Mg, the lines for Laboratories G, H, and I were all somewhat parallel, with G again the lowest; however, here I was the highest. Because Ca did not vary with ash, comparison of laboratories on this basis was difficult, although the relative magnitude of the values for Ca was similar to that for Mg.

Laboratory G, which used AACC procedure 40-41, was consistently low in all four minerals because of their incomplete solubility in the 50% HCl used to extract the ash. Currently, AACC

TABLE II
Flour Ash and Protein Correlations with Flour Mineral Content

|	**Flour Type**	**Fe**	**Mg**	**Ca**	**Zn**
Flour Ash	Hard	0.66[a]	0.71[a]	0.08	0.80[a]
	Soft	0.62[a]	0.68[a]	0.37	0.70[a]
	Bread	0.58[a]	0.65[a]	-0.25	0.63[a]
	Family	0.66[a]	0.66[a]	0.41	0.80[a]
	Hearth	0.30	0.68	0.40	0.82[a]
	Cake	0.41	0.70	0.24	0.61
	Cookie/ cracker	0.49	0.30	0.58	0.31
	All	0.70[a]	0.77[a]	0.16	0.79[a]
Flour protein	Hard	0.70[a]	0.61[a]	-0.33[b]	0.59[a]
	Soft	0.45[a]	0.53[a]	0.18	0.58[a]
	Bread	0.66[a]	0.61[a]	-0.46	0.45
	Family	0.55[a]	0.50	-0.34	0.47
	Hearth	0.61	0.55	-0.36	0.35
	Cake	0.36	-0.42	-0.13	-0.17
	Cookie/ cracker	0.08	0.25	0.17	0.13
	All	0.64[a]	0.74[a]	-0.12	0.59[a]

[a] $P = 0.01$ level.
[b] $P = 0.05$ level.
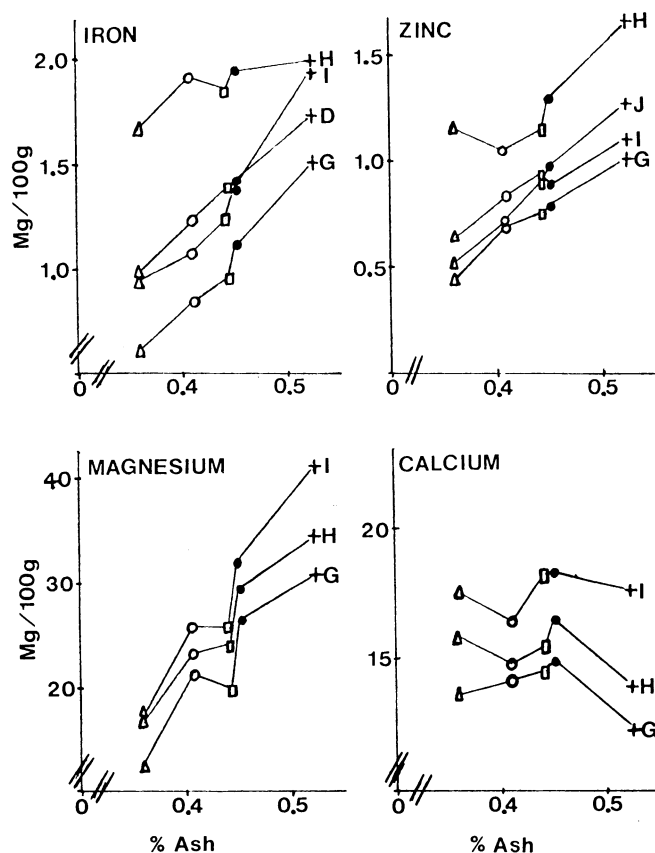
Fig. 1. Relationship of mineral levels of flour types to ash percentages. Flour type codes: Δ = cake, o = family, □ = cookie/cracker, ● = bread, + = hearth. To the right of each curve is the letter identifying the laboratory from which the data were obtained.

methods 40-41A and 40-70 for determining minerals by atomic absorption spectrophotometry call for dissolving the ash in concentrated HCl. Furthermore, losses due to volatilization or to possible incorporation of minerals in the ashing vessel as well as losses due to transfer can be quite serious with dry ashing.

The correlations between laboratory results (Table III) suggested that the method used by Laboratory H may be responding differently to soft wheat flours than to hard wheat flours for Fe and Zn. At higher mineral levels, the technique produced data that agree more closely with those obtained with other digestion methods, as shown by a study (Lorenz and Loewe 1977) involving the mineral analysis of the wheats from which the flours in the present study were milled.

In light of these differences, the Fe and Zn results of flours from Laboratory H were omitted from the correlation coefficients in Tables II and IV.

An analysis of variance of the Fe results on the 17 Canadian samples from the different laboratories showed an interlaboratory standard error of 20%. When the results from Laboratories G and H were omitted, the error was reduced to 10%, which is probably as good as can be expected for Fe determinations in flour. The interlaboratory error points out the need for use of a single digestion and extraction procedure in determining mineral components of cereals. Use of a standard reference material (Wolf

1977) to calibrate the method would also help improve reliability.

Effect of Wheat Mineral Content

The mineral content of the parent wheats from which these flour samples were milled was reported separately (Lorenz and Loewe 1977). Variations in the mineral levels of the wheats were fairly consistent; coefficients of variation were 13, 19, 18, and 15% for Fe, Ca, Mg, and Zn, respectively. In the flours, the coefficients of variation were 33, 18, 26, and 29%, respectively. Ca was the only mineral whose variability failed to increase on milling.

Mineral levels in flour averaged 32, 44, 21, and 29% of the levels in wheat for Fe, Ca, Mg, and Zn, respectively. Average correlation coefficients between the mineral contents of wheat and flour are shown in Table IV.

The ash content of wheat had no influence on the mineral content of flour. The protein content of wheat, on the other hand, showed significant correlations (negative for Ca) with the levels of all flour minerals in hard wheat flours but not in soft wheat flours. This relationship was partly because higher-protein hard wheats were milled into higher-ash flours (r = 0.48, P = 0.01); higher flour ash levels were directly related to the levels of Fe, Mg, and Zn (Table II).

Although the total mineral content of wheat (as measured by ash content) had little effect on flour mineral levels, the levels of Fe, Ca, and Zn in wheat did have such an effect. This is especially true for Ca; correlations between the wheat and flour contents of this mineral were significant in all five flour types. The correlation between Fe and Zn levels in wheat and flour are of a smaller magnitude and significant for hard wheat only. Although the level of Mg in wheat had little influence on the Mg content of flour, it did correlate somewhat with the Fe and Zn contents of soft wheat flours. Other cross-correlations were seen for hard wheat Fe on flour Mg and Zn and on flour Ca (negative r) as well. In addition, hard wheat Ca (negative r) and hard wheat Zn were correlated with flour Fe and Mg.

Effect of Milling Variables

The flour extrusion rate showed a small correlation with Mg (r = 0.24, P = 0.05) and Zn (r = 0.21, P = 0.05) levels in flour but not with Fe or Ca. The patent percentage correlated well with Mg (r = 0.53, P = 0.01) and Zn (r = 0.54, P = 0.01), less with Ca (r = 0.29, P = 0.01), and not significantly with Fe (r = 0.21).

Correlations with Flour Ash and Protein

Fe, Mg, and Zn levels in both hard and soft wheat flours are

TABLE III
Correlation Coefficients of Mineral Assays Between Laboratories

Mineral	Laboratories	All Flours	Hard Wheat Flours	Soft Wheat Flours
Fe	G and H	0.51[a]	0.53[a]	0.39
	G and D	0.51[a]	0.56[a]	0.21
	H and D	0.02	0.03	−0.16
	D and I	0.48[a]	0.58[a]	0.07
Mg	G and H	0.72[a]	0.57[a]	0.66[a]
	G and I	0.86[a]	0.77[a]	0.77[a]
	H and I	0.71[a]	0.61[a]	0.53[b]
Ca	G and H	0.49[a]	0.47[a]	0.57[a]
	G and I	0.56[a]	0.51[a]	0.72[a]
	H and I	0.65[a]	0.69[a]	0.52[b]
Zn	G and H	0.52[a]	0.65[a]	0.12
	G and J	0.86[a]	0.81[a]	0.87[a]
	H and J	0.54[a]	0.65[a]	0.04

[a] P = 0.01.
[b] P = 0.05.

TABLE IV
Correlation Coefficients Between Wheat and Flour

Flour	Type	Wheat					
		Protein	Ash	Fe	Mg	Ca	Zn
Protein	Hard	0.95[a]	0.18	0.53[a]	0.18	−0.45[a]	0.35[b]
	Soft	−0.12	−0.33	−0.03	0.25	−0.09	−0.20
	All	0.96[a]	−0.04	0.51[a]	0.08	−0.24[b]	0.30[b]
Ash	Hard	0.48[a]	0.25	0.37[b]	0.10	−0.11	0.41[b]
	Soft	−0.41	−0.16	−0.14	0.24	0.03	−0.19
	All	0.57[a]	0.04	0.34[a]	0.15	−0.09	0.24[b]
Fe	Hard	0.63[a]	0.21	0.41[a]	0.21	−0.29[b]	0.36[a]
	Soft	0.18	0.14	0.17	0.29	0.07	0.11
	All	0.58[a]	0.08	0.30[b]	0.24[b]	−0.18	0.27[b]
Mg	Hard	0.50[a]	0.07	0.41[a]	0.14	−0.28[b]	0.31[b]
	Soft	−0.36	−0.17	0.18	0.36	0.31	−0.20
	All	0.64[a]	−0.07	0.45[a]	0.19	−0.14	0.25[b]
Ca	Hard	−0.40[b]	0.06	−0.31[b]	0.18	0.64[a]	−0.13
	Soft	−0.21	−0.10	−0.23	0.32	0.65[a]	0.16
	All	0.19	0.03	−0.25[b]	0.20	0.64[a]	−0.08
Zn	Hard	0.48[a]	0.26	0.29[b]	−0.11	0.16	0.41[a]
	Soft	−0.21	0.12	0.07	0.40[b]	0.14	0.05
	All	0.46[a]	0.14	0.29[b]	0.24[b]	−0.04	0.29[b]

[a] P = 0.01.
[b] P = 0.05.

highly correlated with flour ash and protein, as shown in Table II. Ca shows a small negative correlation with flour protein in hard wheat flours.

Effect of Location

Statistical evaluation of the effects of location on the mineral composition of the individual flour types was not possible because results from a single flour only were available in many instances. Analysis of variance of the pooled data from all flour types, however, indicated significant differences in Ca due to location; Canadian samples had a lower Ca content. The effects of mill location on Fe, Mg, and Zn levels were insignificant ($P = 0.05$).

SUMMARY AND CONCLUSIONS

The levels of four nutritionally important minerals (Fe, Ca, Mg, Zn) in 63 flour and parent wheat samples, representing commercial flour production for 1975, were determined by different laboratories using standard in-house procedures.

Our primary objective was to determine the mean mineral content and its variability in different types of wheat flour. Even though the mineral composition of wheats and flours has been reported previously, the introduction of new varieties, modifications in agronomic practices, and changes in milling procedures warrant periodic analyses. This type of information was needed to assess the levels of minerals to add to flour for meeting the fortification standards proposed by the U.S. National Research Council (NAS/NAR 1974) and the expanded Canadian enrichment standards (Ranum 1980).

Analytical methodology also has changed. Atomic absorption spectroscopy, used by all but one of the laboratories, has become the dominant method for determining the mineral content of foods. However, an assortment of mineral extraction methods were employed by the different laboratories. This caused considerable differences in the results, especially for the low levels of Fe and Zn in unenriched flour. Interlaboratory error cannot be eliminated, a fact that should be considered when regulating fortification programs, but it can be reduced by specifying a single extraction procedure and by employing flour standards of certified mineral content to calibrate the entire procedure.

This study also produced information on what factors affect or are associated with the natural content of each mineral in wheat flour. Ca followed an entirely different pattern from that of Fe, Mg, and Zn: 1) Ca showed the highest mineral retention after milling and, unlike the other three minerals, its variability in flour did not increase over that in wheat. 2) Dependence of flour mineral content on wheat mineral content was highest for Ca. 3) Ca levels in flour showed little correlation with flour ash content; the other three minerals were highly correlated with ash. 4) Ca levels in flour were negatively correlated with flour protein content; the other minerals were positively correlated with protein. 5) Only Ca differed significantly because of location.

Most of these findings can be explained by the distribution of Ca within the wheat kernel. About 50% of the Ca is in the endosperm and 25% in the aleurone layers; less than 10% of the Mg and Zn is in the endosperm, with 70 and 50%, respectively, in the aleurone (Pomeranz 1971). Ash, protein, Mg, and Zn levels would be expected to increase as more of the aleurone is included in flour due to an increased extraction rate or patent percentage. The levels of Fe, Mg, and Zn in flour were closely related, with one exception, Mg and Zn correlated significantly with extraction rate and patent percentage but Fe did not.

This study determined the total amounts of minerals in flour. However, the possibility that part of this total amount may not be nutritionally available is suggested for Fe, at least, by the lower values reported by Laboratory G, which used 50% HCl rather than concentrated HCl to extract the flour ash. If a mineral in ash is insoluble in 50% HCl, a question arises as to its extractability under simulated gastric conditions (90-min extraction at 37°C with 0.5% pepsin in 0.83% HCl), as used in Fe bioavailability studies by Ranhotra et al (1971, 1973). If a naturally occurring form of a mineral in flour can be extracted only under the most severe in vitro conditions (such as an Fe silicate, for example) it is likely to be poorly bioavailable. Much work has been done on comparative bioavailability of added Fe; perhaps more should be done to clarify the chemical changes in naturally occurring Fe during in vitro and in vivo extraction methods. Comparative bioavailability studies of naturally occurring Fe from selected fractions of the wheat kernel could also be productive.

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