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Iron Binding of Wheat Bran at Human Gastric pH

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In vitro measurements on several wheat brans demonstrate that iron binding in ~0.1 N HCl is dependent upon time and concentration of iron in solution and is similar for all brans examined. Ferric iron is bound more than ferrous iron during exposure times of ≤4 h; at longer times, >16 h, there is little difference in amounts bound. Binding of ferric iron by wheat brans can be greatly altered by various treatments and may involve mechanisms other than interaction with phytate. The iron content of wheat bran remnants recovered after passage through the human gastrointestinal tract is equivalent to or less than that associated with initial pericarp tissue.

Research efforts currently are directed to examination of effects of dietary fiber consumption upon human health and nutrition. Reports claim both beneficial and adverse effects of dietary fiber in human diets. One area of major interest is the relationship between fiber consumption and mineral requirements. Earlier work had established that iron balance was lowered when whole-meal wheat bread was eaten rather than white bread (Widdowson and McCance, 1942). Recent works have amply verified that wheat bran in the diet lowers iron absorption (Björn-Rasmussen, 1974; Simpson et al., 1981). Thus, Simpson et al. report a sharp decrease in iron absorbed from a light meal that contains 12 g of wheat bran. However, reports from longer term studies show that a modest increase of about 25 g/day of wheat bran added to a low-fiber diet may increase the apparent requirements of humans for several minerals but not for iron (Sandstead et al., 1979). A discussion of the complex multitude of factors involved in physiological mechanisms and chemical properties concerned with iron absorption is presented in a review by Forth and Rummel (1973). A recent review addresses aspects of iron fortification of foods, bioavailability, and behavior in food systems (Lee and Clydesdale, 1979). Clinical studies provide ample evidence that a variety of diet components, including fruit juices and egg protein, also affect iron absorption in humans (Elwood et al., 1968; Monsen and Cook 1979; Rossander et al., 1979). Thus, wheat bran is one of many items in the diet that may influence mineral absorption.

Because wheat bran is a natural, whole diet component and is being used as a supplementary dietary fiber source, we consided it worthwhile to examine some iron binding properties of this food ingredient. Recent work has convincingly demonstrated that various dietary fiber components and sources, including wheat brans, do bind minerals in vitro under a variety of conditions (Reinhold et al., 1976;, 1981; Ismail-Beiji et al., 1977; Reilly, 1979; Camire and Clydesdale, 1981). Although dietary fiber components have the ability to act as a mineral sink, it is not yet directly demonstrated whether or not this binding is sufficiently strong under physiological conditions to be a major mechanism by which mineral bioavailability is reduced. In this work are examined some aspects of iron binding behavior of wheat brans under simulated human gastric conditions of low pH and then are considered a few treatments that affect iron binding. This report concludes with some data that allow comparison of the iron binding content of as-is bran pericarp with that of bran remnants retrieved after having been baked in bread, consumed, and passed through the human digestive system.

MATERIALS AND METHODS

Brans of Waldron variety, a hard red spring wheat grown in North Dakota, and durum wheat were obtained from the Spring and Durum Wheat Quality Laboratory, North Dakota State University, Fargo. AACC wheat bran, a blend made from soft white winter wheats, was purchased from the American Association of Cereal Chemists. Eagle variety, a hard red winter wheat, was purchased from the Department of Grain Science and Industry, Kansas State University, Manhattan. All brans used in these binding experiments were of similar particle size distributions obtained by sieving to pass a No. 18 U.S. standard sieve (0.98-mm opening) onto a No. 30 U.S. standard sieve (0.52-mm opening). A few measurements were made on similar distributions of a commercial dry-milled corn bran.

Measurements of tracer counts were made in a Tracor analytic automatic gamma system equipped with a TN-1710 multichannel analyzer. Activities were kept at a low level, with counting periods of 10 min used for sample counting. Control blanks that did not contain bran substrates were run with all measurements.

Experiments were designed to obtain balance measurements of radioactivities under conditions of high gastric acidity. Solutions were made 0.1 N in HCl to contain 10 mequiv/L of K^+ added as KCl and 30 mequiv/L of Na^+ added as NaCl. Radioactive iron, ^{59}Fe, for tracer use was purchased either as $FeCl_3$ in 0.1 N HCl (Amersham, Arlington Heights, IL) or as $FeSO_4$ in 0.05 M H_2SO_4 (New England Nuclear, Boston, MA). Iron in the appropriate form of $FeCl_3$ or $FeSO_4$ was added as a carrier

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to all radioactive solutions. The weight ratio of radioactive iron to nonradioactive carrier iron was never more than 10^{-8} and 10 mL of these solutions contained 4×10^{-7} mg of ^{59}Fe. Solutions were prepared to contain total iron concentrations from 10 to 2400 mg/L.

Portions of untreated bran, 0.5 g dry weight, were weighed into 150-mL beakers. Ten milliliters of iron-containing solution was added to each beaker, which was covered with aluminum foil and placed in a constant-temperature bath at 37.5 °C for the desired time. Except during periods of overnight runs, the beakers were gently swirled a few times every hour. During this incubation treatment, the wheat bran mixtures separated into a layer of dense particles containing pericarp material, a grayish white precipitate, and a usually turbid supernatant. After incubation for the desired time, the beaker was swirled; the grayish white precipitate became suspended in the supernatant, which was removed with a Pasteur capillary pipet, placed in a test tube, and centrifuged at 900*g* for 10 min. This centrifugation yielded a clear supernatant, which transferred with the pipet and stored. The bran pericarp remaining in the beaker was washed with a 10-mL portion of 0.1 N HCl; the beaker was again swirled, and the supernatant was withdrawn and transferred to the tube containing the centrifuged precipitate. This mixture was stirred with a Vortex mixer to wash the precipitate. The tube contents were centrifuged again, and the supernatant wash was collected for counting. The pericarp material and grayish white precipitate were washed separately 3 times in this manner, given a final wash with ethanol, and air-dried. The bran pericarp, precipitate, and washes were all counted for ^{59}Fe γ activity, and the solids were weighed.

The iron content in the as-is bran and in bran remnants retrieved from fecal material was determined by using atomic absorption procedures of Garcia et al. (1974). Bran pericarp remnants from bran that had been baked in bread, passed through the human digestive system, and retrieved from feces were collected on 40-mesh sieves as described previously (Dintzis et al., 1979). The phytate content of brans and recovered pericarp exposed to iron concentrations of 100 mg/L or less was determined by the HPLC method of Graf and Dintzis (1982). Phytate in samples exposed to greater concentrations of iron was determined by a modified procedure in which 100–200 μmol of disodium ethylene diamine tetraacetate (EDTA) was added to the 20 mL of 0.5 N HCl extraction solution. Use of EDTA in ion-exchange phytate analysis has been examined by Ellis and Morris (1983). Because of concern that residual EDTA would harm the C_{18} reverse-phase, HPLC column, phytate was determined in EDTA-treated samples by measuring phosphorus in the eluted sample as described by Ellis and Morris (1983). In this work iron solutions were not buffered; therefore, slight differences in pH occurred because of interactions with substrate and iron. Thus, the pH of an AACC bran mixture containing 4 mg of Fe^{3+}/10 mL was 1.36, whereas that of a Waldron bran mixture was 1.44.

The diet regimen, metabolic unit procedures, and methods used to collect and process human fecal samples from which bran remnants were retrieved have been described previously (Sandstead et al., 1979; Tucker et al., 1981).

RESULTS

Weights of recovered pericarp material from the 0.5-g samples were of the order of 0.34–0.37 g, whereas weights of recovered white precipitate were 0.05 g or less per sample. Hence, one could accurately estimate a specific binding for the recovered pericarp but not for the precipitate, which had greater variation because of its low weight and presumed losses during handling. Some results of bran exposure to 0.1 N HCl containing added iron and tracer ^{59}Fe are shown in Table I. In part A, weight recovery values are presented for two brans as a function of incubation time and exposure to ferrous or ferric iron. The amount of recovered pericarp is relatively insensitive to both incubation times examined and the form of iron used here. At short incubation times, the amount of precipitate is significantly greater when exposure is to ferric iron. Total solids recovery varied from 73 to 83%. Low-power microscope examination of eagle bran revealed a marked decrease in the amount of white endosperm particles remaining in the pericarp recovered after a 4-h incubation in 4 mg of Fe^{3+}/10 mL of solution.

Table I. Percent Recovery of Weight and Radioactivity

(A) Weight,[a] %

	time in simulated gastric juice	
	2 h	30 h
(1) AACC bran (n = 3)		
pericarp tissue	73.3 ± 0.1[b]	69.6 ± 0.3[b]
	72.2 ± 0.2[c]	70.5 ± 0.5[c]
centrifuged ppt	9.91 ± 0.12[b]	10.12 ± 0.23[b]
	6.77 ± 0.12[c]	9.23 ± 0.38[c]
total recovered solids	83[b]	80[b]
	79[c]	80[c]
(2) Waldron bran (n = 5)		
pericarp tissue	72.5 ± 0.9[b]	71.0 ± 0.6[b]
	71.1 ± 1.5[c]	71.4 ± 1.2[c]
centrifuged ppt	6.04 ± 0.56[b]	5.05 ± 0.98[b]
	1.53 ± 0.11[c]	3.68 ± 0.36[c]
total recovered solids	79[b]	76[b]
	73[c]	75[c]

(B) Radioactivity[d] of ^{59}Fe

	pericarp tissue	ppt	supernatant and washes
AACC bran	32[b]	62[b]	6[b]
	14[c]	4[c]	82[c]
Waldron bran	37[b]	57[b]	6[b]
	15[c]	3.4[c]	82[c]

[a] Values in table corrected to a dry weight basis; sample weight of 0.50 g. [b] Incubated in 10 mL containing 4 mg of Fe^{3+} as $FeCl_3$. [c] Incubated in 10 mL containing 4 mg of Fe^{2+} as $FeSO_4$. [d] Four-hour incubation is simulated gastric juice, 4 mg of iron/10 mL.

The recovery of radioactivity was at least 97%. Part B of Table I displays typical values of activity found in two brans after a 4-h exposure. Most of the ferric iron is bound, but much of the ferrous iron is not. A higher percentage of ferric ion activity resides in the precipitate rather than in the residual pericarp tissue. A minor amount of added ferrous iron is found in the precipitate, and the percentage of ferrous activity in the pericarp is about half that of the ferric activity. Thus, much of the ferrous iron remains in solution while most of the ferric iron does not.

Binding of iron to AACC bran pericarp is displayed in Figure 1 as a function of time and exposure to two iron concentrations. Bound iron increases with time and with an increase in solution iron concentration. At short exposure times, ferrous iron is bound appreciably less than ferric iron; at longer exposure times, the amounts bound are similar.

Ferric iron binding of four wheat brans incubated 4 h is examined in Figure 2 and Table II as a function of iron concentration. A range of bound-iron values is displayed at each evaluated iron concentration. An initial steep rise in binding is followed by a region at higher iron concentrations where binding increases much more slowly. Details of this binding are given in Table II, part A, where

Table II. Distribution of Added Iron and Phytate[a]

mg of Fe^{3+} in 10 mL		bran[b] AACC	durum	Eagle	Waldron
		(A) Milligram of Added Iron Bound in Sample			
0.1	RP[c]	5.2×10^{-2}	5.6×10^{-2}	5.3×10^{-2}	5.8×10^{-2}
	ppt	1×10^{-4}	7×10^{-4}	5×10^{-4}	6×10^{-4}
		(5.2×10^{-2})[d]	(5.6×10^{-2})	(5.3×10^{-2})	(5.8×10^{-2})
0.25	RP	0.16	0.16	0.16	0.17
	ppt	7.5×10^{-4}	4.3×10^{-3}	2.8×10^{-3}	2.8×10^{-3}
		(0.16)	(0.16)	(0.16)	(0.17)
0.40	RP	0.28	0.25	0.25	0.28
	ppt	8.4×10^{-3}	2.5×10^{-2}	9.6×10^{-3}	5.6×10^{-3}
		(0.29)	(0.28)	(0.26)	(0.29)
1.0	RP	0.60	0.49	0.49	0.58
	ppt	0.19	0.31	0.20	0.18
		(0.79)	(0.80)	(0.69)	(0.86)
4.0	RP	1.2	1.0	0.96	1.1
	ppt	2.6	2.2	2.2	2.5
		(3.8)	(3.2)	(3.2)	(3.6)
8.0	RP	2.9	3.2	3.1	
	ppt	3.4	2.2	3.1	
		(6.3)	(5.4)	(6.1)	
12.0	RP	4.3	4.5	4.8	
	ppt	2.8	1.4	2.3	
		(7.1)	(5.9)	(7.1)	
24.0	RP	7.2	5.5	6.7	
	ppt	0.37	0.46	0.48	
		(7.6)	(6.0)	(7.2)	
		(B) Phytate Content in Milligrams			
0.0	ppt	<0.2	<0.2	<0.2	<0.2
	untreated bran control	19 ± 1	14 ± 1	18 ± 1	24 ± 1
1.0	ppt	1.0 ± 0.1	2.2 ± 0.4	2.6 ± 0.2	2.2 ± 0.1
8.0	ppt	2.3 ± 0.4	2.6 ± 0.2	5.6 ± 0.1	4.7 ± 0.5

[a] All values obtained from 0.50-g samples (dry weight) incubated 4 h at 37.5 °C in simulated gastric juice. Measurements done at least in duplicate. [b] Endogenous iron content of whole bran fractions are listed in Table IV, part B. [c] RP = recovered pericarp, average weight ~0.36 g; ppt = recovered nonpericarp precipitate. [d] Parentheses indicate total bound added iron.

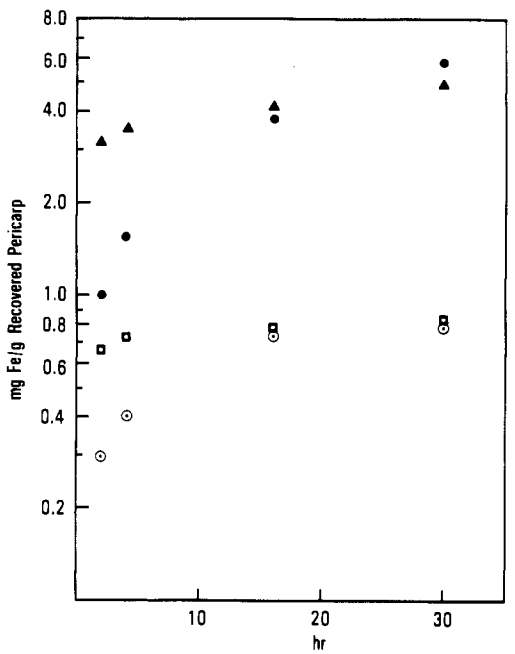

Figure 1. Time dependence of AACC bran pericarp iron binding. Initial 18–30-mesh distribution of bran incubated in solution of 0.1 N HCl at 37.5 °C, pH ~1.3–1.4. (⊙) 0.4 mg of Fe^{2+}/10 mL; (●) 4.0 mg of Fe^{2+}/10 mL; (□) 0.4 mg of Fe^{3+}/10 mL; (▲) 4.0 mg of Fe^{3+}/10 mL.

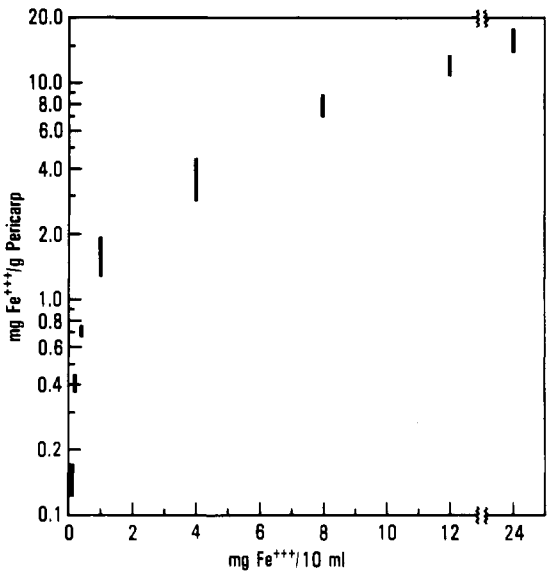

Figure 2. Iron bound by wheat bran pericarp. Four-hour exposure at 37.5 °C, 0.1 N HCl, pH 1.3–1.5. Note the change in scale for 24 mg of Fe^{3+}/10 mL.

measured tracer iron counts are used to calculate amounts of bound iron. One observes that the ferric iron is distributed unevenly between pericarp tissue and precipitate. Iron bound by pericarp tissue increases continuously with solution iron concentration, but iron bound by the precipitate goes through a maximum in the vicinity of 8 mg of Fe^{3+}/10 mL. The amount of iron bound by wheat bran pericarp was still increasing with iron concentration at 12 mg of Fe^{3+}/10 mL. Therefore, the iron concentration was doubled, and an estimated average binding limit of about 16 mg of Fe^{3+}/g of recovered pericarp was obtained (Figure 2), or about 0.29 mmol of iron/g of recovered pericarp. At concentrations of 4 mg of Fe^{3+}/10 mL, an average of about 0.03 g of precipitate is collected after a 4-h incubation, and an average of 2.4 mg of iron is bound by this material to yield a value of ~80 mg of Fe^{3+}/g of precipitate. This value is significantly higher than the binding value of ~3.5

Table III. Treatment Effects on Bran Properties

mg of Fe^{3+}/10 mL of simulated gastric juice		mg of Fe^{3+}/g of pericarp: 4-h incubation[a]	pretreated[b] and then 4-h incubation	demineralized[c]
		(A) Ferric Iron Binding		
		(1) Waldron Bran		
0.10	A[d]	0.17 (n = 2)	0.16 ± 0.01 (n = 3)	
	B	0.13		
	C	0.12 (n = 2)		0.13 ± 0.01 (n = 4)
0.25	A	0.47	0.39 ± 0.03 (n = 3)	
	C	0.37 (n = 2)		
0.40	A	0.74 (n = 2)		
	C	0.69 (n = 2)	0.56 ± 0.01 (n = 3)	0.30
1.0	A	1.6		
	C	1.9 (n = 2)		0.40 ± 0.02 (n = 3)
4.0	A	3.0 ± 0.1 (n = 10)	5.54 ± 0.12 (n = 5)	
	B	3.6 ± 0.1 (n = 3)		
	C	4.3 ± 0.1 (n = 4)		0.52 ± 0.03 (n = 4)
		(2) Other Brans		
0.4	AACC	0.75 (n = 2)	0.53	
	durum	0.69	0.49	
	eagle	0.72	0.55	
4.0	AACC	3.3	5.45 ± 0.19 (n = 3)	
	durum	2.9	4.7	
	Eagle	2.8 (n = 2)	5.3	

(B) Percent Phytate

	in whole bran, untreated	in recovered pericarp: 4-incubation, no added iron	4-h incubation with 4 mg of Fe^{3+}/10 mL	4-h incubation in acetate buffer, pH 4.5	overnight in distilled water
AACC	3.8	1.4	1.4	0.46	
Eagle	3.5	1.3	1.3	0.29	0.50

[a] Treatment carried out at 37.5 °C, in 0.1 N HCl for 4 h unless otherwise specified. [b] First incubation in 0.1 N HCl at 37.5 °C for 4 h, washed, and air-dried in a hood. [c] Incubated in acetate buffer at pH 4.5 for 24 h at ambient temperatures. [d] A, B, and C are different batches of Waldron bran.

mg of Fe^{3+}/g of recovered pericarp obtained from the same treatment.

In part B of the table are displayed the phytic acid contents of centrifuged precipitates collected from solutions of two different concentrations of added iron. The control solutions, which contained no added iron, generated precipitates that contained at least a factor of 10 less phytate than did precipitates generated from the other two solutions. The increase from 1.0 to 8.0 mg of Fe^{3+}/10 mL caused increased phytate content in the precipitates from three of the brans but not durum bran. Release of materials from the whole bran particle into the supernatant also was influenced by the presence of added iron. Thus, average weights of 21, 29, and 34 mg, respectively, were obtained on AACC bran precipitates obtained from 10-mL solutions containing 0.0, 1.0, and 8.0 of mg of Fe^{3+}. Corresponding weight values for Eagle bran centrifuged precipitates were 19, 32, and 31 mg. Quantities of phytate associated with the precipitates were not uniform, especially at the higher iron concentration. Thus, with solutions of 8 mg of Fe^{3+}/10 mL about 30% of the phytate was associated with the centrifuged precipitate of Eagle bran while the corresponding value for AACC bran was 12%.

How different treatments affected the ferric iron binding ability of bran and what variations might occur within different batches of the same bran variety were examined. Data in Table III demonstrate that binding may be affected dramatically by treatment. Results of lowered phytate content obtained on demineralized brans and brans incubated overnight in distilled water are similar to those reported by Morris and Ellis (1980). Type C Waldron bran is from a different crop year than brans A and B, which are from the same crop year and differ in that B has been stored longer and therefore has aged more than A and has somewhat different milling characteristics. Soaking the bran overnight in acetate buffer at pH 4.5 (demineralization) greatly reduces binding of ferric iron. Pretreating the brans by soaking them in 0.1 N HCl for 4 h seems to generate an effect that is dependent upon iron concentration, as is noted with the Waldron bran. This trend is verified in part A, 2, of Table III, where it is shown that this pretreatment decreases binding at 0.4 mg of Fe^{3+}/10 mL and increases it at 4.0 mg of Fe^{3+}/10 mL.

The phytate content of recovered pericarp for two brans, listed in part B of Table III as a function of various treatments, is displayed in Figure 3 as a function of bound iron resulting from a 4-h incubation in simulated gastric juice solution. Note that the amount of phytate remaining in the pericarp is essentially independent of the ferric ion concentration up to a concentration of 4 mg of Fe^{3+}/10 mL (Figure 3). Exposure to 8 mg of Fe^{3+}/10 mL does tend to further lower the phytate content.

Remnants of pericarp from bran that had been baked in bread and fed to human volunteers maintained on strictly controlled diets were obtained for this study through a cooperative association with the Human Nutrition Center in Grand Forks, North Dakota. In part A of Table IV are presented some estimates of ferric iron bound by brans retrieved from human feces collected on 40-mesh sieves, washed (Dintzis et al., 1979), and later incubated 4 h in 0.1 N HCl containing 4.0 mg of Fe^{3+}/10 mL. Baking and passage through the human digestive system appear to have a variable effect upon the in vitro iron binding ability of the brans.

In part B of Table IV are listed some values of iron contents of as-is bran and retrieved bran remnants. Examination of amounts of starch and evaluation of protein contents in terms of recovered amino acids indicate a combined weight loss of about 25%, averaged over the wheat brans, between initial and retrieved bran. An es-

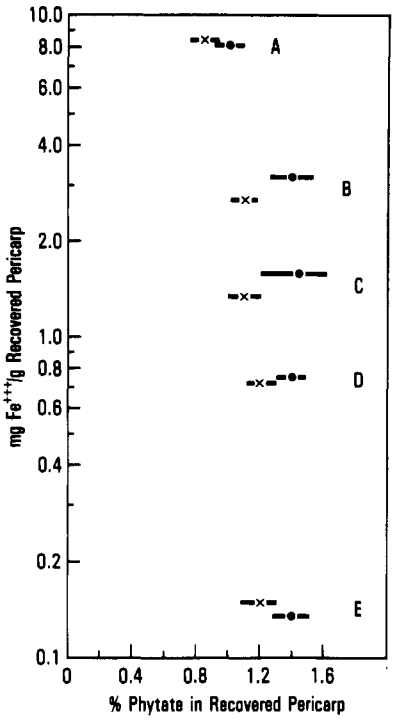

Figure 3. Phytic acid remaining in recovered bran pericarp calculated as dry weight percent of whole bran sample. Four-hour exposure in 0.1 N HCl, 37.5 °C, pH 1.3–1.5. AACC bran (●); Eagle bran (×). Iron concentration of solutions in mg of Fe^{3+}/10 mL: A = 8.0; B = 4.0; C = 1.0; D = 0.4; E = 0.1.

timated 30% weight loss occurs when the original brans are incubated 4 h in 0.1 N HCl at 37.5 °C. Therefore, an adjusted value, indicated by parentheses in the table, is an attempt to exclude from the starting bran adhering endosperm, which is removed by the process of baking and digestion and is no longer present in the retrieved pericarp. This adjusted value attributes all minerals in the starting bran fraction to the pericarp material. Starch and labile protein contents of the dry-milled corn brans were less than 5%, and therefore, no correction is made for these materials. Iron content values in retrieved bran were obtained from fecal matter of different individuals. These estimates indicate no significant increase in the iron content of wheat bran pericarp after passage through the human digestive system. However, increased amounts of iron were associated with dry-milled corn bran after passage through the human GI tract.

DISCUSSION

Data in Table I demonstrate the preference of wheat brans to bind ferric rather than ferrous iron at low gastric pH. One effect of incubating wheat brans in these simulated gastric fluid solutions is to partition the bran into three components: pericarp tissue with still adhering endosperm, a grayish white precipitate, and solubles remaining in the supernatant. The relatively consistent percent weight recovery with time indicates an absence of severe acid hydrolysis. The results in part B of the table clearly demonstrate that components in wheat brans bind ferric iron by at least a factor of 10 more than they bind ferrous iron.

The behavior at longer incubation times (Figure 1), in which amounts of ferrous and ferric iron bound become similar, is not likely to be an artifact caused by oxidation of ferrous ion. Nojeim and Clydesdale (1981) have shown that at pH ≤3.0, ferrous iron is stable for 48 h. Some of the incubation beakers were covered with aluminum foil and the air space above the mixture was flushed with N_2 before the foil around the beaker was sealed. After 30-h incubation in simulated gastric fluid, the binding values were the same for N_2-flushed samples and those left exposed to the atmosphere. The lower binding values of ferrous iron to bran at short incubation times and the high percentage that remains in solution after 4 h (Table I, part B) are compatible with the known greater bioavailability of ferrous iron supplementation compared to ferric iron (Cook et al., 1973).

Table IV. Effects of Baking and Passage through Human Digestive Systems upon Iron Content

(A) Binding during Exposure to Simulated Gastric Conditions[a]

bran[b]	mg of Fe bound/g of recovered pericarp: prior to baking[c]	mg of Fe bound/g of recovered pericarp: retrieved from feces[d]
AACC	3.3 ± 0.3	1.0
		1.9
durum	3.1 ± 0.2	1.7
		1.0
		0.7
Eagle	2.8 ± 0.1	1.9
		3.3
Waldron	3.0 ● 0.1	1.6
		3.3
		3.5
		3.7
corn bran, dry milled	0.24 ± 0.16	0.09
		0.10
		0.14

(B) Iron Content Estimates

bran	μg of Fe/g of substrate: prior to baking[c]	μg of Fe/g of substrate: retrieved from feces[e]
AACC	133 ± 19 (190)[f]	169 ± 22
durum	127 ± 4 (181)	117 ± 34
Waldron	209 ± 14 (299)	208 ± 50
corn bran, dry milled	13.5 ± 1.6	50 ± 12

[a] Incubated in the presence of 4.0 mg of Fe^{3+}/10 mL, 4 h at 37.5 °C. [b] 18–30 mesh sieved distributions of each bran. [c] Each value is the average of at least three independent measurements. Endogenous iron content of Eagle bran is 121 ± 10 μg of Fe/g. [d] Each value is the average of three measurements on retrieved bran obtained from a 6-day fecal collection of one human subject. [e] For wheat bran remnants averages are duplicate values from three differemtn volunteers. For corn bran remnants the average represents nine measurements associated with five volunteers. [f] Parentheses indicate values adjusted for loss of endosperm.

Data in Table II, part A, and the display in Figure 2 illustrate the similar behavior of four different wheat brans. The rate of increase in amount of iron bound is seen to be greatest at concentrations of <1.0 mg of Fe^{3+}/10 mL. The system is not a simple one. One complication is that the amount of adhering endosperm is influenced by iron concentration, and as indicated previously, this effect seems most pronounced in the range of zero to 1.0 mg of Fe^{3+}/10 mL of added iron. An approximate value of 0.15 mg of Fe^{3+} bound/g of recovered pericarp at an exposure concentration of 0.1 mg of Fe^{3+}/10 mL is of the same order of magnitude as ferrous iron binding by neutral detergent fiber treated tissues of maize and wheat measured at pH 6.45 and 25 °C (Reinhold et al., 1981).

Significant amounts of phytate can be associated with the centrifuged precipitate as indicated in part B, Table II. This is not surprising since treatment of plant tissues with HCl is an accepted procedure to extract phytate (Makower, 1970; Harland and Oberleas, 1977) and the ferric phytate complex is known to be insoluble at acid pH in the presence of a moderate excess of ferric iron. The

presence of phytic acid provides at least a partial explanation for the high percentage of iron bound by the precipitate in mixtures containing $FeCl_3$ compared to those containing $FeSO_4$ (part B, Table I). The decrease in precipitate bound iron at concentrations greater than 8 mg of Fe^{3+}/10 mL (part A, Table II) is compatable with the reported solubility of ferric phytate complexes at high ferric iron concentrations (Anderson, 1963).

The ability of different treatments to alter ferric ion binding (Table III, part A) is consistent with reports of different treatments affecting ferrous iron binding as well as binding of other minerals (Camire and Clydesdale, 1981). At this time we do not known why the two treatments, a 4-h exposure to 0.1 N HCl at 37.5 °C or overnight incubation in pH 4.5 acetate buffer at ambient temperature, have such different effects on iron binding at the higher ferric concentrations. Apparently, exposure to dilute HCl has altered pericarp composition so as to allow a greater loading of ferric iron onto the tissue. All treatments listed in part B of Table III reduce phytate content. Incubation in acetate buffer at pH 4.5, 37 °C, is a rapid method to do this. We note that 4-h incubation in 0.1 N HCl at 37.5 °C, with or without 4 mg of Fe^{3+}/10 mL, reduces phytate less than does the presumed effect of endogenous phytase activity at pH 4.5 in acetate buffer.

Binding of iron by phytate is one mechanism to explain our data, but it is not the only one. In Figure 3 is presented the relationship between phytate content and iron bound in recovered pericarp. As a result of 4-h exposures to concentrations from 0.1 to 1.0 mg of Fe^{3+}/10 mL in 0.1 N HCl, the phytate content of recovered bran pericarp is relatively constant, whereas the amount of bound iron increases by factors that average about 20. If one assumes a residual phytate molecule in the pericarp is able to bind four ferric ions, as a phytate molecule in solution would do, then calculation reveals that bound iron could be accounted for by the remaining phytate in recovered pericarp tissue exposed to solutions containing 4.0 mg of Fe^{3+}/10 mL or less iron (i.e., 1.0 mg of phytate may bind ~0.34 mg of Fe^{3+}). Binding of ferric iron at concentrations of 8 mg of Fe^{3+}/10 mL or higher must involve other mechanisms. Thus, remaining phytate in recovered AACC and Eagle bran pericarp incubated in 4 mg of Fe^{3+}/10 mL, 1.3 and 1.1%, respectively (Figure 3), might bind up to ~4.4 and 3.7 mg of Fe^{3+}/g of RP. However, data from Table II indicate that after incubation with solutions containing 8 mg of Fe^{3+}/10 mL, recovered AACC and Eagle bran pericarp bind about 8.1 and 8.4 mg of Fe^{3+}/g, respectively.

Results of in vitro studies on wheat brans and other dietary fiber components provide overwhelming evidence of the potential for these materials to behave as mineral sinks. A recent paper showed that the amount of iron solubilized from different foods into gastric fluid obtained from 16 human volunteers was decreased when wheat bran was added to many of the foods tested (Lock and Bender, 1980). However, many complications associated with such potential behavior are known, and some factors that would affect the ability of dietary fiber to act as a mineral binding agent have been examined (Reinhold et al., 1981). Data in part A of Table IV represent an opportunity to compare in vitro binding of bran pericarp before and after baking in bread and passage through the human digestive system. We observed that results for wheat brans are quite scattered, that binding is sometimes strongly affected, and that by comparison ferric iron binding by dry-milled corn bran can be much lower. Three of the Waldron bran and one of the Eagle bran samples retrieved from fecal matter show enhanced binding, whereas comparable samples of AACC and durum bran have diminished binding.

Data in part B of Table IV leads to conclusions for the wheat brans that are reasonable clear—under realistic dietary conditions no binding of iron by the retrieved wheat bran remnants was detected. Comparison of adjusted values [Table IV (B), in parentheses] with iron contents of retrieved wheat brans shows no increase for AACC bran and indicates lower values for durum and Waldron brans. Thus, although in vitro binding of ferric iron by wheat brans is well demonstrated, direct measurements on retrieved wheat brans do not seem to support a concept of lowered iron bioavailability occurring by the mechanism of wheat bran pericarp tissue acting as an iron sink. Retrieved dry-milled corn bran does contain more iron than it started with, and we will need additional data before making comment on this observation. Studies are in progress to examine the binding of various minerals by brans under realistic human dietary conditions.

Registry No. Fe, 7439-89-6; HCl, 7647-01-0; phytate, 83-86-3.

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