Iranian Flat Breads: Relative Bioavailability of Iron[1]

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ABSTRACT

The iron in five types of Iranian flat breads (Barbari, Lavash, Taftoon, Sangak, and Village) and in the corresponding fermented doughs was assessed for its efficacy in promoting hemoglobin synthesis in hemoglobin-depleted rats. Bioavailability of iron in these breads differed significantly, ranging from 53 to 95% (relative to iron in ferrous sulfate) as calculated by the modified AOAC method and from 63 to 100% as calculated by methods based on gain in hemoglobin. No direct relationship of these values to protein, phytate, or dietary fiber content of breads was observed. Comparison of breads with doughs showed that the baking process appreciably improved the bioavailability of iron in Barbari, Taftoon, and Sangak breads and decreased it in Lavash and Village breads.

Various types of flat breads are traditionally produced and consumed in Iran. These breads provide as much as 90% of the daily caloric and protein needs for a major segment of the population (Faridi et al 1981). They also provide a substantial amount of various micronutrients in the diet, including iron.

The physiological availability of iron in bread and other cereal-based foods is generally considered low in comparison to iron in foods of animal origin (Monsen et al 1978). The availability of iron among various cereal-based foods also differs appreciably depending on the presence or absence of certain dietary (and nondietary) factors that either enhance or hinder iron absorption. Fiber, phytate, proteins, and certain microcomponents, minerals in particular, have variously been implicated as adversely affecting the absorption of iron (Cook et al 1973, Ismail-Beigi et al 1976, Monsen et al 1978, Reinhold et al 1975). Using rats as the test model, five different types of Iranian flat breads were tested for their efficacy in meeting the body's need for iron. By comparing fermented doughs with breads, the effect of baking on iron availability was also examined.

MATERIALS AND METHODS

The five test breads and corresponding doughs were prepared according to the formulas and procedures shown in Table I and detailed elsewhere (Faridi et al 1981). Breads (oven-dried at 50° C) and doughs (freeze-dried) for all analyses were finely ground and stored in airtight plastic bags under refrigeration.

The biological value of iron in these samples (relative to the value of iron in ferrous sulfate) was determined based on the hemoglobin (Hb) depletion-repletion method of the AOAC (1975). Because of lack of available material, test diets were formulated at only one iron level.

Weanling, male, Sprague-Dawley rats, housed individually in stainless steel cages and under controlled environment, were fed the low-iron (basal) diet (Table II) for four weeks. At that time, their Hb levels had dropped to an average of 5.6 g/dl. The depleted rats were then randomly divided into 14 groups (Hb = 5.62 ± 0.03 g/dl), with 10 rats per group. Then groups were placed on the test diets (Table III), which contained 9 ppm iron each from added breads or doughs. The other four groups were placed on the reference diets (Table III), which contained Fe at 0, 6, 9, and 12 ppm from ferrous sulfate added to the low-iron diet. Bread or dough in low-iron test diets was added at the expense of sucrose. The amount of casein in the diets was adjusted so that all diets contained 17% protein. During the two-week repletion period, weekly weight gain and daily diet intake records were kept. Diet and deionized water were offered ad libitum throughout.

Standard AACC (1976) methods were used to determine moisture, ash, protein, and lipid contents of breads and doughs. Dietary fiber was determined by the AACC (1976) modified method. Crude fiber was determined using the AOAC method (1975). Phytate phosphorus was determined by the method of Makower (1970). Iron in breads and doughs (Table I) and in diets

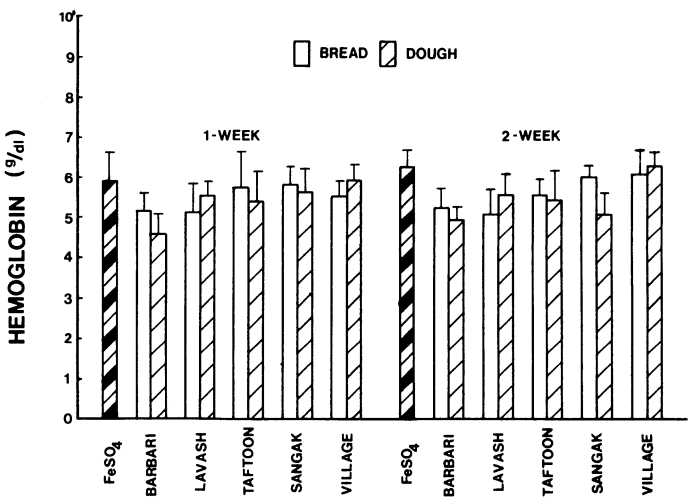

Fig. 1. Hemoglobin levels at one and two weeks of the repletion phase.

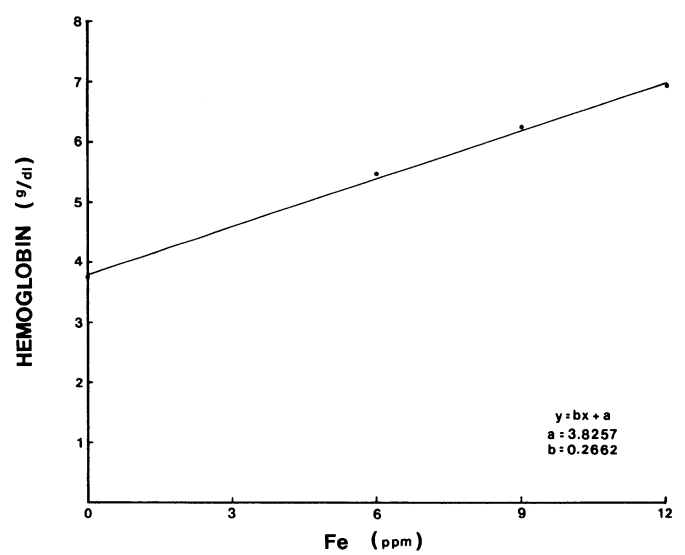

Fig. 2. Regression of hemoglobin levels on dietary iron (as ferrous sulfate) levels.

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TABLE I
Formulation and Composition of Iranian Flat Breads

	Name of Bread and Extraction Rate of Flour				
	Barbari, 78	Lavash, 82	Taftoon, 84	Sangak, 87	Village, 97
Formula					
Wheat flour (g)	100	100	100	100	100
Compressed yeast (g)	1	0.5	0.5	0.125	...
Sour dough (g)	...	...	...	20	...
Salt (g)	2	2	1	1	1
Soda (g)	0.35	0.25	0.25	...	...
Date syrup (g)	...	...	1.5	...	...
Water (ml)	60	45	60	85	48
Baking Variables					
Fermentation time (min)	120	90	60	120	90
Baking temperature (° F)	500	630	600	520	410
Baking time (min)	12.0	1.3	2.5	5.0	3.0
Composition[a]					
Moisture (%)	5.6 (3.3)	6.2 (3.6)	5.8 (4.0)	9.9 (5.7)	5.7 (2.8)
Protein (N × 5.7, %)	8.3 (8.5)	8.9 (8.8)	8.6 (9.0)	8.5 (9.0)	9.2 (9.7)
Ash (%)	2.6 (2.7)	2.6 (2.7)	1.7 (1.7)	1.6 (1.6)	2.1 (2.1)
Ether extract (%)	0.4 (0.6)	0.4 (0.7)	0.3 (0.8)	0.4 (0.8)	0.7 (1.3)
Crude fiber (%)	0.1 (0.1)	0.3 (0.2)	0.4 (0.3)	0.8 (0.7)	1.6 (1.5)
Dietary fiber (%)	0.6 (0.5)	1.3 (1.2)	2.0 (1.8)	3.3 (3.2)	6.0 (5.9)
Phytate P (mg/100 g)	2.4 (1.8)	55.4 (53.1)	52.5 (58.9)	3.1 (1.5)	185.9 (185.9)
Iron (ppm)	13.1 (12.1)	17.7 (16.6)	19.3 (18.7)	21.3 (21.1)	30.8 (31.5)

[a]Values within parentheses refer to doughs.

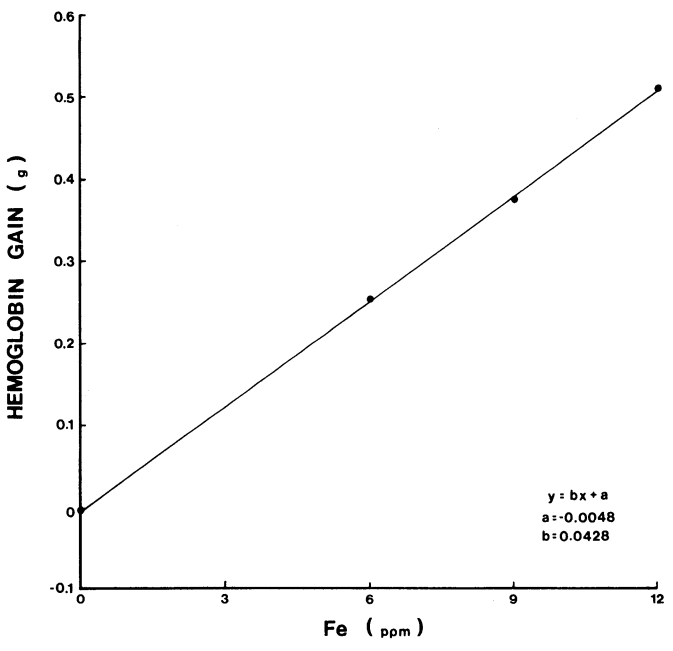

Fig. 3. Regression of hemoglobin gain on dietary iron (as ferrous sulfate) levels.

was determined, following dry ashing, by atomic absorption spectrophotometry using an IL (Instrumentation Laboratories, Inc.) model 251 spectrophotometer. Hb was determined on tail blood by the cyanmethemoglobin method (Crosby et al 1954).

RESULTS AND DISCUSSION

The five bread types tested (Table I) represent the most widely-consumed varieties in Iran. As traditionally practiced, flours of variable extraction rates were used in the preparation of these breads. Because the breads contained no added iron, their iron contents (Table I) typified values normally associated with flours of various extraction rates (Ranum et al 1980). Bread A (Barbari), made with white flour (78% extraction), contained the lowest amount of iron. Because of this, the iron content of test diets could not exceed 9 ppm. One of the reference diets (Table III) also contained 9 ppm iron in place of the proposed (AOAC 1975) 24 ppm iron. The 9 ppm iron represents about one fourth of the requirement (NAS/NRC 1978) for rats.

TABLE II
Composition of Basal, Low-Iron Diet[a] (g/100 g)

Ingredients	
Casein	20.0
Vitamins[b]	2.2
Trace minerals[c]	0.5
Corn oil	5.0
NaCl	0.5
KCl	0.5
$NaH_2PO_4 \cdot H_2O$	2.0
$CaCO_3$	2.0
DL-methionine	0.1
Sucrose	67.2
Iron (ppm)	1.9

[a]The standard (AOAC 1975) diet was used except that cornmeal and gelatin were not included.
[b]Vitamin diet fortification mixture from ICN Pharmaceuticals (ascorbic acid, 0.1%).
[c]Containing (in sucrose base): Mg, 40 mg; Cu, 0.5 mg; I, 0.015 mg; Mn, 5 mg; and Zn, 1.2 mg.

Figure 1, which shows the extent of Hb repletion at the end of one and two weeks, clearly indicates that the test diets differed appreciably in their capacity to promote Hb synthesis. Table III gives more detailed information on this. In the two-week period, while rats on the low-iron reference diet (diet K) became severely more anemic, all those fed 9 ppm iron diets showed only slight changes (decrease or increase) in Hb levels. Where a slight decrease occurred, it apparently resulted from a rapid expansion of the blood volume (and consequent dilution of the Hb synthesized) rather than from failure to synthesize Hb. Where a higher dietary iron level was provided (reference diet N), the Hb level increased appreciably in spite of presumed dilution.

The body weight gain of rats on the 9-ppm iron diets (diets A–J and M) did not differ significantly. Although this should make direct comparison of Hb levels (expressed as g/dl) between diets more meaningful, the observed differences in diet, and hence in iron, intakes appear to temper this.

TABLE III
Relative Bioavailability of Iron in Iranian Flat Breads[a]

	Test Diet													
	A	B	C	D	E	F	G	H	I	J				
	Bread					Dough (Fermented)					Reference[b] Diet			
	Barbari	Lavash	Taftoon	Sangak	Village	Barbari	Lavash	Taftoon	Sangak	Village	K	L	M	N
Diet content														
Casein (g)	13.27	14.84	15.31	15.76	16.84	12.59	14.42	14.93	15.50	16.76	20.00	20.00	20.00	20.00
Bread or dough (g)	68.81	50.85	46.68	42.31	29.22	74.38	54.18	48.26	42.65	28.57				
Sucrose (g)	5.12	21.51	25.21	29.13	41.14	0.23	18.60	24.01	29.05	41.87	67.20	67.20	67.20	67.20
Others[c] (g)	12.80	12.80	12.80	12.80	12.80	12.80	12.80	12.80	12.80	12.80	12.80	12.80	12.80	12.80
Dietary iron (ppm)	9	9	9	9	9	9	9	9	9	9	0	6	9	12
Body weight gain (g)	84 ±8	79 ±6	86 ±8	89 ±7	85 ±8	85 ±9	78 ±7	78 ±7	85 ±5	86 ±11	48 ±7	72 ±8	78 ±6	91 ±5
Intake														
Diet (g)	183 ±9	185 ±15	184 ±16	207 ±13	193 ±13	172 ±13	181 ±11	169 ±14	187 ±11	187 ±13	154 ± 8	188 ±19	190 ±15	201 ±22
Iron (mg)	1.65 ±0.08	1.67 ±0.13	1.65 ±0.14	1.86 ±0.12	1.74 ±0.12	1.55 ±0.12	1.63 ±0.10	1.52 ±0.13	1.69 ±0.10	1.68 ±0.12	...	1.13 ±0.12	1.71 ±0.14	2.41 ±0.26
Hemoglobin (g/dl)														
0 day	5.63 ±0.69	5.61 ±0.63	5.58 ±0.69	5.65 ±0.69	5.60 ±0.65	5.63 ±0.64	5.67 ±0.46	5.60 ±0.54	5.59 ±0.59	5.62 ±0.67	5.60 ±0.91	5.65 ±0.76	5.57 ±0.89	5.62 ±0.58
14 days	5.25 ±0.47	5.09 ±0.62	5.57 ±0.41	6.03 ±0.31	6.10 ±0.61	4.95 ±0.36	5.60 ±0.52	5.45 ±0.75	5.08 ±0.55	6.31 ±0.37	3.78 ±0.82	5.49 ±0.38	6.27 ±0.43	6.95 ±0.37
Gain[d]														
Hemoglobin (g)	0.269 ±0.061	0.237 ±0.072	0.321 ±0.024	0.388 ±0.045	0.380 ±0.071	0.234 ±0.031	0.285 ±0.059	0.272 ±0.059	0.252 ±0.041	0.411 ±0.073	...	0.254 ±0.060	0.377 ±0.070	0.511 ±0.070
Hemoglobin (g/mg Fe)	0.163 ±0.030	0.141 ±0.040	0.196 ±0.024	0.209 ±0.026	0.219 ±0.043	0.151 ±0.017	0.177 ±0.041	0.180 ±0.040	0.150 ±0.024	0.244 ±0.033	...	0.225 ±0.050	0.222 ±0.040	0.212 ±0.030
Relative bioavailability														
AOAC[e]	59	53	73	92	95	47	74	68	52	104	...	...	...	...
Hb gain[e] (g)	71	63	84	102	100	62	75	72	66	108	...	...	...	...
Hb gain (g/mg Fe)	73	64	88	94	99	68	80	81	68	110	...	...	100	...

[a]Each value represents the average (± standard deviation) of 10 rats.
[b]Ferrous sulfate (Fe, 29.9%) added to low-iron diet.
[c]As in basal diet (Table II).
[d]In 14 days; calculation based on blood volume (6.7 ml/100 g) of rats and their Hb concentration at 0 and 14 days.
[e]Calculated from the standard curves (Figs. 2 and 3).

To minimize the effect of differences in rat body weights and iron intakes, the relative biological values (RBV) of iron in the test diets were calculated by three different methods. The AOAC (1975) calculation method used regression equations developed from the reference diet data (Table III). Using the regression equation for Hb level (Fig. 2), the RBV of iron in test diets equaled [Hb (g/dl) − a]/[b × dietary Fe (ppm)] × 100. When, in the second method, the gain in Hb was considered (Fig. 3), RBV of iron in test diets equaled [Hb gain (g) − a]/[b × dietary Fe (ppm)] × 100. The third method compared directly the Hb gain (g/mg Fe consumed) on reference diet M (= 100) to gains on test diets.

Regardless of the method of calculation, the RBV of iron in test diets differed significantly ($P<0.01$). The modified AOAC (1975) method, which used the regression equation instead of the slope ratio, appeared to underestimate the RBV of iron in breads, especially those made with low-extraction flours.

A number of bread components—phytate, fiber, and protein, in particular—have variously been implicated as adversely affecting the availability of iron (Cook et al 1973, Ismail-Beigi et al 1976, Reinhold et al 1975). These, and probably other components, apparently interacted in some fashion to affect iron availability because differences between bread types were appreciable. On the other hand, no direct relationship between iron availability and these bread components emerged (Tables I and III), and thus their exact involvement is difficult to assess. The same was observed earlier (Ranhotra et al 1979) in studies with variety breads commercially produced in the United States.

The process of baking appeared to either improve (Barbari, Taftoon, and Sangak breads) or not improve (Lavash and Village breads) the RBV of iron. The improvement observed for Sangak bread (diet D) was not only most striking but also somewhat puzzling because Sangak dough (diet I) contained virtually no phytate (intermediate hydrolytic products were not measured), apparently as the result of intense hydrolytic activity during fermentation (Ranhotra et al 1974). Most likely, the chemical form of iron and/or its solubility changed dramatically during the baking process.

Among the breads, iron in Village bread (extraction rate, 97%) showed the highest RBV. Although Village bread contained the highest (Table I) amount of phytate, most of the iron probably existed as monoferric phytate, which is reported (Ellis and Morris 1979) to be quite available to the rat.

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