Iron Bioavailability and Utilization in Rats Are Lower from Lime-Treated Corn Flour than from Wheat Flour When They Are Fortified with Different Sources of Iron[1]

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ABSTRACT Although iron bioavailability from wheat flour fortified with iron has been widely studied, the bioavailability of lime-treated corn flour has not been evaluated sufficiently. We compared iron bioavailability and utilization of lime-treated corn flour and wheat flour supplemented with various iron sources. Bioavailability and utilization were determined in Sprague-Dawley rats using the iron balance and hemoglobin depletion-repletion methods. Rats were iron depleted by feeding them a low iron, casein diet for 10 d. During the repletion period, the rats were fed diets based on lime-treated corn flour or wheat flour, both supplemented with ferrous fumarate, ferrous sulfate, ferric citrate and reduced iron for 14 d. Hemoglobin was determined at the end of depletion and repletion periods. The phytate concentration was lower in wheat flour (114 mg/100g) than in lime-treated corn flour (501 mg/100g). Iron bioavailability and utilization by rats were higher from fortified and unfortified wheat flour than from the lime-treated corn flour counterparts. Iron utilization was greater in rats fed wheat flour supplemented with ferrous sulfate, followed by fumarate and citrate than in rats fed reduced iron. In lime-treated corn flour, iron utilization by rats fed unfortified flour and flour fortified with reduced iron did not differ, but utilization was higher in rats fed corn flour fortified with iron sulfate, fumarate and citrate than with reduced iron. We conclude that fortification of lime-treated corn flour with reduced iron has no effect on iron bioavailability or utilization, probably due to the high phytate content. Other iron compounds must be selected to fortify lime-treated corn flour when intended for public nutrition programs. J. Nutr. 133: 154–159, 2003.

KEY WORDS: • *lime-treated corn flour* • *wheat flour* • *iron utilization* • *iron bioavailability* • *rats*

Food iron fortification is a strategy used worldwide in public nutrition intervention to fight iron deficiency. Iron deficiency, with and without anemia, is the most prevalent nutritional problem globally, but particularly in less developed nations where food consumption is based on cereals and legumes. These foods have a high phytate content (1,2), which is a strong inhibitor of nonheme iron absorption (3,4). Wheat flour and its products are the more frequently fortified foods, mainly with reduced iron, which has low bioavailability (5,6). Studies in Venezuela have reported that fortification of wheat and corn flour with ferrous fumarate is more successful than with other iron sources (7,8).

Recently, the Mexican federal government launched a fortification program of wheat flour and lime-treated corn flour with reduced iron. Lime-treated corn flour is used to make tortillas, the most frequently consumed staple in Mexico (9). Although both wheat and corn are cereals, they go through different processes before they are ready for consumption. Wheat flour is obtained by grinding the grain after losing its external husk, where most of the phytate is contained (10). Then, it is supplemented with ascorbic acid to improve its baking quality, thus improving iron absorption (11). In contrast, lime-treated corn flour is obtained after maize is heated in an alkali solution (calcium hydroxide) at 90–92°C until soft (12). Although this procedure reduces its native phytate content by 16–28% (537–812 mg/100 g), it remains high (420–700 mg/100 g) (13,14). Although calcium concentrations increase to values higher than 200 mg/100 g (13), a concentration that is lower than values reported by others, it still might inhibit iron absorption (15–17).

On the basis of previous measurements of phytate found in lime-treated corn flour in our laboratory, we predict that the above-mentioned public nutrition intervention in Mexico would have a poor effect on iron status of the population because of low bioavailability of reduced iron. This investigation compares the bioavailability and utilization of several chemical compounds of iron added to lime-treated corn flour and to wheat flour. Thus, this information will help to identify an iron fortifier with better bioavailability than reduced iron.

MATERIALS AND METHODS

Experimental design and composition of the diets. Diets were based on commercially available wheat and lime-treated corn flours with and without fortification with hydrogen-reduced iron, 325 mesh.

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Similarly, wheat and lime-treated corn flours were fortified in our laboratory by adding 30 mg iron/kg flour to unfortified flours in the form of ferrous sulfate, ferrous fumarate and iron ammonia citrate to match the iron content to that of commercial flours fortified with reduced iron. **Table 1** shows the composition of the diets. Casein, free of vitamins (Sigma, St. Louis MO), was used as the protein source to prepare a low iron diet. Diets were prepared with 95% lime-treated corn flour and 82% wheat flour (70–72% extraction), with protein contents of 8 and 9%, respectively. This concentration of protein was similar to that of the low iron, casein diet. The proximate composition of flours was assessed using techniques described by the AOAC (18). Diet samples were ashed by calcination to determine iron content by atomic absorption spectrometry (Perkin Elmer Analyst 300, Norwalk, CT), and the phytate content by the method described by Frübek et al. (19). All experimental procedures involving laboratory animals were performed in accordance to the Mexican Official Regulations NOM-062-Z0–1999, which are essentially in line with NIH guidelines for experimentation in rats.

The hemoglobin (Hb)[3] depletion-repletion method was used to determine iron bioavailability and utilization. Iron balance, the percentage of absorption and protein efficiency were evaluated during the repletion phase. Male and female Sprague-Dawley rats, 21–23 d old, recently weaned, were selected and collocated in individual stainless steel cages with metal-grid floors. Rats were fed the casein diet (9% protein and 1.4 mg iron/100 g) for 10 d, and deionized water was provided throughout the experiment period (24 d). After the depletion phase, the rats were weighed and a blood sample was obtained from the tail vein to measure Hb concentration (initial Hb). Hemoglobin concentration was measured in a portable photometer (Hemo Cue, Angel Holm, Sweden). The anemic rats were assigned to 10 groups ($n = 6$), with similar Hb concentration. A sample size of 6 rats per group was calculated to have a power of 0.80 to detect differences of 0.20 mg of absolute iron utilization with a SD of 0.11. Each group was fed one of 10 experimental diets during the repletion period. During this phase, rats were fed diets containing wheat or lime-treated corn flour fortified with different chemical forms of iron. The control diets consisted of unfortified wheat or lime-treated corn flours. During this period, the weight and food intake of the rats were recorded daily considering food spills. Feces were collected daily and separated from spilt food with a nylon sieve; urine was not collected because it contains negligible Fe. The feces were dried, weighed, ground and analyzed for Fe according to the same methodology used for the diets. At the end of the repletion period, a blood sample was obtained from the tail vein to determine Hb concentration (final Hb).

Indices of iron bioavailability and utilization. Hemoglobin and iron determination were used to estimate the following variables (20–22).

1) Percentage of bioavailability, calculated as hemoglobin regeneration efficiency (HRE):

$$\%\text{HRE} = [\text{mg Fe Hb (final)} - \text{mg Fe Hb (initial)} \times 100]/\text{mg Fe consumed}$$

2) Iron utilization:

$$\text{Iron utilization (mg)} = (\%\text{HRE} \times \%\text{iron in the diet})/100$$

3) Iron content of Hb. Calculated assuming a total blood volume of 6.7% of the rat body weight, and an average iron content of Hb of 0.335 (21)

$$\text{mg Fe Hb} = [\text{body weight (g)} \times \text{Hb (g/L)} \times 6.7 \times 0.335]/10{,}000$$

4) Relative biological value (RBV):

$$\text{RBV} = (\text{iron utilization from the test sample} \times 100)/\text{iron utilization from wheat flour fortified with FeSO}_4$$

TABLE 1

Composition of low-iron casein, lime-treated corn flour and wheat flour diets

Ingredient	Casein	Lime-treated corn flour	Wheat flour
		g/kg	
Casein (90% protein)	100		
Lime-treated corn flour		953	
Wheat flour			820
Corn oil	80	—	67
Cornstarch	710	—	54
Mineral mixture[1,2]	48	37	44
Vitamin mixture[3]	10	10	10
Water	42	—	—
Cellulose	10	—	5

[1] Iron free.

[2] The mineral mixture supplied the following g/kg: calcium carbonate, 292.9; calcium phosphate, dehydrated, 4.3; cupric sulfate, pentahydrated, 2.44; magnesium sulfate, heptahydrated, 99.8; potassium phosphate, monobasic, 343.1; sodium chloride, 250.6; zinc chloride, 0.2; selenium oxide, 0.006; potassium iodine, 0.005; ammonium molibdate, 0.025; manganese sulfate, 1.21; sucrose, 5.414.

[3] Teklad test diet, (40060). The vitamin mixture supplied the following (g/kg): *p*-aminobenzoic acid, 11.0132; ascorbic acid coated (97.5%), 101.6604; biotin, 0.0441; cyanocobalamin, 2.9736; calcium pantothenate, 6.6079; choline dehydrogen citrate, 349.6916; folic acid, 0.1982; inositol, 11.0132; menadione, 4.9559; niacin, 9.9119; pyridoxine · HCl, 2.2026; riboflavin, 2.2026; thiamin · HCl, 2.2026; vitamin A palmitate, (500,000 IU/g) 3.9648; cholecalciferol (500,000 IU/g) 0.4405; vitamin E acetate (500 IU/g), 24.2291; cornstarch, 466.6878.

5) Iron absorption:

$$\text{Iron balance} = \text{mg Fe intake} - \text{mg Fe in feces}$$

$$\%\text{Fe absorption} = [(\text{mg Fe intake} - \text{mg Fe in feces}) \times 100]/\text{mg Fe intake}$$

6) Protein efficiency ratio (PER) (23)

$$\text{PER} = \text{g weight gain/g protein intake}$$

Statistical analysis. The Minitab statistical software (Minitab 12, State College, PA) was used for statistical analyses. Data are presented as means ± SD, $n = 6$. The General Linear Model and Bonferroni's test were used for comparisons among groups. A correlation between the iron content of diets and iron bioavailability was determined with Pearson correlation analysis. Differences were considered significant at $P < 0.05$ (24).

RESULTS

The protein content of wheat flour was greater than that of lime-treated corn flour ($P < 0.05$) (**Table 2**). In contrast, the fiber and phytate contents in lime-treated corn flour were almost three- to fourfold higher than those values in wheat flour.

The PER of lime-treated corn flour, with or without supplementation, was higher than the PER of wheat flour (**Table 3**, $P < 0.05$), but the iron source added to the diets did not influence the PER. There were no differences in food intake among groups fed wheat and lime-treated corn flours. The food intake of rats fed flours fortified with ferric citrate was higher than that of groups fed unfortified diets and those fortified with reduced iron ($P < 0.05$).

Rats fed diets with unfortified flours excreted less iron and had the lowest iron balance among the groups ($P < 0.05$).

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[3] Abbreviations used: Hb, hemoglobin; HRE, hemoglobin regeneration efficiency; PER, protein efficiency ratio; RBV, relative biological value.

TABLE 2

Chemical composition of unfortified wheat flour (WF) and lime-treated corn flour (LCF) and these flours fortified with different iron compounds

Samples	Moisture	Ash	Protein	Fat	Crude fiber	Carbohydrates	Phytates
				g/100 g sample			
LCF	8.7	1.3	8.6	4.3	1.5	75.6	0.503
LCF + reduced iron	9.9	1.3	7.6	4.2	1.3	75.7	0.500
LCF + ferrous sulfate	8.5	1.2	8.3	4.2	1.8	76.0	0.494
LCF + ferrous fumarate	8.5	1.3	8.2	4.3	1.7	76.0	0.492
LCF + ferric citrate	8.4	1.3	7.8	4.2	1.6	76.7	0.514
Mean ± SD	8.8 ± 0.6	1.3 ± 0.0	8.1 ± 0.4	4.2 ± 0.1	1.6 ± 0.2	76.0 ± 0.4	0.500 ± 0.009
WF	10.1	0.6	10.2	1.5	0.5	77.1	0.125
WF + reduced iron	10.1	0.6	10.3	1.4	0.4	77.2	0.110
WF + ferrous sulfate	8.5	0.7	11.0	1.6	0.5	77.7	0.105
WF + ferrous fumarate	8.6	0.7	11.1	1.5	0.5	77.6	0.123
WF + ferric citrate	8.4	0.6	10.6	1.6	0.4	78.4	0.107
Mean ± SD	9.1 ± 0.9	0.6 ± 0.1	11 ± 0.4	1.5 ± 0.1	0.5 ± 0.1	77.6 ± 0.5	0.114 ± 0.009

However, their iron balance was still positive (**Table 4**). Iron balance in the groups that consumed diets supplemented with iron sulfate, fumarate and citrate was higher than that of rats fed flours supplemented with reduced iron ($P < 0.01$). The percentage of iron absorbed was higher for the groups fed wheat flour than for those fed lime-treated corn flour. The highest absorption was observed in the group of rats fed the unfortified wheat flour diet and the lowest in the groups fed lime-treated corn flour with and without fortification with reduced iron. Analyzing separately the groups fed lime-treated corn flour, iron absorption was higher for the rats that consumed diets fortified with iron sulfate, fumarate and citrate than for rats that consumed unfortified diets and those fortified with reduced iron.

Initial and final body weight, Hb and iron in Hb are presented in **Table 5**. The Hb concentration after depletion was 57 ± 17 g/L. After repletion, rats fed wheat flours gained more Hb than the groups fed lime-treated corn flours ($P = 0.02$, **Table 6**). Rats fed diets supplemented with iron sulfate, fumarate and citrate gained more Hb than those fed unfortified diets or diets fortified with reduced iron ($P < 0.05$, Table 6). The percentage of iron bioavailability, expressed as HRE, was higher for unfortified flours than for fortified lime-treated corn flour and wheat flour ($P < 0.01$). The highest bioavailability was in the rats fed unfortified wheat flour, and the lowest in rats fed lime-treated corn flour fortified with reduced iron. In general, iron utilization was lower in rats fed lime-treated corn flours than in rats fed wheat flours. Rats fed flours fortified with iron sulfate, fumarate or citrate had higher utilization than rats fed unfortified flours or flours fortified with reduced iron (Table 6).

The RBV, relative to rats fed wheat flour fortified with iron sulfate, was higher in general for the rats fed wheat flours than for those fed lime-treated corn flours, probably because the former had a lower phytate concentration (114 vs. 501 mg/100 g, respectively).

DISCUSSION

In this study, iron bioavailability was higher in the rats that consumed unfortified diets than in those fed diets fortified with

TABLE 3

Protein efficiency ratios (PER) in iron-depleted rats fed diets based on unfortified wheat flour (WF) and lime-treated corn flour (LCF), and these flours fortified with different iron compounds for 2 wk[1]

Diet	Protein	Food intake	Weight gain	PER[2]
	g/100 g sample	*g*	*g*	
LCF	7.2	85.6 ± 22.7	9.5 ± 6.2[a]	1.55 ± 0.51[a]
LCF + reduced iron	7.6	112.6 ± 19.1	15.6 ± 5.0[a]	1.79 ± 0.47[a]
LCF + ferrous sulfate	7.3	118.8 ± 16.2[bc]	16.2 ± 5.7[a]	1.84 ± 0.44[a]
LCF + ferrous fumarate	7.2	143.2 ± 21.9[bc]	17.2 ± 4.2[a]	1.68 ± 0.32[a]
LCF + ferric citrate	7.7	137.9 ± 13.2[bc]	17.6 ± 4.6[a]	1.84 ± 0.34[a]
Mean				1.74 ± 0.12
WF	9.6	112.0 ± 20.2	13.6 ± 1.5	1.28 ± 0.11
WF + reduced iron	8.4	108.8 ± 13.8	9.9 ± 4.7	1.10 ± 0.55
WF + ferrous sulfate	8.5	110.7 ± 14.5[bc]	12.8 ± 3.6	1.35 ± 0.34
WF + ferrous fumarate	8.7	116.9 ± 6.5[bc]	11.7 ± 2.6	1.16 ± 0.30
WF + ferric citrate	9.2	145.2 ± 23.6[bc]	16.2 ± 3.5	1.22 ± 0.24
Mean				1.22 ± 0.10

[1] Values are mean ± SD, $n = 6$.

[2] PER = g gained weight/g protein intake. [a] $P < 0.05$ comparing fortified and unfortified LCF with fortified and unfortified WF; [b] $P < 0.05$ compared with unfortified flours; [c] $P < 0.05$, compared with reduced iron.

TABLE 4

Iron balance in anemic rats fed diets based on unfortified wheat flour (WF) and lime-treated corn flour (LCF) and these flours fortified with different iron sources for 2 wk[1]

Diet	Fe in diet	Fe intake	Fe excreted	Fe balance[2]	Fe Absorption[3]
	mg/100 g		*mg*		*%*
LCF	2.52	2.68 ± 0.65[a]	1.90 ± 0.40[a]	0.78 ± 0.33[a]	28.9 ± 5.8[a]
LCF + reduced iron	5.39	6.07 ± 1.02[ab]	4.35 ± 0.87[ab]	1.72 ± 0.44[ab]	27.9 ± 4.7[a]
LCF + ferrous sulfate	5.62	6.68 ± 0.91[ab]	3.90 ± 0.57[ab]	2.78 ± 0.44[abc]	41.5 ± 3.5[a]
LCF + ferrous fumarate	5.62	8.05 ± 1.23[abc]	4.77 ± 0.65[ab]	3.28 ± 0.64[abc]	40.6 ± 2.5[a]
LCF + ferric citrate	5.93	7.58 ± 0.78[abc]	4.64 ± 0.62[ab]	2.94 ± 0.34[abcd]	39.0 ± 3.8[a]
WF	2.23	2.50 ± 0.45	1.09 ± 0.35	1.41 ± 0.15	56.9 ± 6.1
WF + reduced iron	5.42	5.43 ± 1.38[b]	2.84 ± 0.80[b]	2.59 ± 0.71[b]	47.8 ± 5.2
WF + ferrous sulfate	5.63	6.23 ± 0.82[b]	3.42 ± 0.77[b]	2.81 ± 0.20[bc]	45.6 ± 5.7
WF + ferrous fumarate	5.90	6.90 ± 0.38[bc]	3.46 ± 0.38[b]	3.44 ± 0.51[bc]	49.7 ± 5.7[c]
WF + ferric citrate	4.12	5.98 ± 0.97[bc]	3.55 ± 0.58[b]	2.43 ± 0.45[bcd]	40.6 ± 2.8

[1] Values are mean ± SD, $n = 6$.

[2] Iron balance = g iron intake − g iron in feces.

[3] % Fe absorption = (mg Fe intake − mg Fe in feces × 100)/mg Fe intake. [a] $P < 0.05$ comparing fortified and unfortified LCF with fortified and unfortified WF; [b] $P < 0.05$ compared with unfortified flours; [c] $P < 0.05$, compared with reduced iron; [d] $P < 0.05$ compared with ferrous fumarate.

iron. This finding is contradictory to those reported by others who found a better iron bioavailability from diets with high iron content (25). This difference may be explained by the manner in which iron bioavailability was evaluated. As suggested by Layrisse et al. (8), the percentage of iron absorbed decreases as iron intake increases, but the total iron absorbed is higher. Similar results were reported by Cook et al. (26), who found in humans that those fed bread fortified with increasing amounts of iron (1, 3 and 5 mg) had lower percentages of iron absorbed, but their absolute absorption increased in response to increasing iron intakes. In our study, we also found an inverse correlation between diet iron content and bioavailability ($r = -0.83$, $P < 0.05$), and a direct relationship between the gain in Hb and iron utilization ($r = 0.93$, $P < 0.05$). These results suggest that this method is adequate for determining the effect of food fortification because the rats that were fed wheat flour fortified with ferrous sulfate had low iron bioavailability, but significantly higher utilization compared with rats fed unfortified flour. Further, most of the rats recovered their Hb status after repletion (Hb >120 g/L) except for those fed unfortified flours or flour fortified with reduced iron.

We confirmed that iron absorption and bioavailability do not reflect appropriately the effect of iron supplementation. Thus, we decided to use iron utilization as the outcome variable because it evaluates the efficiency of Hb regeneration (see Methods section). Iron utilization from unfortified lime-treated corn flour or this flour fortified with reduced iron was not different (RBV = 51 and 55% respectively). These values are slightly higher than those found by Fritz et al. (5) in nonlime-treated corn flour added with reduced iron. This is important because reduced iron is currently used to fortify lime-treated corn flour in public nutrition programs of Mexico to decrease the prevalence of iron deficiency anemia. In contrast, lime-treated corn flour fortified with citrate had a RBV of 81%, and with fumarate of 70% relative to wheat flour fortified

TABLE 5

Body weights, hemoglobin (Hb) and hemoglobin iron content in anemic rats fed diets based on unfortified wheat flour (WF) or lime-treated corn flour (LCF) and these flours fortified with different iron sources for 2 wk[1]

	Weight		Hemoglobin		Fe in Hb[2]	
Diet	Initial	Final	Initial	Final	Initial	Final
	g		*g/L*		*mg*	
LCF	60 ± 9	69 ± 14	60 ± 21	110 ± 24[a]	0.87 ± 0.4[a]	1.78 ± 0.6[a]
LCF + reduced iron	56 ± 8	72 ± 10	57 ± 14	108 ± 16[a]	0.73 ± 0.2[a]	1.74 ± 0.3[a]
LCF + ferrous sulfate	60 ± 4	76 ± 5	56 ± 18	125 ± 6[abc]	0.76 ± 0.2[a]	2.15 ± 0.2[ab]
LCF + ferrous fumarate	65 ± 6	82 ± 10	56 ± 17	135 ± 12[abc]	0.83 ± 0.3[a]	2.51 ± 0.5[abc]
LCF + ferric citrate	55 ± 9	75 ± 5	56 ± 11	142 ± 8[abc]	0.68 ± 0.2[a]	2.38 ± 0.2[abc]
WF	60 ± 8	74 ± 9	57 ± 15	109 ± 16	0.77 ± 0.3	1.85 ± 0.6
WF + reduced iron	72 ± 8	82 ± 6	57 ± 15	123 ± 8	0.94 ± 0.4	2.26 ± 0.3
WF + ferrous sulfate	62 ± 9	75 ± 10	57 ± 13	156 ± 9[bc]	0.80 ± 0.3	2.61 ± 0.4[b]
WF + ferrous fumarate	80 ± 7	91 ± 6	60 ± 17	140 ± 9[bc]	1.09 ± 0.3	2.90 ± 0.2[bc]
WF + ferric citrate	80 ± 10	97 ± 12	60 ± 24	145 ± 17[bc]	1.06 ± 0.4	3.16 ± 0.6[bc]

[1] Values are mean ± SD, $n = 6$.

[2] Fe in Hb was estimated as described in the material and methods section: mg Fe Hb = (body weight × Hb × 6.7 × 0.335)/10000. [a] $P < 0.05$ comparing fortified and unfortified LCF with fortified and unfortified WF; [b] $P < 0.05$ compared with unfortified flours; [c] $P < 0.05$, compared with reduced iron.

TABLE 6

Hemoglobin regeneration efficiency (HRE), iron utilization and relative biological value (RBV) in anemic rats fed diets based on unfortified wheat flour (WF) or lime-treated corn flour (LCF) and these flours fortified with different iron sources for 2 wk[1]

	Gain					
Diet	Weight	Hb	Iron Hb	HRE[2]	Iron utilization[4]	RBV[3]
	g	*g/L*	*mg*	*%*	*mg*	
LCF	9 ± 8^{a}	50 ± 11^{a}	0.91 ± 0.3^{a}	33.8 ± 4.6^{a}	0.85 ± 0.11^{a}	51
LCF + reduced iron	16 ± 5^{a}	51 ± 14^{a}	1.01 ± 0.2^{a}	17.2 ± 3.5^{ab}	0.92 ± 0.19^{a}	55
LCF + ferrous sulfate	16 ± 6^{a}	69 ± 18^{abc}	1.39 ± 0.2^{abc}	21.0 ± 3.5^{ab}	1.18 ± 0.20^{abc}	71
LCF + ferrous fumarate	17 ± 4^{a}	79 ± 18^{abc}	1.68 ± 0.4^{abc}	20.8 ± 2.7^{ab}	1.17 ± 0.15^{abc}	70
LCF + ferric citrate	20 ± 5^{ab}	86 ± 12^{abc}	1.70 ± 0.2^{abc}	22.5 ± 2.8^{abc}	1.34 ± 0.16^{abc}	81
WF	14 ± 2	52 ± 11	1.08 ± 0.4	41.4 ± 6.1	0.92 ± 0.14	55
WF + reduced iron	10 ± 5	66 ± 16	1.32 ± 0.3	22.8 ± 6.4^{b}	1.24 ± 0.34	75
WF + ferrous sulfate	12 ± 4	99 ± 12^{bc}	1.81 ± 0.2^{bc}	29.5 ± 4.0^{b}	1.66 ± 0.23^{bc}	100
WF + ferrous fumarate	12 ± 3	80 ± 16^{bc}	1.81 ± 0.3^{bc}	26.2 ± 4.6^{b}	1.55 ± 0.27^{bc}	93
WF + ferric citrate	16 ± 4^{b}	85 ± 22^{bc}	2.10 ± 0.7^{bc}	34.7 ± 6.9^{bc}	1.43 ± 0.28^{bc}	86

[1] Values are mean ± SD, $n = 6$.

[2] % HRE = [mg Fe hemoglobin (final) − mg Fe hemoglobin (initial) × 100]/mg Fe consumed.

[3] RBV = Iron utilization from the test sample × 100/iron utilization from wheat flour fortified with $FeSO_4$. $^{a}P < 0.05$ comparing fortified and unfortified LCF with fortified and unfortified WF; $^{b}P < 0.05$ compared with unfortified flours; $^{c}P < 0.05$, compared with reduced iron.

[4] Iron utilization (mg) = %HRE × % iron diet/100.

with ferrous sulfate. Therefore, it is clear that reduced iron is of little use as a fortifier of lime-treated corn flour because corn flour fortified with reduced iron that is not lime treated also has low iron utilization (5). There is enough evidence to suggest a change of iron compound to ferrous sulfate, ferrous fumarate or ferric citrate. Effects of the fortifier on the organoleptic characteristics of the product during the required shelf life must be assessed before any decision is made.

Our results confirmed the findings reported by others that ferrous sulfate is well utilized when added to wheat flour (5). However, it is not a suitable source of iron fortification because it easily oxidizes the food matrix, affecting its shelf-life and acceptability in storage (27). Fortunately, we found that flours fortified with iron fumarate and citrate were also well utilized and are less likely to oxidize the food matrix before use.

The validity of the depletion/repletion method was verified by Forbes et al. (6) and by Hurrell et al. (27) in rats to predict iron bioavailability. In these experiments, the depletion/repletion model defined the adequacy of the level of depletion when Hb was reduced at least 50%, which, in our case occurred in 10 d. There is great variability in the duration of such a period in the literature (7–40 d), (5,6,21,22,25,27), and none of the studies used indices of iron status such as serum iron, ferritin or others as indicators of depletion. Further, the vitamin mix was calculated to ensure that folic acid and vitamin B-12 intakes met recommendations. Thus, such a reduction in Hb ensures a robust indicator of iron deficiency and provides enough sensitivity to detect changes during the repletion period.

We demonstrated a better iron absorption from wheat flour diets than from lime-treated corn flour diets. The difference may be explained by the high phytate content in lime-treated corn flour (threefold greater than in wheat flour). As previously reported, phytate is one of the most potent inhibitors of intestinal iron absorption (11,28). A note of caution about the interpretation of these results is in order: the diets used here were based on raw flours. Thus, we did not take into account the decrease in phytate activity caused by higher temperatures during baking or cooking. Future experiments using bread and tortillas baked with these flours are warranted.

LITERATURE CITED

1. Lehrfeld, J. (1989) High performance liquid chromatography analysis of phytic acid in pH stable, microporus polymer column. Cereal Chem. 66: 510–515.
2. Chitra, U., Vimala, V., Shing, U. & Geervani, P. (1995) Variability in phytic acid content and protein digestibility of grain legumes. Plant Foods Hum. Nutr. 47: 163–172.
3. Hallberg, L., Rossander, L. & Skamberg, A. B. (1987) Phytates and the inhibitory effect of bran on iron absorption in man. Am. J. Clin. Nutr. 45: 988–996.
4. Brune, M., Rossander-Hulton, L., Hallberg, L., Gleerup, A. & Sandberg, P. (1992) Iron absorption from bread in humans: inhibiting phosphates with different numbers of phosphate groups. J. Nutr. 122: 442–449.
5. Fritz, J. C., Pla, G. W., Roberts, T., Bohene, J. W. & Hove, E. (1970) Biological availability in animals of iron from common dietary sources. J. Agric. Food Chem. 18: 647–651.
6. Forbes, A. L., Adam, C. E., Arnaud, R. F., Chichester, C. O., Cook, J. D., Harrison, B. N., Hurrell, R. F., Khan, S. G., Morris, E. R., Tanner, J. T. & Whittaker, P. (1989) Comparison of in vitro animal and clinical determinations of iron bioavailability: International Nutritional Anemia Consultative Group-Task Force report on iron bioavailability. Am. J. Clin. Nutr. 49: 225–238.
7. Layrisse, M., Chavez, J. F., Mendez-Castellano, H., Bosch, V., Tropper, E., Basardo, B. & González. E. (1996) Early responses to the impact of iron fortification in the Venezuelan population. Am. J. Clin. Nutr. 64: 903–907.
8. Layrisse, M. & Garcia-Casal, M. N. (1997) Strategies for the prevention of iron deficiency through foods in the household. Nutr. Rev. 55: 233–239.
9. Figueroa, J. (1999) La tortilla vitaminada. Av. Perspect. (Mex.) 18: 149–158.
10. Tabekhia, M. M. & Donnelly, B. J. (1982) Phytic acid in durum wheat and its milled products. Cereal Chem. 59: 105–107.
11. Cook, J. D., Reddy, M. B., Burry, J., Juillerat, M. A. & Hurrell, R. F. (1997) The influence of different cereal grains on iron absorption from infant cereal foods. Am. J. Clin. Nutr. 65: 964–969.
12. Bressani, R. (1990) Chemistry, technology and nutritive value of maize tortillas. Foods Rev. Int. 6: 225–264.
13. Urizar, A. L. & Bressani, R. (1999) Efecto de la cocción con cal (nixtamalización) sobre el contenido de ácido fítico y hierro disponible. Ciencia en acción (Guatemala) No. 8. http//www.uvg.edu. gt/facs/boletines-ii/cea8a. htm (accessed: December 6, 2001).
14. Wyatt, C. J. & Triana-Tejas, A. (1994) Soluble and insoluble Fe, Zn, Ca and phytates in foods commonly consumed in northern Mexico. J. Agric. Food Chem. 42: 2204–2209.
15. Wienk, K. J. H., Marx, J.J.M., Lemmens, A. G., Brink, E. J., Vandermer, R. & Beynon, A. C. (1996) Mechanism underlying the inhibitory effect of high calcium carbonate intake on iron bioavailability from ferrous sulfate in anemic rats. Br. J. Nutr. 75: 109–120.
16. Hallberg, L., Brune, M., Erlandsson, M., Sandberg, S., Rosander-Hulten, L. (1991) Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. Am. J. Clin. Nutr. 53: 112–119.
17. Cook, J. D., Dassenko, S. A. & Whttaker, P. (1991) Calcium supplementation: effect on iron absorption. Am. J. Clin. Nutr. 53: 106–111.

18. Association of Official Analytical Chemists (1984) Official Methods of Analysis (Williams, S., ed.), 14th ed. AOAC, Arlington, VA.

19. Frubbeck, K. G., Alonso, R., Marzo, F. & Santidrian, S. (1995) A modified method for the indirect quantitative analysis of phytate in foodstuffs. Anal. Biochem. 225: 206–212.

20. Thannoun, A. M., Mahoney, A. W., Hendricks, D. G. & Zhang, D. (1987) Effect of meat-bread mixtures on bioavailability of total dietary iron for anemic rats. Cereal Chem. 64: 399–403.

21. Campos, M. S., Pallares, I., Moratalla, A., Lopez-Aliaga, I., Gomez-Ayala, A. E., Hartiti, S., Alferez, M.J.M., Barrionuevo, M. & Lisbona, F. (1996) Bioavailability of Fe, Ca, P and Mg in Fe-deficient rats treated with different sources of dietary iron. Nutr. Res. 16: 683–696.

22. Santos, V. D., Bianchi, M.L.P., Latunde-Dada, & Danfluzzi, J. C. (1996) Bioavailability of iron from home prepared weaning foods. Nutr. Res. 16: 1601–1605.

23. Pellet, P. L. & Young, V. R. (1980) Nutritional Evaluation of Protein. The United Nations University Food and Nutrition Bulletin Supplement No. 4 (The United Nations University, Tokyo).

24. Dawson-Saunders, B. & Trapp, R. G. (1994) Basic and Clinical Biostatistics. Appleton and Lange, Stamford, CT.

25. Ologunde, M. O., Morris, J. B., Sheppard, R. L., Folabi, A. O. & Oke, O. L. (1994) Bioavailability to rats of iron from fortified grain amaranth. Plant Foods Hum. Nutr. 45: 191–200.

26. Cook, J. D., Minnich, V., Moore, C. V., Rasmussen, A., Bradley, W. B. & Finch C. A. (1973) Absorption of fortification iron in bread. Am. J. Clin. Nutr. 26: 861–872.

27. Hurrell, R. F., Furniss, D. E., Burri, J., Whittaker, P., Lynch, S. R. & Cook, J. D. (1989) Iron fortification of infant cereals: a proposal for the use of ferrous fumarate or ferrous succinate. Am. J. Clin. Nutr. 49: 1274–1282.

28. Saha, P. R., Weaver, C. M. & Mason, A. C. (1994) Mineral bioavailability in rats from intrinsically labeled whole-wheat flour of various phytate levels. J. Agric. Food Chem. 42: 2531–2535.