

# Color, Flavor, and Iron Bioavailability in Iron-Fortified Chocolate Milk

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## ABSTRACT

Chocolate milks fortified with nine iron compounds commonly used for food fortification and with ferripolyphosphate and ferripolyphosphate-whey protein complex were evaluated for changes in color and flavor. Sodium ferric pyrophosphate, ferripolyphosphate, and ferripolyphosphate-whey protein complex produced little or no off-color change in the products initially and after 2 wk of storage. All other added compounds resulted in initial and persistent off-colors determined by a Color Difference Meter and a panel of judges. Flavor evaluation by experienced judges showed that ferric compounds produced little or no off-flavors in chocolate milks initially or after holding at 4°C for 7 and 14 days. However, ferrous compounds produced off-flavors initially, but flavor scores improved after milks were held at 4°C for 14 days.

The bioavailability of iron in two iron fortified chocolate milks least affected in color and flavor was determined by rat feeding. Chocolate milks fortified with ferripolyphosphate-whey protein complex and sodium ferric polyphosphate were dried and mixed with a low iron ration to provide varied iron fortification. In a hemoglobin depletion-repletion bioassay with weanling rats, ferripolyphosphate-whey protein complex was utilized

as well as ferrous sulfate but sodium ferric polyphosphate only 35% as well as ferrous sulfate.

## INTRODUCTION

The nutrient commonly below its recommended allowance in diets of women and children is iron. Girls and women from age 9 to 54 yr receive from their diets an average of 20% less than the recommended allowance of iron, with many below 30% or more (1); below age 9 dietary iron is 11 to 50% below the recommended daily allowance.

Iron is always in milk, the usual range being from 100 to 900 µg per liter (19); therefore, milk would have to be enriched to be a dietary source of iron. Enrichment of milk products with needed iron without detectable change in color or flavor is a solution to this problem. Iron enriched milks generally require special processing techniques to prevent off-flavor development caused by iron's catalytic effect on oxidation of milk fat. To prevent off flavors, members of this laboratory have recommended elevated pasteurization temperatures (77°C for 16 s rather than 71°C for 16 s) when whole milk is enriched with ferric iron salts and deaeration of milk prior to addition of ferrous iron salts (5). Kurtz et al. reported that in the preparation of iron enriched nonfat dry milk it was preferable to concentrate the skim milk before adding ferric compounds and subsequently drying the concentrate (12). When these iron enriched milks are used as coffee whiteners or are flavored with chocolate, the resulting beverages develop undesirable colors.

In 1942 Kinder et al. (11) proposed that foods containing cocoa and chocolate are suited to be fortified with iron because the added iron remains completely available and the cocoa and chocolate contain natural antioxidants to inhibit development of oxidative rancidity. As a result they might serve as good carriers of added iron. They also reported that when iron was added to

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a cocoa-milk ration, the added iron was as available in the presence of cocoa as when added to a whole milk ration.

An iron enriched chocolate milk might be particularly helpful for children whose diets are deficient, because many children prefer the chocolate flavored product and chocolate milks are used widely in institutional feeding programs (7). This study was to evaluate a series of iron salts to determine which compounds could be used most successfully in preparation of iron enriched chocolate milk.

## EXPERIMENTAL PROCEDURE

### Materials

Fresh raw whole milk was standardized to 3.5% milk fat. The chocolate powder was Non-Settling Dairy Powder No. 62, Robert A. Johnston Co.<sup>5</sup> Iron compounds (Table 1)<sup>6</sup> for evaluation were chosen from those commonly used for food fortification or for therapeutic treatment of iron deficiency anemia (15). They were purchased from commercial suppliers, except ferripolyphosphate (FIP) and ferripolyphosphate whey protein complex (FIP-PRO), which were prepared at the Eastern Regional Research Center, Philadelphia, PA (9). Most compounds in this study have been tested for bioavailability by the Animal Hemoglobin Repletion Test (2) and with the exception of sodium ferric pyrophosphate (SFP) have been readily available when tested alone or added to foods (6, 10). The expected compound has a low biological value when tested alone, but this value has increased when the compound has been incorporated in processed foods (17, 18).

Equipment for processing was conventional dairy plant equipment.

### Methods

*Sample Preparation.* Iron compounds were dissolved or suspended in water prior to being added to milk. Iron concentrations were 10 mg/ml of solution added at 10 or 20 mg/.95 liters

<sup>5</sup> Reference to brand or firm name does not constitute endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

<sup>6</sup> Abbreviations in Table 1 are used throughout text.

of milk. Sugar and chocolate mix were dry blended together and dissolved in the milk 10 min after addition of the iron compound. The mix was held for 10 min, pasteurized in a "tubular" heat exchanger at 81°C for 16 s, cooled to 63°C, and passed through a homogenizer valve. The chocolate milk was cooled, discharged into sample containers, and stored at 4°C. Samples were evaluated for color and flavor after 1, 7, and 14 days. A control sample containing no added iron was processed similarly.

### *Preparation of Dried Chocolate Milks and Iron Fortified Diets for Bioavailability Studies.*

A control lot (no added iron) of chocolate milk was prepared by heating raw, standardized (3.5% fat) milk to 66°C, adding sugar and chocolate powder, pasteurizing at 71°C for 30 min, cooling to 63°C, and passing through a homogenizing valve. The milk then was concentrated in a falling-film evaporator to 45% total solids. After overnight refrigeration, the concentrate was prepared for freeze-drying by bubbling nitrogen into the well stirred concentrate, then spreading in thin layers in trays. Drying was achieved in a high vacuum shelf drier. The product was comminuted and stored in sealed containers.

Two lots of dehydrated iron fortified chocolate milks also were prepared similarly, one lot containing sodium ferric pyrophosphate and the other ferripolyphosphate-whey protein complex. Each lot was calculated to contain 160 ppm iron dry weight. The compounds were added to the whole milks before being blended with sugar and chocolate powder.

Iron fortified diets were prepared with iron fortified chocolate milk added to a commercial low iron ration in the ratio of 1:2. To reduce the iron in the diet and keep the ratio of chocolate milk to low iron ration the same, fortified chocolate milk was diluted with unfortified chocolate milk. A basal diet fed to all rats during the depletion period was made from unfortified chocolate milk and low iron ration. The reference diet containing ferrous sulfate was made with the iron compound added at 3 levels of fortification directly to a basal diet.

*Color Evaluation.* A Hunter Color Difference Meter was used to obtain a measure of the degree of off-color produced by addition of iron to milk in the presence of chocolate. The color coordinates of this color meter are  $L$  = visual light-

TABLE 1. Iron compounds to fortify chocolate milks.

Iron compound	Abbreviations	Relative biological value <sup>a</sup>
Sodium ferric pyrophosphate	SFP	19
Ferripolyphosphate	FIP	60
Ferripolyphosphate whey protein complex	FIP-PRO	92
Ferric ammonium citrate	FAC	107
Ferric choline citrate	FCC	102
Ferric citrate	FC	...
Ferric glycerophosphate	FGP	93
Ferrous fumarate	FF	95
Ferrous gluconate	FG	97
Ferrous lactate	FL	...
Ferrous sulfate	FS	100

<sup>a</sup>Relative biological value = percent of response in hemoglobin and hematocrit obtained with an equal quantity of iron furnished by  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ .

ness,  $a$  = redness-to-greenness, and  $b$  = yellowness-to-blueness (8). The instrument was standardized with a cocoa standard reference tile (Gardner Laboratory, Tile #G538). Coordinates for the tile are  $L = 43.3$ ,  $a = 10.9$ , and  $b = 8.0$ . The color meter was operated with a wide aperture and small area of illumination. Chocolate milk samples contained in optical beakers supplied with the instrument were read at room temperature ( $\sim 27^\circ\text{C}$ ) immediately after being poured. Readings represent means of two readings per sample.

Subjective color evaluations of the iron enriched chocolate milks were by a panel of 20 to 25 judges (13, 14). Panels of 7 to 10 members also made other color evaluations. Evaluation procedures, offered in separate sessions to reduce interaction of judgment from different questionnaires, consisted of triangle difference testing and ranking by color preference without knowledge of the identity of the control sample. Visual comparison was in a room illuminated with Hg-Ar fluorescent lights. White paper cups placed on a pale green background were used as sample containers. Judges were instructed to rate samples only as required on the questionnaire.

*Flavor Evaluation.* The iron fortified choco-

late milks were evaluated for flavor in panel sessions by 10 judges in a special room illuminated with sodium vapor lamps to minimize effects of color variations. Panelists were requested to rate samples on the 9-point Hedonic scale (16) (like extremely to dislike extremely) and describe any other off-flavors. Taste panels usually consisted of 4 to 7 coded samples including a control (no added iron) chocolate milk prepared from the same batch of milk as the iron enriched samples.

*Bioavailability Studies.* The hemoglobin repletion test for the determination of iron bioavailability was used. Male weanling rats of the Sprague-Dawley strain weighing from 45 to 55 g were housed individually in stainless steel cages and were fed 4 wk on a low iron diet containing 16.7 ppm iron. Diet and distilled water were supplied ad libitum.

After the rats were fed the basal diet for 4 wk, hemoglobin in the blood taken from their tails was determined by the cyanomethemoglobin method (3) with the Unopette system.<sup>7</sup> The rats then were grouped so the average hemoglobin for each group was approximately the same. One group was contained on the basal diet while the other groups were fed an experimental diet containing added iron. Test diets were fed for 10 days after which hemoglobin again was determined.

Data were analyzed by a slope ratio technique, and comparisons of the slopes of the

<sup>7</sup>Becton-Dickinson Company, Rutherford, NJ.

TABLE 2. Hunter color coordinates of iron fortified chocolate milks.

Iron <sup>a</sup> compound	L		a		b		ΔE	
	1 day	14 days						
	None	43.3	42.0	8.8	8.7	9.0	9.4	.37
Sodium ferric pyrophosphate	43.0	42.6	9.0	9.0	9.1	9.8	.10	.22
Ferripolyphosphate <sup>b</sup>	43.4	42.0	8.8	8.6	9.0	9.2	.20	3.00
Ferrous fumarate	43.3	40.8	8.8	6.4	8.8	7.9	6.18	6.40
Ferrous sulfate <sup>c</sup>	39.1	37.4	5.5	5.5	5.9	6.3	5.74	6.10
Ferric ammonium citrated	39.6	38.0	5.5	5.3	6.1	6.3		

<sup>a</sup>Iron added at 10 mg/.95 liter.

<sup>b</sup>Ferripolyphosphate whey protein complex.

<sup>c</sup>Ferrous lactate.

<sup>d</sup>Ferric citrate, ferric choline citrate, and ferrous glycerophosphate.

lines relating response per unit of added iron provided a measure of the relative bioavailability of iron in the fortified chocolate milk.

## RESULTS AND DISCUSSION

### Color Evaluation

Variations in color of iron enriched chocolate milks as determined by the Hunter coordinates are in Table 2. These data are recorded for only five of the iron compounds. Color coordinates of the other compounds were essentially the same as one of those listed (see footnote, Table 2, for groupings). Initially the *L* for samples containing SFP, FIP, and ferrous fumarate (FF) were not changed appreciably from the control. However, pronounced darkening occurred in samples to which other ferrous and ferric compounds had been added. After 2 wk only a slight darkening occurred in the control, SFP, and FIP samples. A much more pronounced darkening occurred in all other ferrous and ferric compounds.

The Hunter *a* showed no change from control initially for samples containing SFP, FIP, and FF, but there was a decrease in redness of samples containing other added compounds. The *a* tended to decrease on aging with the greatest decrease in redness in the FF sample.

The Hunter *b* showed no change initially for samples containing SFP, FIP, and FF but decreased for other ferrous and ferric salts, giving a corresponding decrease in yellowness of the samples. After 2-wk storage, the *b* increased in all samples and the control, except for the sample containing ferrous fumarate, which continued to decrease. The most significant color change with time resulted from the addition of FF as shown by the change in its total color difference,  $\Delta E$ . This was calculated from the Hunter-Schofield equation (14)

$$\Delta E = [\Delta L^2 + \Delta a^2 + \Delta b^2]^{.5}$$

The  $\Delta L$ ,  $\Delta a$ , and  $\Delta b$  represent differences between the *L*, *a*, and *b* of samples and control. The SFP and FIP had little color change initially or after 14 days of storage. However, ferrous sulfate (FS) and ferric ammonium citrate (FAC) produced a significant color change in the chocolate samples initially but little additional change after 14 days.

The triangle difference test was used to ascertain whether small differences in color detected by the color difference meter would be detected by panel members. Panelists also were instructed to indicate whether the odd sample was lighter and whether it was preferred over the other samples. Results in Table 3 compare only those samples that closely resemble the control, in that they produced little off-color. Two amounts of iron, 10 and 20 mg/.95 liter, were studied over 14 days. As the iron increased and as FIP chocolate milks aged, there was a definite darkening of the products as evidenced by increases in  $\Delta L$  and  $\Delta E$ , which were detectable in most instances by panelists (Table 3). However, in chocolate milk enriched with 20 mg FIP/.95 liter (14 days) the darkened effect was sufficient to cause panelists to express a definite preference for the control sample.

To ascertain further effect of this off-color development in the chocolate milks, in separate sessions panelists ranked samples for color preference only. Twenty-five judges ranked five coded samples (control, FIP, FIP-PRO, FAC, and ferrous lactate (FL)) 1st, 2nd, 3rd, 4th, and 5th choice. Total scores were analyzed by analysis of variance. The difference between the color of samples was highly significant ( $F=20.73$ ). Application of Duncan's multiple range (4) to the sample scores (Table 4) showed that FAC and FL were significantly different from the other samples (different letters a and b) at 5%. Also panelists showed no clear preference for first choice among the other three samples (same letter c, c, c). Nearly all panelists preferred the lighter colored samples to darker samples.

The results indicated excellent agreement as measured by the (photoelectric) color difference meter and the panel's preference for samples light reddish-brown rather than dark greenish-purple.

#### Flavor Evaluation

Flavor scores of control chocolate milk and iron fortified chocolate milks after processed and stored at 4°C for 1, 7, and 14 days are in Table 5. All samples were tempered to ~ 23°C before evaluation. All samples containing ferric compounds (SFP, FIP, FIP-PRO, ferric citrate (FC), ferric choline citrate (FCC),

TABLE 3. Triangle difference test comparing control and iron-containing chocolate milks.

Iron sample	mg/ liter	Age- days	$\Delta L$	$\Delta E$	Number of judges	Judges correct	Level of signifi- cance (%)	Number of judges preferring lighter sample
Ferripolyphosphate	10	1	.25	.27	20	12	5	6
Ferripolyphosphate	20	1	.35	.45	20	18	.1	8
Ferripolyphosphate	10	14	.55	.59	23	14	1	8
Ferripolyphosphate	20	14	.95	1.28	23	19	.1	16
Ferripolyphosphate-whey protein complex	10	1	.45	.60	20	9	NS	3
Sodium ferric pyrophosphate	20	14	.60	.78	20	9	NS	5

TABLE 4. Comparison of color preference scores by Duncan's multiple range.

Samples	Means
Ferric ammonium citrate	4.44 <sup>a</sup>
Ferrous lactate	3.68 <sup>b</sup>
Ferripolyphosphate-whey protein complex	2.48 <sup>c</sup>
Ferripolyphosphate	2.20 <sup>c</sup>
Control (no iron)	2.20 <sup>c</sup>

<sup>a,b,c</sup>Means followed by different letters are significantly different from each other at 5%.

and (FAC)) as compared to the control produced little or no off-flavor in the chocolate milks initially or after 7 and 14 days. Initially, all ferrous samples except FF scored appreciably lower than the control. These low initial scores of the ferrous samples probably were caused by milk not being deaerated prior to addition of iron salts (thereby producing oxidized off-flavor criticisms). The intensity of this defect decreased with aging with a corresponding increase in flavor scores after 7 and 14 days. Flavor score of FF decreased from 7.8 initially to 6.6 after 14 days. Samples containing ferric compounds (SFP, FIP, FIP-PRO, FC, FAC, FCC, and ferric glycerophosphate (FGP) produced little or no off-flavors in the chocolate milks.

#### Bioavailability Studies

Since FIP-PRO and SFP produced the least color and flavor changes, they were evaluated for bioavailability.

Compositions of basal and low iron diets are in Table 6. Data for diets and hemoglobin analysis appear in Table 7. The three fortifications were approximately 40, 20, and 10 ppm. Since they actually varied, calculations were on the measurements in Table 7. Hemoglobin increased in all cases where iron-fortified diets were fed; the degree of increase related to the type and amount of iron fed.

Results of iron biological availability assay are in Figure 1 and Table 8. Figure 1 shows the slope ratio analyses of the iron biological availability assay. In the hemoglobin depletion-repletion bioassay, changes in rat hemoglobin per unit of determined iron were used as assay criteria. The slope of the line is an indication of the rate of recovery from the hemoglobin depleted state for each test diet.

Calculated relative biological values with 95% confidence limits and analysis of variance for data that fit the slope ratio model are in Table 8. The biological availability of FIP-PRO was essentially equal to the reference salt, ferrous sulfate. However, the SFP was significantly lower ( $P < .05$ ), and only 35% was available as ferrous sulfate.

These findings are consistent with relative biological values reported in the literature.

TABLE 5. Mean flavor scores<sup>a</sup> of pasteurized iron-fortified chocolate milks.

Iron compound 10 mg/.95 liter	Age of samples (days)		
	1	7	14
None (control)	7.6	7.6	7.8
Sodium ferric pyrophosphate	7.9	7.6	7.5
Ferripolyphosphate	7.6	7.6	7.6
Ferripolyphosphate-whey protein complex	7.5	7.4	7.6
Ferric ammonium citrate	7.2	7.3	7.6
Ferric choline citrate	7.4	7.3	7.2
Ferric citrate	7.2	7.6	7.6
Ferric glycerol phosphate	7.2	7.6	7.3
Ferrous fumarate	7.8	7.5	6.6
Ferrous gluconate	5.6	7.0	6.8
Ferrous lactate	4.4	5.0	7.1
Ferrous sulfate	5.8	7.0	7.1

<sup>a</sup>Nine point Hedonic scale.

TABLE 6. Composition of diets fed to rats.

Diet	% Fat	% Solids	% Protein
Basal	14.5	95	22.5
Low iron <sup>a</sup>	14	93	27

<sup>a</sup>Low iron ration (ICN #102199) consists of 14% vegetable oil, 27% vitamin-free casein, 56% corn starch, 3% Hubbell-Mendell-Wakeman salt mixture without ferric phosphate, and vitamin fortification mixture (vitamin A concentrate 4.5 g, vitamin D concentrate .25 g, alpha tocopherol 5.0 g, ascorbic acid 45.0 g, inositol 5.0 g, choline chloride 75.0 g, monodione 2.25 g, p-aminobenzoic acid 5.0 g, niacin 4.5 g, riboflavin 1.0 g, pyridoxine hydrochloride 1.0 g, thiamine hydrochloride 1.0 g, calcium pantothenate 3.0 g, biotin 20 mg, folic acid 90 mg, and vitamin B<sub>12</sub> 1.35 mg).

Values of 92 and 95 have been reported for FIP-PRO (10). Whereas Fritz and Pla (6) reported values of 12 and 14 for SFP, Theuer et al. (17, 18) showed that the availability of SFP is increased by processing from 15 to 66 in soy isolate formulas and from 40 to 60 in milk based formulas. They attribute the improvement in availability to factors that include other ingredients and the heat applied during sterilization. The lower value of their milk base formula is similar to our value of 35 in choco-

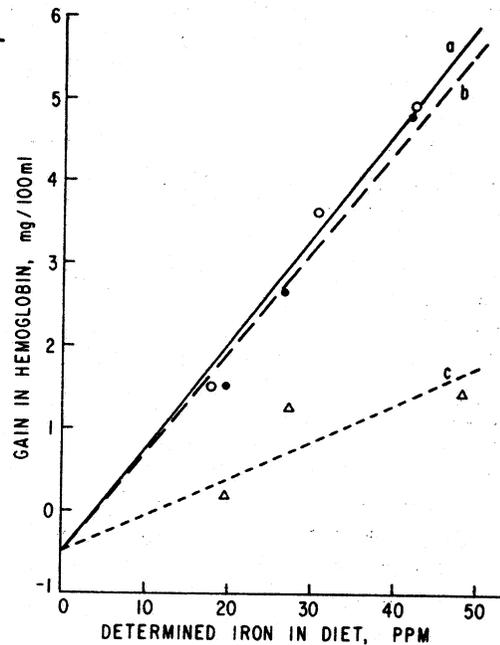


Figure 1. Slope ratio analyses of iron biological availability assay. a, Ferrous sulfate; b, ferripolyphosphate-whey protein complex; c, sodium ferric pyrophosphate.

late milk, a product which has not received sterilizing treatment.

The fortification of chocolate milk would

TABLE 7. Iron for diets and rats fed iron-fortified chocolate milk diets.

Diet	Iron in diet (ppm)		Hemoglobin (mg/100 ml)	
	Added	Determined	Depletion	Repletion
Basal	None	16.7	6.628	6.635
Ferripolyphosphate-whey protein complex	40	41.6	6.650	11.345
	20	26.6	6.619	9.275
	10	19.7	6.620	8.151
Sodium ferric pyrophosphate	40	48.0	6.552	7.994
	20	27.2	6.590	7.837
	10	19.6	6.639	6.839
Ferrous sulfate	40	42.1	6.549	11.495
	20	30.6	6.589	10.117
	10	17.9	6.606	8.887

TABLE 8. Biological availability of iron in iron-fortified chocolate milks.

Iron preparation	RBV <sup>a</sup>	95% Confidence interval
Ferripolyphosphate-whey protein complex	95	85-105
Sodium ferric pyrophosphate	35 <sup>b</sup>	26-44

	df	Sum of squares	F
Analysis of variance			
Regression	3	281.09	51.4 <sup>c</sup>
Blanks	1	1.07	1.60
Intersection	3	3.97	1.98
Curvature	2	3.06	2.29
Between doses	9	289.19	48.1 <sup>c</sup>
Error	90	60.12	
Total	99	349.31	

<sup>a</sup>Relative biological value as compared to FeSO<sub>4</sub>.

<sup>b</sup>Significant with  $P < .05$ .

<sup>c</sup>Significant with  $P < .01$ .

appear feasible because of its availability as well as color and flavor. Our results bear out earlier findings (11) that cocoa does not affect bio-availability adversely, and chocolate milk may be a good vehicle for iron fortifying compounds.

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