Ferripolyphosphate–Whey Protein Powders. Their Potential as Nutritional Iron Supplements

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Ferripolyphosphate, an aqueous complex of ferric chloride and sodium polyphosphate, when added to acid whey at pH 4.6, precipitated the proteins. The product, a powder free of lactose and milk salts, contained approximately 10% Fe, 13% P (or 30% P_2O_5), and 50% protein. In bioassay, ferripolyphosphate–protein powder was 92–100% efficient, relative to ferrous sulfate, in restoring hemoglobin levels of iron-depleted rats and chicks. The iron in sterile whole milk concentrates fortified with ferripolyphosphate–protein was 84–107% bioassimilable. Whole milk containing ferripolyphosphate–protein for iron enrichment required no change in pasteurization temperature to prevent lipolytic rancidity. Taste panel tests showed that milks enriched with ferripolyphosphate–protein were of the same beverage quality as the controls. No abnormal effects were found in rats fed a dietary intake of 720 ppm of iron ad libitum from ferripolyphosphate–protein for a 90-day period.

The preparation of ferripolyphosphates and ferripolyphosphate–whey protein powders has been described (Jones et al., 1972) and these materials are now under study as nutritional iron supplements for flour, milk, baby cereals, and other foods.

The technical criteria for utility as an iron fortification agent are (1) that the iron ingested be absorbed and well utilized by the body, and (2) that the iron compound have no adverse effect on product flavor, color, or stability. Iron compounds presently in use, such as $FeSO_4$ and reduced iron, rarely fulfill both requirements. A frequent observation is that an iron compound which is sufficiently soluble so that it can be absorbed by the body will usually cause some type of processing difficulty.

The present report describes experiments which were carried out with ferripolyphosphate–whey protein powder and ferripolyphosphate, solid and aqueous, to determine bioavailability of iron, toxicity, and effect on product flavor. This information is valuable in determining the efficacy of these products as iron fortification agents.

EXPERIMENTAL SECTION

Preparation of Ferripolyphosphates. Ferripolyphosphate (hereafter referred to as FIP), a complex of ferric ions with a long-chain polyphosphate, can be prepared as a solid gel or an aqueous complex (Hazel et al., 1968). The solid gel was generated by combining 2 vol of 0.5 *M* ferric chloride and 1 vol of a 3 *M* solution of sodium phosphate glass, $\bar{N}$ = 11 (Calgon, Calgon Corporation, Pittsburgh, Pa.), at 10°. The slurried precipitate was freed of salt by dialysis and finally lyophilized. The aqueous FIP complex was obtained by dissolving the solid gel in excess 3 *M* polyphosphate solution. The aqueous complex may contain various Fe/P ratios depending on the amount of polyphosphate used. The aqueous complex used in these experiments was made with 1 vol of ferric chloride and 2 vol of phosphate, contained 1% Fe(III), and had a mole ratio Fe/P = 1/12.

Preparation of Ferripolyphosphate–Protein Powder. A solution of FIP, mole ratio Fe/P = 1/12, which is aged for at least 2 weeks, will precipitate the proteins of whey (Jones et al., 1972). One volume of the FIP solution was added to 4 or 5 vol of acid whey at pH 4.6. The precipitate formed was dialyzed extensively against distilled water and lyophilized, producing a white, finely divided powder of variable composition depending on the protein content of the whey and the age of the aqueous complex.

The experiments reported in this paper were conducted with FIP (solid gel), 19% iron, FIP (aqueous), 1% iron, and FIP–protein powders, 8–12% iron, prepared from acid whey and FIP (aqueous).

Bioassimilability Tests. Assimilability of the iron was tested both in rats and chicks. Materials tested were FIP (solid gel), FIP (aqueous), and FIP–protein powder, as well as lyophilized samples of sterile concentrated whole milks which had been fortified with FIP–protein powder at a level of 10 mg of iron per quart of milk, reconstituted.

The test animals were depleted on a low-iron diet until hemoglobin determinations indicated that severe anemia had been produced. Sprague-Dawley male rats, 21-days old, were depleted for 3 weeks and day-old White Plymouth Rock chicks were depleted for 2 weeks. The animals were then placed on the test diet for 15–18 days. At the end of this period, individual blood samples were drawn (rat tail vein and chick wing vein) and the responses in hemoglobin were compared between test animals that (1) received known quantities of iron from ferrous sulfate and (2) animals that received similar quantities of iron from the test samples.

To determine the assimilability of iron from FIP after processing, a series of sterile concentrated milks was prepared, some of which were fortified with FIP–protein at the level of 10 mg/qt reconstituted. Fresh, raw whole milk was pasteurized at 75° for 20 min, homogenized in a Manton-Gaulin Laboratory Homogenizer for 20 min at 2500 psi, and concentrated by rotary evaporation at 53°. Between ⅓ and ½ of the water was removed. Sterilization was by autoclaving at 15 psi and 120°. FIP–protein was added to the milks at various stages of the processing. Column 2 of Table II identifies each milk concentrate as to its manner of preparation.

The sterile concentrates gelled during autoclaving and were resuspended in water and then lyophilized. The solids were added to the diets of depleted rats. Five groups of four male rats, depleted for 3 weeks, received the basal diet plus sufficient milk solids-plus-FIP to bring the iron level to 20

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Table I. Iron Availability of Ferripolyphosphate Samples Fed to Iron Depleted Rats

Group[a]	Iron supplement	Mean body wt. gain, g, ± SD	Mean Hb gain, g %	Hb gain per mg of Fe intake ± SD	% availability of Fe
1	FIP–protein	119 ± 9	6.6	1.12 ± 0.13	92
2	FIP (aq)	128 ± 13	7.6	1.12 ± 0.05	93
3	FIP (solid)	118 ± 12	4.6	0.73 ± 0.09	60
4	Ferrous sulfate	122 ± 5	7.8	1.22 ± 0.07	100

[a] Four weanling male Sprague-Dawley strain rats per group, 21 days on low-iron depletion diet, then fed repletion diets containing 20 ppm of iron from supplement indicated for 18 days.

Table II. Summary of Mean Iron Availability Values of Iron-Fortified Milk Concentrate Solids

Group	Dietary iron supplement[a,b]	Body wt. gain,[c] g, SD	Hb gain, g %	Hb gain/mg of Fe intake[c] ± SD	% availability of Fe
1	Basal (no iron)	73 ± 6[d]	−0.4		
2	+ Ferrous sulfate	107 ± 3	6.4	1.30 ± 0.10	100
3	+ Milk no. 1 (PH)(no iron)	65 ± 6[d]	−0.5		
4	+ Milk no. 2 (PHCS) + ferrous sulfate	118 ± 9	6.0	1.20 ± 0.10	93
5	+ Milk no. 3 (FPHCS)	112 ± 11	5.6	1.10 ± 0.04	85
6	+ Milk no. 4 (PFHCS)	106 ± 6	5.1	1.09 ± 0.21	84
7	+ Milk no. 5 (PHFCS)	117 ± 10	6.2	1.24 ± 0.23	95
8	+ Milk no. 7 (PHCF)	111 ± 7	6.4	1.24 ± 0.08	95
9	+ Milk no. 8 (PHF)	107 ± 20	6.6	1.39 ± 0.27	107

[a] Four male rats per group, depleted for 3 weeks on low-iron basal diet, then fed supplements for 15 days. [b] F, FIP–protein added; P, pasteurized; H, homogenized; C, concentrated; S, sterilized. [c] Data ± standard deviation. [d] $P < 0.01$ (compared with group 2).

ppm (Table II, groups 5–9). Group 3 received basal diet plus milk solids with no iron; group 2 received ferrous sulfate in the basal diet; group 4 received ferrous sulfate and milk solids in the basal diet; and group 1 received only the basal diet. (The composition of milk solids is 23–31% protein, 31–38% fat, 31–38% lactose, and 7% salts [Ca, K, Na, phosphate, citrate]. The basal rat diet consisted of 20% crude casein, 60% corn starch, 5.0% nonnutritive bulk Alphacel (Nutritional Biochemicals Co.), 10% corn oil, 1.0% vitamin mixture, and 4.4% Fe-free mineral mix. The basal diet contained approximately 5 ppm of Fe. Alphacel was omitted in later assays because of its sigificant iron content.)

Rat Toxicity Studies. Three separate 3-month feeding trials were conducted to detect possible toxic effects in rats. In two studies, FIP–protein was fed ad libitum at dietary levels of 720 and 1000 ppm of iron; in a third study, FIP and $FeSO_4$ were fed at a level of 10,000 ppm, or 1% of the diet. The last was done in order to reach a level of dietary iron which would probably result in hemosiderosis, the deposition of iron in various organs and tissues. Prior to autopsy, blood samples were taken for hematology. Composite urine samples were also analyzed. After 3 months, all rats were autopsied and various organs and tissues were weighed and preserved in formalin for microscopic examination.

Enrichment of Pasteurized Whole Milk with Ferripolyphosphate Compounds: Effect on Flavor. Taste panel evaluation of iron-enriched whole milks has shown that ferric compounds present before pasteurization at levels of 10–40 mg/qt result in lipolytic rancidity when milk is pasteurized at minimum to moderate temperatures (below about 79°) (Edmondson et al., 1971). Ferric iron increases the heat resistance of milk lipase. It was desired to know if FIP compounds promote development of either rancid or oxidized off-flavors. Fresh whole milk was fortified at the level of 10 mg of iron/qt with FIP–protein powder, FIP (aqueous), and ferric ammonium citrate. The last mentioned compound, although it increases the heat resistance of lipase, was found to be an acceptable additive provided an elevated pasteurization temperature was used (Edmondson et al., 1971). After addition of the iron compounds, the milk was pasteurized at 78° for 15 sec. The milk was judged by a trained ten-member taste panel after 1 day, 1 week, and 2 weeks.

RESULTS AND DISCUSSION

Bioassimilability Tests. In the studies conducted with weanling rats, the trial was terminated after 18 days and rat tail blood hemoglobin values were obtained. Summarized in Table I are the calculated iron availability values, based on ferrous sulfate as the standard (100%). The FIP–protein and FIP (aqueous) were nearly as good as ferrous sulfate (92 and 93%) whereas the FIP (solid gel) was considerably lower (60%). The same order of assimilability, although quantitatively lower, was found in chicks (FIP–protein, 89%; FIP (aqueous), 83%; FIP (solid), 52%).

Shown in Table II are the mean body weight gains, hemoglobin gains, and percent iron availabilities for rats fed the iron-fortified milk concentrate solids for a period of 15 days. Groups 1–4 served as standards for comparison of the fortified milk samples. Confirming the previous bioassay of the FIP–protein powder, three of the milk samples (5, 7, and 8) had iron availability values that were slightly greater than the value for milk sample 2 plus $FeSO_4$ (93%). The values for milk samples 3 and 4 were only slightly (not significantly) below the value for sample 2 plus $FeSO_4$.

It may be noted that no attempt was made to maintain the same proximate analysis of each diet fed. Hence, all diets did not contain the same percentages of casein, fat, and lactose. At high levels lactose may cause digestive disturbances but there was no indication of diarrhea or poor growth in these rats.

In all trials, the iron derived from FIP–protein remained highly assimilable. We may conclude from the data that this processing protocol had little effect on the assimilabil-

Table III. Final Body Weights, Organ Weights, and Feed Efficiencies of Rats Fed FIP-Protein

Diet[a]	Final body wts, g	Organ weights in g/100 g body weight					Feed efficiency, gain/feed
		Liver	Kidneys	Spleen	Heart	Testes	
1. Corn–soy basal[b]	388 ± 57	3.46 ± 0.22	0.76 ± 0.05	0.13 ± 0.02	0.33 ± 0.03	0.85 ± 0.12	0.137 (4 weeks) 0.067 (91 days)
2. FIP–protein[c]	451 ± 25[d]	3.51 ± 0.18	0.69 ± 0.05[d]	0.14 ± 0.02	0.33 ± 0.03	0.69 ± 0.10[d]	0.148 (4 weeks) 0.075 (91 days)

[a] A 98-day assay, 6 male young S-D rats/group, mean initial weight = 85 g. [b] Level of iron in diet was 50 ppm. [c] Level of iron in diet was 720 ppm. [d] $P < 0.05$.

Table IV. Body Weights and Organ Weights of Rats Fed Iron Compounds Containing 1000 ppm of Iron

Diet[a]	Final body wt ± SD	Organ weights as g/100 g body weight						
		Liver	Spleen	Kidneys	Heart	Testes	Adrenals[b]	Thyroids[b]
Control	452 ± 57^{Aa}[c]	3.19 ± 0.11^{Aa}	0.16 ± 0.01^{Ab}	0.65 ± 0.03^{Aa}	0.29 ± 0.03^{Aa}	0.75 ± 0.10^{Aa}	10.6 ± 1.2^{Aa}	4.6 ± 0.9^{Aa}
FIP–protein	425 ± 55^{Aa}	3.14 ± 0.15^{Aa}	0.16 ± 0.01^{Ab}	0.68 ± 0.04^{Aa}	0.31 ± 0.03^{Aa}	0.81 ± 0.06^{Aa}	11.7 ± 1.7^{Aa}	5.7 ± 0.9^{Aa}
FIP (solid)	424 ± 29^{Aa}	3.20 ± 0.24^{Aa}	0.18 ± 0.02^{Aa}	0.64 ± 0.06^{Aa}	0.31 ± 0.03^{Aa}	0.80 ± 0.05^{Aa}	10.8 ± 1.6^{Aa}	5.2 ± 1.3^{Aa}

[a] Eight male weanling S-D rats per group for 91 days, FIP diets contained 1000 ppm of iron. [b] Data for adrenals and thyroids are in mg/100 g body weight. [c] Duncan multiple range test. The means without a superscript letter in common are significantly different; $P < 0.05$ for lower case; $P < 0.01$ for capitals.

Table V. Body Weights and Organ Weights of Rats Fed Diets Containing 10,000 ppm of Iron

Diet[a]	Final body wt ± SD	Organ weights as g/100 g body weight						
		Liver	Spleen	Kidneys	Heart	Testes	Adrenals[b]	Thyroids[b]
Control	431 ± 41^{Aa}[c]	2.96 ± 0.16^{Bb}	0.14 ± 0.01^{Aa}	0.67 ± 0.03^{Bb}	0.29 ± 0.02^{Aa}	0.77 ± 0.01^{Bb}	11.2 ± 2.3^{Ab}	5.2 ± 0.4^{ABab}
+ $FeSO_4$	271 ± 29^{Bb}	3.63 ± 0.16^{Aa}	0.17 ± 0.02^{Aa}	0.79 ± 0.03^{Aa}	0.30 ± 0.02^{Aa}	1.20 ± 0.14^{Aa}	15.2 ± 1.2^{Aa}	6.0 ± 0.8^{Aa}
+ FIP	457 ± 44^{Aa}	3.08 ± 0.37^{ABb}	0.14 ± 0.02^{Aa}	0.71 ± 0.04^{Bb}	0.30 ± 0.02^{Aa}	0.78 ± 0.07^{Bb}	11.5 ± 1.6^{Ab}	4.6 ± 0.5^{Bb}

[a] Four male weanling S-D rats per group for 106 days, iron diets contained 10,000 ppm of iron. [b] Data as mg/100 g body weight. [c] Duncan multiple range test. The means without a superscript letter in common are significantly different; $P < 0.05$ for lower case; $P < 0.01$ for capitals.

ity of iron derived from FIP–protein. All samples had highly acceptable values which were not influenced by the specific treatment of the milk sample.

Toxicity Studies. Final body weights and organ weights of the rats fed the iron complex at 720 ppm are presented in Table III. All values fall within normal limits. Hematology data, including red and white cell counts, hemoglobin concentrations, and hematocrits, were obtained from blood samples taken when the trial had progressed for approximately 75 days. All values were within normal limits. At 80 days a composited urine sample from three rats in each group was analyzed; no abnormalities were detected. Microscopic examination of preserved tissues evidenced no specific pathology attributable to the iron complex.

For rats maintained on the 1000-ppm diet, hematology and urinalysis data were normal. Final body weights and organ weights are listed in Table IV. Results are normal with the possible exception of the slightly increased spleen weights of the rats fed FIP, at the 95% probability limit. Histochemical examination revealed more splenic iron deposition in the form of hemosiderin in rats from groups fed the FIP compounds than for the control group. The deposition was not considered heavy. Neither iron deposition nor any tissue alteration attributable to the iron-containing diets was found in any other organ or tissue.

For rats fed FIP and $FeSO_4$ at 10,000 ppm, histological examination showed that these animals exhibited lesions consistent with hemosiderosis, or dietary iron overload, as indicated by increased amounts of iron pigment in tissues other than spleen. However, the occurrence of stainable iron was much more pronounced in rats fed $FeSO_4$ than in those fed FIP. In the $FeSO_4$ animals, deposition was found in more organs and in heavier amounts than with the FIP animals. Final body weights and organ weights for these rats appear in Table V. Except for a significantly higher hematocrit value for the group fed $FeSO_4$, there were no significant differences in the total red and white cell counts and hemoglobin values. Urinalysis data were negative with the possible exception of reduced pH values for the rats fed the iron supplements (control, pH 7.7; FIP, pH 6.3; $FeSO_4$, pH 5.8).

Flavor Tests. Figure 1 shows changes in flavor scores of a control sample and the fortified milks during a 2-week storage period at 4°. Scores were assigned between 31 and 40, 40 being the best flavor quality. A score of 35 is considered acceptable beverage quality milk. The scoring system is based on a score-card developed by the American Dairy Science Association in 1963. Each milk was evaluated after 1 day, 1 week, and 2 weeks. Each score is the average of two taste panel trials, ten judges per panel. The graph shows little change in flavor score for FIP–protein fortified milks and the unfortified milk control. Both maintained acceptable beverage quality throughout the storage period. Lower flavor scores were observed when FIP (aqueous) and ferric ammonium citrate were added to whole milks. The scores show nonacceptable beverage quality well before 7 days had elapsed. The panel judged the deterioration in flavor to be due almost entirely to development of lipolytic rancidity. Little or no oxidized flavors were detected in any of the four samples.

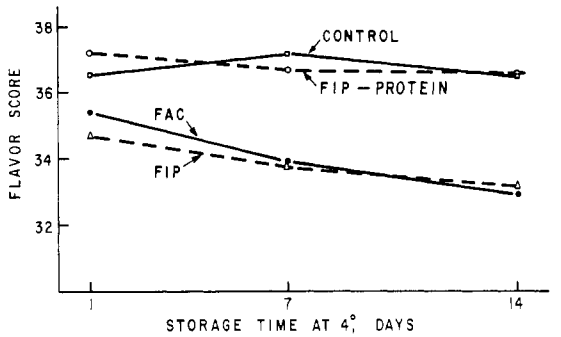

Figure 1. Effect of storage on beverage flavor quality of milks fortified with iron at the level of 10 mg/qt (FIP, ferripolyphosphate; FAC, ferric ammonium citrate).

SUMMARY

The iron from FIP–protein powders and FIP (aqueous) was found to be highly assimilable (92–100%) relative to ferrous sulfate, when fed by direct addition to animal diets. Iron from FIP, solid gel, was less well utilized (50–60%), but nonetheless can be considered a good source of biologically assimilable iron. The iron from FIP–protein remained highly assimilable in sterile concentrated whole milks despite the protocol of its addition.

No toxic or pathological effects were found in rats fed a dietary intake of 720 ppm of iron from FIP–protein for a 90-day period. Slightly increased splenic iron deposition was found when rats were fed 1000 ppm of Fe from FIP and FIP–protein for 90 days. Rats fed 10,000 ppm of Fe from FIP and $FeSO_4$ developed lesions from dietary iron overload, with $FeSO_4$ causing the more severe iron deposition.

FIP–protein, when used to fortify whole milk at 10 mg of iron/qt, maintained a beverage flavor quality comparable to the unfortified control for a period of 2 weeks.

FIP–protein and FIP solid and aqueous may serve as useful iron-enriching additives to dairy products and other commonly used foods such as flour and cereals. Processing trials in these commodities are in progress.

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Separation and Comparative Toxicity of Toxaphene Components

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Components and fractions of toxaphene have been separated by a combination of methods including Sephadex LH-20–methanol column chromatography, thin-layer chromatography, reverse phase thin-layer chromatography, and preparative gas chromatography. Fractions separated by the above methods show varying toxicities to three aquatic organisms, a blue-green alga, brine shrimp, and mosquito larvae. Employing a combination of preparative TLC and GC methods, a toxic fraction of toxaphene 1.87, 1.75, and 1.35 times more toxic than toxaphene to mosquito larvae, brine shrimp, and algae, respectively, has been isolated. The fraction, though it behaves as a single component in various chromatographic systems, was found to consist of two components on the basis of nuclear magnetic resonance (NMR) spectroscopy. They were partially characterized by infrared and mass spectrometry as octachlorobornanes.

Toxaphene is a widely used insecticide. Two-thirds of its production is used for cotton insect control while other uses include vegetables, small grains, soybeans, and control of external insects on livestock. It has also been employed extensively in fish eradication programs (Muirhead-Thomson, 1971). Its annual production is about 50 million pounds (1971 estimate, Environmental Protection Agency, 1972) with a total usage of one billion pounds in the past 25 years. Despite this wide usage, little has been known about the chemistry, toxicity, metabolism, or environmental fate of its components. Only recently a major effort toward answering these questions has been made by Casida et al. (1974), who were successful in isolating and identifying a toxic component of toxaphene, 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane. Also isolated was a $C_{10}H_{10}Cl_8$ component which was more toxic to mice and houseflies than the above component; however, no structure was proposed for the latter component. According to them, at least 175 polychlorinated 10-carbon compounds were recognized by their methods (Holmstead et al., 1974). The components were described as polychlorobornanes, polychlorobornenes, and polychlorotricyclenes with 6 to 10 chlorine atoms per component.

Isolation and identification of all the components of toxaphene would be a monumental task. Therefore, at this stage of our understanding of toxaphene, it is necessary to limit study to the major toxic components. The definition of toxicity, however, is not so simple. The first problem is to define the target species. Casida et al. (1974), for instance, used mice and houseflies as test organisms to bioassay fraction and component toxicity.

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