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Effect of Fiber in Corn Tortillas and Cooked Beans on Iron Availability

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Availability of iron from a diet of corn tortillas and cooked beans with different levels of fiber was determined by hemoglobin repletion. Neutral detergent fiber (NDF) in corn tortillas and cooked beans and soluble, ionizable, and total iron the the test foods were determined. Weanling male rats were fed an iron-free diet until anemic and then divided into groups. The rats were fed diets with 6.5, 10, and 15% NDF and 25, 30, and 35 ppm of total iron. Iron availability was significantly reduced by 15% NDF. The amount of iron in the diet affected iron repletion. A significant correlation between soluble iron at pH 1.35 in vitro and percent efficiency of hemoglobin repletion was obtained. Iron from corn tortillas and cooked beans is approximately 50% less available than ferrous sulfate.

Iron deficiency is a commonly recognized nutritional deficiency in developing as well as in affluent societies (Goodhart and Shils, 1980). Reinhold et al. (1975) suggested that fiber largely determines the availability of bivalent metals in wheat bread. Solomons et al. (1979) proposed that the high consumption of fiber from corn tortilla by rural populations in Guatemala may affect zinc absorption. Incorporation of corn into the diet has been reported to decrease iron absorption. Miller (1978) found that incorporation of iron in hemoglobin was reduced when a corn diet was fed at 2-day intervals with a casein diet. In another study Layrisse et al. (1968) reported a decrease in iron absorption from veal after corn was introduced into the diet of experimental subjects.

Fiber is thought to interfere with iron absorption because of its binding capacity (Reinhold et al., 1975). Ismail-Beigi et al. (1977) reported wheat bran and hemicellulose exhibited high iron binding capacity. More recently Reinhold et al. (1981) found the neutral detergent fiber (NDF) fraction from corn tortilla bound as much as 0.3 mg of iron/g of NDF at pH 6.45. Since corn tortillas along with cooked beans are the main staple of the Mexican diet, the purpose of this study was to investigate the effect of fiber from corn tortillas and cooked beans on iron availability.

MATERIALS AND METHODS

Experimental Animals and Diets. Weanling male 28-day-old Long Evans rats were obtained from Charles River Breeding Laboratories (North Wilmington, MA). The rats were housed individually in stainless steel cages with wire mesh floors. Lighting was automatically controlled to provide 12 h of darkness. Feed (in aluminum cups) and deionized water (in rubber-stoppered glass bottles) were supplied ad libitum. Final weight gain as well as total feed consumption were recorded.

The compositions of the experimental diets are shown in Table I. The diets were formulated by using corn tortillas and cooked beans to provide 66.49 ± 1.98% of the total calories. The experimental diets had three levels of NDF (6.5, 10, and 15%) and three levels of iron (25, 30, and 35 ppm). Corn tortillas and cooked beans were substituted in the diets at the expense of glucose and casein. The amount of cooked beans in each of the experimental diets was held constant to 25.2% while the level of corn tortilla plus cellufil was increased to achieve different NDF levels. The three different iron levels were obtained by supplementing the endogenous iron level of the corn tortilla and the cooked beans with ferrous sulfate heptahydrate to the desired level. At the lowest level of iron, 25 ppm, no ferrous sulfate addition was necessary. Three control diets containing 0, 25, and 35 ppm of iron as ferrous sulfate heptahydrate were used in the study.

The commercially made corn tortillas and the raw beans (*Phaseolus vulgaris*) were obtained from Guadalajara, Mexico. The corn tortillas were sun-dried and ground to pass a 18-mesh screen. The powder was stored at −10 °F until blended into the diet. The beans were soaked overnight in tap water at room temperature (3:1 ratio) and cooked until soft in an open vessel at boiling temperature. The cooked beans and the broth were freeze-dried and ground to pass a 20-mesh screen (Oregon Freeze Dried Foods, Inc., Albany, OR). The remaining ingredients used in the diet were all purchased from United States Biochemical Corp. (Cleveland, OH). After the diets were completely mixed, the material was placed in plastic bags and held at −10 °F until fed.

Iron Availability. Iron availability was assessed by the depletion–repletion method (Fritz and Pla, 1972). The rats were fed an iron-free diet for 51 days. At the end of this period the hemoglobin concentration in whole blood was

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Table I. Percent of Ingredients in Experimental Diets

diet	corn tortilla	cooked beans	casein (vitamin free)	glucose monohydrate	corn oil	vitamin[a] mixture	cellufil	mineral[b] mixture	$FeSO_4 \cdot 7H_2O$
control 0			20	69.6	5.0	3.5		2.20	no
control 25			20	69.6	5.0	3.5		2.20	yes
control 35			20	69.6	5.0	3.5		2.20	yes
15 NDF 35[c]	40	25.2	6.34	10.22	3.72	3.5	8.82	2.20	yes
15 NDF 30	40	25.2	6.34	10.22	3.72	3.5	8.82	2.20	yes
15 NDF 25	40	25.2	6.34	10.22	3.72	3.5	8.82	2.20	no
10 NDF 35	45	25.2	4.76	12.41	3.61	3.5	3.52	2.20	yes
10 NDF 30	45	25.2	4.76	12.41	3.61	3.5	3.52	2.20	yes
10 NDF 25	45	25.2	4.76	12.41	3.61	3.5	3.52	2.20	no
6.5 NDF 35	50	25.2	4.18	11.82	3.51	3.5		2.20	yes
6.5 NDF 30	50	25.2	4.18	11.82	3.51	3.5		2.20	yes
6.5 NDF 25	50	25.2	4.18	11.82	3.51	3.5		2.20	no

[a] AIN Vitamin mixture 76, U.S. Biochemical Corp., Cleveland, OH 44122. [b] AIN Mineral mixture 76, U.S. Biochemical Corp., Cleveland, OH 44122. [c] 15 NDF 35 = percent NDF (neutral detergent fiber) and ppm of iron.

measured according to the cyanomethemoglobin method (Crosby et al., 1954). The anemic animals were divided into groups of three animals each. A control group was maintained on the iron free diet. The remaining groups of animals were placed on one of the nine experimental diets and two groups of animals on the control diets of 25 and 35 ppm of iron. After a 14-day repletion period the hemoglobin concentration was again determined. At the termination of the experimental feeding period, the animals were killed by placing them in a CO_2 chamber.

Proximate Composition. The proximate composition of corn tortilla and raw and cooked beans was determined by AOAC (1980) methods. NDF in the above samples was determined according to the method described by Reinhold and Garcia-Lopez (1979). The acid detergent fiber (ADF) was determined by the method of Goering and Van Soest (1970).

Total, Soluble, and Ionizable Iron. Total iron content of the different materials was determined by a procedure similar to that described by Simpson and Blay (1966). Two grams of sample was weighed into a 100-mL volumetric flask to which 40 mL of concentrated HCl was added. The flask was then heated to boiling for 30 min. The samples were cooled, made to volume with deionized water, and filtered through filter paper (Whatman No. 1). The clarified solution was analyzed in an atomic absorption spectrophotometer (Perkin-Elmer 303) by using 40% HCl standards.

The soluble and ionizable iron content of the corn tortillas, cooked beans, and all the test diets was determined by using the method of Narasingas Rao and Prabhavathi (1979). Two grams of dry sample powder and 25 mL of pepsin–HCl solution (0.5% pepsin in solution in 0.1 N HCl) were mixed. The pH of the solution was adjusted to 1.35 by the addition of 4 N HCl. The solutions were incubated at 37 °C for 90 min in a water bath shaker. After the incubation period the samples were centrifuged for 45 min at 1935*g* and 10 °C. Finally, the supernatant was filtered through Whatman No. 44 filter paper. The soluble iron was determined in the supernatant by atomic absorption spectroscopy. The ionizable iron was measured according to the procedure described by Henry et al. (1974). It was necessary to modify the procedure by adding an equal volume of chloroform to the supernatant to clarify the solution. The solution was thoroughly mixed and centrifuged for 15 min at 1935*g*, and the upper layer was extracted and used for the ionizable iron determination.

Inhibition of the Maillard reaction was accomplished by washing 30 g of the raw bean powder with distilled water and soaking overnight in a 0.002% solution of sodium metabisulfite. After being soaked, the beans were cooked by boiling for 1 h. An additional 100 mL of 0.02% sodium metabisulfite was added during cooking. The cooked beans were then dried and ground to pass a 120-mesh screen.

Table II. Composition of Corn Tortillas and Raw and Cooked Beans (Average of Triplicates)

item	protein	% dry wt crude fat	% dry wt dry matter	NDF[a]	ADF[b]
raw beans		1.37		5.80	5.06
cooked beans	27.88	1.76	98.80	15.75	6.96
corn tortillas	11.60	1.89	90.11	6.53	

[a] NDF = neutral detergent fiber. [b] ADF = acid detergent fiber.

Bean proteins were extracted from raw beans by washing the raw bean powder with 2% NaCl. To 10 g of the powder, 150 mL of distilled water was added, and the slurry was mixed for 30 min at room temperature and then centrifuged for 20 min at 9990*g* at 5 °C. The supernatant was discarded. The residue was then washed twice with 100 mL of 2% NaCl solution and once with 100 mL of distilled water. The residue was cooked in a microwave oven for 1 min, dried overnight, and ground to pass a 120-mesh screen.

Extraction and identification of leucoanthocyanins in the NDF fraction of cooked beans were accomplished by using a solution of 25 mL of concentrated HCl in 500 mL of 1-butanol. The NDF fraction (125 mg) was placed into a test tube and 8 mL of the 1-butanol–HCl solution added. The tubes were mixed thoroughly and heated to 90 °C for 40 min in a water bath. The tubes were cooled and centrifuged in a clinical centrifuge, and absorbance of the supernatant at 550 nm was determined (Swain and Hillis, 1959).

The presence of starch material in the NDF fraction was determined by placing a portion of the NDF fraction in a test tube containing 5 mL of distilled water and heating for 15 min in a boiling water bath. A few drops of iodine solution (2%) were added. Starch will give a characteristic blue color.

The data were statistically analyzed by analysis of variance (ANOVA), correlation coefficient, and multiple regression according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The protein, crude fat, dry matter, NDF, and ADF contents of the diet ingredients are shown in Table II. The protein concentration (Kjeldahl $N \times 6.25$) of 27.88% in cooked beans is in agreement with values reported by Sgarbieri et al. (1979) and with those reported by Kon (1979). This value is higher than the 18.60–23.0% range

Table III. Form of Iron and Effect on Efficiency of Iron Incorporation into Hemoglobin (Average of Triplicates)

	mg/100 g dry wt				
diet	total Fe (A)	soluble Fe[a] (B)	ionizable Fe[a] (C)	insoluble Fe[b] (A − B)	% efficiency ± SD
control 35	3.64	1.81	0.64	1.83	47.67 ± 8.10
control 25	1.57	1.53	0.44	0.04	43.00 ± 8.70
control 0	1.43	1.09	0.53	0.34	0.42 ± 1.10
15 NDF 35[c]	5.51	1.85	1.56	3.66	15.85 ± 5.40
15 NDF 30	5.09	1.70	1.18	3.38	18.07 ± 14.10
15 NDF 25	3.93	1.57	0.82	2.26	11.70 ± 1.40
10 NDF 35	5.02	1.55	1.19	3.47	25.61 ± 6.10
10 NDF 30	4.78	1.31	0.97	3.47	22.33 ± 0.30
10 NDF 25	4.18	1.34	0.87	2.84	16.15 ± 3.70
6.5 NDF 35	4.89	1.14	0.88	3.75	20.75 ± 11.30
6.5 NDF 30	4.85	1.02	0.79	3.83	13.79 ± 2.80
6.5 NDF 25	3.81	1.52	1.24	2.29	15.77 ± 14.80

[a] Determined at pH 1.35. [b] Difference between soluble at pH 1.35 and total iron. [c] 15 NDF 35 = 15% NDF and 35 ppm of Fe.

reported by Elias et al. (1979) in Guatemalan varieties of beans (*P. vulgaris*). The protein concentration in corn tortilla was found to be 11.60%. This value is higher than the 10.50% value reported by Robson et al. (1976) for corn; however, some variation is expected to occur with the type and variety of corn used in making the tortilla. The crude fat percentage of 1.76% for cooked beans agrees with the value reported by Watt and Merrill (1963). No values have been reported for the crude fat content in corn tortillas. The level of NDF in corn tortillas was 6.53%, which is similar to the level previously reported by Reinhold and Garcia-Lopez (1979). A concentration of 5.80% was observed in the raw beans. After cooking the level increased to 15.75%. This increase was attributed to the production of insoluble proteins and other nondigestible products such as those produced by the Maillard reaction during cooking (Van Soest, 1965).

When the Maillard reaction was inhibited during the cooking process, the NDF value was decreased to 10.47%. If the proteins were extracted before cooking, the NDF value was 12.44%. The NDF fraction of cooked beans contained as much as 1.16% total nitrogen. Although these values are lower than the value reported for total NDF, it is difficult to conclude with certainty that the Maillard reaction and the production of insoluble proteins during cooking are responsible for the total increase in the NDF level in cooked beans. Later experiments in which different enzyme concentrations (amylase) as well as lower temperatures and longer periods of digestion (from 1 to 24 h at 40 °C) were used showed a range of values from 9.12 to 10.50% with an average of 9.88%, suggesting that the conditions during starch digestion are critical and possibly the high values could be due to incomplete digestion even though the samples were shown to be free of starch by the iodine test after digestion.

Other compounds such as leucoanthocyanin pigments could also be involved in the increase due to cooking. Cooked beans were found to contain leucoanthocyanins (7.70 units of absorbance at 550 nm/g of dry sample) when they were extracted with a butanol–HCl solution. Phenolic-type products were also detected in both cooked and raw NDF fractions. These compounds could contribute to the observed increases in the NDF fraction of cooked beans.

Monte and Maga (1980) reported a similar increase in the insoluble matter after pinto beans were cooked. They reported an increase of approximately 20% in insoluble matter. Similar effects have been observed by Matthee and Appledorf (1978) in legumes.

The cooked beans had a total iron content of 5.12 mg/100 g. Sgarbieri et al. (1979) reported a value of 5.59 mg/100 g and Augustin et al. (1980) a value of 5.84 mg/100 g. One might expect some variability among different varieties and growing conditions. The corn tortillas contained 2.66 mg/100 g total iron. Robson et al. (1976) reported 2.8 mg/100 g in maize. Layrisse et al. (1968) reported 2.2 mg/100 g total iron in corn. Hunt et al. (1978) reports a value almost double in corn tortillas made in Los Angeles, CA, that reported by other workers. This variability could be due mainly to differences in varieties and methods of preparation rather than to a disagreement in the results obtained. The soluble iron in cooked beans was 2.04 mg/100 g. Wallis and Jaffe (1977) found a range of 0.47–3.39 mg/100 g in beans. Cooked beans contained 1.14 mg/100 g ionizable iron. No values have been reported in the literature for ionizable iron in cooked beans.

Corn tortillas contained almost half of the total amount of iron present in beans; however, the majority of this iron is in the soluble and ionizable state, 2.12 and 1.84 mg/100 g, respectively. Figure 1 shows soluble, ionizable, and insoluble iron as a percentage of total iron for cooked beans and corn tortillas. It has been pointed out recently that in addition to the total iron content of food, the form of that iron is also important in terms of availability (Lee and Clydesdale, 1980; Narasingas Rao and Prabhavathi, 1979). Thus it appears even though the level of iron is low in corn tortillas it may be readily utilized. A significant linear relationship ($P < 0.1$) was found between the total iron concentration in the diet and the percent efficiency of iron availability (Table III). A good correlation ($r = 0.43$), significant at the 5% level, was obtained between soluble and ionizable iron at pH 1.35 (Table III). The ionizable

Table IV. Effect of Fiber on Iron Availability

diet	initial hemoglobin, g/100 mL	final hemoglobin, g/100 mL	total Fe, mg/100 g of dry liver	wt gain/feed consumption	RBV[a]
control 35	10.31 ± 0.6	15.87 ± 0.9	47.25 ± 17.0	0.2 ± 0.1	
control 25	10.67 ± 1.30	15.11 ± 0.6	24.60 ± 5.6	0.2 ± 0.2	
control 0	10.48 ± 1.1	9.60 ± 2.20	23.66 ± 5.0	0.2 ± 0.1	
15 NDF 35[b]	9.74 ± 3.0	11.58 ± 1.60	23.46 ± 7.6	0.21 ± 0.05	44.33 ± 27.00
15 NDF 30	9.93 ± 1.7	12.08 ± 1.50	22.04 ± 18.7	0.17 ± 0.06	50.91 ± 17.80
15 NDF 25	9.98 ± 2.4	10.84 ± 1.60	19.69 ± 9.2	0.16 ± 0.07	56.91 ± 17.10
10 NDF 35	9.74 ± 1.4	13.26 ± 1.30	33.27 ± 13.1	0.19 ± 0.02	64.21 ± 6.00
10 NDF 30	9.80 ± 1.3	12.35 ± 1.00	37.01 ± 19.9	0.20 ± 0.05	56.49 ± 13.60
10 NDF 25	10.50 ± 1.4	12.24 ± 1.50	31.33 ± 8.4	0.17 ± 0.02	64.34 ± 11.10
6.5 NDF 35	10.34 ± 1.2	12.73 ± 1.30	31.85 ± 6.1	0.17 ± 0.07	58.64 ± 10.00
6.5 NDF 30	10.07 ± 1.0	10.52 ± 1.1	30.41 ± 9.9	0.19 ± 0.07	40.41 ± 12.50
6.5 NDF 25	10.22 ± 2.4	10.85 ± 5.0	24.55 ± 7.8	0.18 ± 0.02	57.80 ± 9.80

[a] Relative biological value, mean ± SD, adjusted for body weight. [b] Percent NDF and ppm of iron.

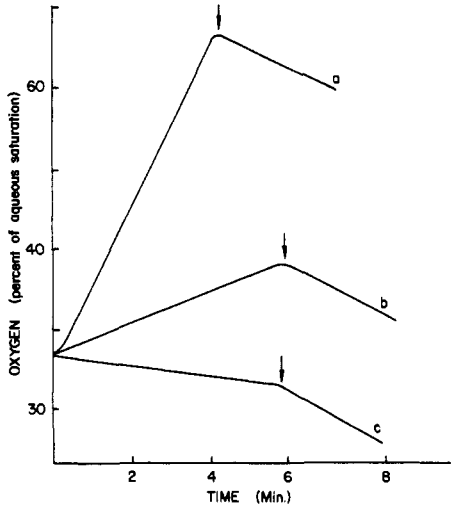

Figure 1. Form of iron in corn tortilla and cooked beans as a percent of total iron.

iron value is lower than the soluble iron value, which could suggest the presence of other iron complexes that are available to the rat. The percent efficiency of iron incorporation into hemoglobin for the experimental and basal diets range from 11.70 ± 1.40% for the lowest efficiency to 25.61 ± 6.1% for the highest percent efficiency, with a mean of 17.78%. The percent efficiency for the basal diet containing 35 and 25 ppm of ferrous sulfate was 47.67 ± 8.1 and 43.0 ± 8.7 and 0.42 ± 1.1% for the iron-free diet. Sgarbieri et al. (1979) had previously reported a 30% efficiency for ferrous sulfate containing diets.

The effect of fiber on iron availability is shown in Table IV. The 15% NDF level of fiber in the experimental diets significantly lowered final hemoglobin levels ($P < 0.001$). Considering the RBV value for ferrous sulfate to be 100, the values for the test diets range from 40.41 ± 12.50 to 64.34 ± 11.10, with a mean RBV value of 55.0 ± 19.10. Miller (1976) reported a 55 RBV value for raw corn, and Sgarbieri et al. (1979) reported a range in RBV values from 13.75 to 17.50 for dry beans. Values have not been reported for a combined diet of beans and corn tortillas.

The results from this study indicated different levels of fiber from corn tortillas significantly affect iron bioavailability in rats as measured by the iron repletion method. The amount of iron in the diet and its form effect the efficiency of incorporation of iron into hemoglobin. Total iron and soluble iron content of test diets correlate with percent efficiency. In vitro determination of soluble iron may be useful in predicting iron availability for different foods. The majority of iron in corn tortillas is soluble and therefore may be readily available. Beans contain a much higher level of total iron but the greatest proportion is insoluble. Because of the potential high availability of the iron in corn tortillas, as the amount was increased in the diet (as a means of increasing fiber) it may have in fact increased the amount of highly available iron.

Cooking increases the NDF level in beans. This increase is due to the production of insoluble proteins, melanoidin-type compounds, and possible insoluble pigments present in the beans' seed coat.

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