The Effect of Dry Heat on the Bioavailability of Iron in Soy Flour

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Bioavailability of iron in soy flour was investigated by the Hemoglobin Regeneration Efficiency (HRE) procedure in 50 three-month-old Sprague-Dawley rats. Rats weighing 250 ± 7 g and with a mean hemoglobin level of 12.9 g/dl were randomly assigned to one of five treatment groups: baseline (BL), unheated soy flour (UH), soy flour heated at 225°F for either 10 min (H10), 30 min (H30), or 120 min (H120). The animals were fed diets (46 ppm iron) containing the soy flour for 21 days. HREs of UH, H10, H30, H120 diets were 17.6, 16.8, 17.7 and 16.8%, respectively. Apparent iron absorption from the UH, H10, H30 and H120 diets was 94.7, 94.3, 93.9 and 94.3%, respectively. Serum iron was significantly lower ($p<.001$) and total iron binding capacity was significantly higher ($p<.001$) in rats fed the H120 diet. Iron concentrations in the liver, spleen, heart and kidney were significantly lower in rats fed H30 or H120 diets. These results suggest that prolonged heating of soy flour may reduce iron bioavailability and result in depletion of iron stores.

KEY WORDS: Dry heat, hemoglobin regeneration efficiency, iron bioavailability, soy flour.

The United States leads the world in the production of soybean with an increase in production from 1,216 million bushels in 1974 to 1,548 million bushels in 1988 (1). More than 50% of this production is used for oil, about 30% for export and the remaining for planting seed, livestock feeding and human consumption (2,3). It is estimated that less than 1% of the total soybean production in the United States is used for human consumption (3). Soybean protein has several important food uses in the form of soy flour (a valuable ingredient for bakery products), soy protein concentrate and isolated soy proteins. The bioavailabilities of essential trace elements, such as iron, in these soy products are especially important because soy protein is often used for nutritional supplementation.

In mature soybeans, nonheme iron exists mainly as a complex with phytate in the form of monoferric, diferric or tetraferric phytates (4), as ferric citrate (5) or as a phytate-protein complex (6), while in immature soybean seeds iron is found primarily in the ferrous state (5). Soybeans are a significant dietary source of nonheme iron in many developing countries (2,3). Absorption of nonheme iron is inhibited by carbonates, phytates, fiber, tannin, phosphates, EDTA, the presence of other trace minerals such as zinc and copper and other chelators (7). Ascorbic acid and some other organic acids such as citric, lactic, succinic, and tartaric acids may enhance nonheme iron absorption (7).

Food processing may either enhance or reduce the absorption of nonheme iron. Fontaine and coworkers (8) noted that at pH 7 and above soy proteins form phytic acid-protein-mineral complexes that may adversely affect mineral bioavailability, especially that of iron and zinc. Incomplete digestion of these complexes to free amino acids may occur resulting in inefficient absorption of amino acids and minerals.

Cook *et al.* (9) observed that the absorption of extrinsically labeled nonheme iron from a soy protein isolate (Supra-710) was only 0.5% compared to 2.5% for albumin and 2.7% for casein. Rodriguez *et al.* (10) studied the effects of heat and partial phytate removal on chick hemoglobin repletion and reported that heating soy protein isolates at 120°C for 20 min improved iron bioavailability by 65 to 77%, while removal of phytate (75% reduction in phytate) improved bioavailability by 6 to 11%. This suggests that both heating and phytate removal promote more complete digestion of protein and protein-iron-phytate complexes and release of iron from soy protein isolate.

Heat treatment of bovine hemoglobin, beef and ferrous sulfate-based diets also increased the efficiency of bovine hemoglobin and heme iron conversion into hemoglobin by anemic rats (11). Rackis and coworkers (12) observed maximum nutrient value of soy protein when the soy protein was heated with steam for about 30 min or was autoclaved at 3.3 kg pressure for 15–20 min.

Research on the effect of dry heating of soy flour on iron bioavailability in soy flour-based diets has not been previously studied. In addition, the effect of heating time on iron bioavailability has received limited attention. Given the many uses of the soybean, research on the bioavailability of trace elements, especially iron, is important.

The purpose of this study was to examine the effects of dry heating on the bioavailability of iron in soy flour. Iron bioavailability in soy-based diets was determined by the hemoglobin regeneration efficiency procedure (13). Iron concentrations in the rat liver, spleen, gastrocnemius muscle, heart and kidney were also determined to assess iron nutriture of animals fed heated soy flour (14).

EXPERIMENTAL PROCEDURES

Fifty, three-month-old male Sprague-Dawley rats weighing 250 ± 7.1 g were fed a diet low in iron (10.5 ppm) for 5 wk. Baseline hematocrit and hemoglobin levels averaged 50 ± 1.24% and 22 ± 1.09 g/dl, respectively. A total of approximately 5 mL of blood was drawn from the orbital venous plexus (15) of each rat on two occasions at 3-day intervals which resulted in a 57% reduction in hemoglobin levels, from 22.4 ± 1.09 g/dl to 15.1 ± 1.04 g/dl and to 12.99 g/dl. Rats weighing 440 ± 11.2 g were then randomly assigned to one of five groups. The animals assigned to the baseline diet were then sacrificed at the start of the experimental period to obtain baseline values for the parameters that were being assayed. The composition of the five diets is presented in Table 1. The soy flour (Soya Fluff 200 W, Central Soya Co., Inc., Fort Wayne, IN) was processed from defatted soybeans under medium steam heat and had a residual trypsin inhibiting activity of 40 units/g and a nitrogen solubility index of 40–50% (Central Soya Co., Inc.). It was heated in an oven at 225°F in steel pans for 10, 30 or 120 min and was allowed to cool

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TABLE 1

Composition of the Diets

Dietary components	Casein	UH[a]	H10[b]	H30[c]	H120[d]
Casein[e]/ soy flour[f]	20.0	20.0	20.0	20.0	20.0
Corn starch	35.0	35.0	35.0	35.0	35.0
Sucrose	30.0	30.0	30.0	30.0	30.0
Corn oil[g]	10.0	10.0	10.0	10.0	10.0
Vitamin mix[h]	1.0	1.0	1.0	1.0	1.0
Mineral mix[i] (no iron)	3.5	3.5	3.5	3.5	3.5
DL Methionine[j]	0.3	0.3	0.3	0.3	0.3
Choline bitartrate[j]	0.2	0.2	0.2	0.2	0.2
Energy (kcal/100 g)	430	442	442	442	442
Iron (ppm)	10.5	46.5	45.1	46.1	47.3

[a]UH = unheated soy flour.
[b]H10 = Soya Fluff 200W, additional heating for 10 min.
[c]H30 = Soya Fluff 200W, additional heating for 30 min.
[d]H120 = Soya Fluff 200W, additional heating for 120 min.
[e]ANRC Vitamin Free Casein, Nutritional Biochemicals Corp., Cleveland, OH.
[f]Soya Fluff 200W, Central Soya Co., Inc., Fort Wayne, IN.
[g]Mazola, Best Foods, CPC International Inc., Englewood Cliffs, NJ.
[h]AIN Vitamin Mixture 76, Nutritional Biochemicals Corp., Cleveland, OH.
[i]AIN Mineral Mixture 76, Nutritional Biochemicals Corp., Cleveland, OH.
[j]Nutritional Biochemicals Corp., Cleveland, OH.

to room temperature prior to making the diets. Flour depth during heating was about 2.5 cm. Animals were fed the respective diets and distilled water *ad libitum* for 3 wk. The iron content of the diets was determined by the wet ashing procedure (14) as read on a Perkin-Elmer 2100 Atomic Absorption Spectrophotometer (Norwalk, CT).

At the end of the 3 wk, the animals were anesthetized and fasting blood was collected by cardiac puncture for the analysis of hemoglobin, hematocrit (16), serum iron and total iron binding capacity (TIBC) by means of a kit (Stanbio Laboratory Inc., San Antonio, TX). Fecal samples were collected for seven days (from day 13 to day 19 of the study), weighed and stored at −18°C. Iron concentrations in the heart, liver, spleen, kidney and gastrocnemius muscles and fecal samples were determined by the wet-ashing procedure (14) as read on the Perkin-Elmer 2100 Atomic Absorption Spectrophotometer (Norwalk, CT). Iron concentrations in the tissues and fecal samples were calculated on a wet-weight basis.

Apparent iron absorption was calculated from the following equation:

$$[(\text{intake of iron} - \text{fecal iron})/\text{intake of iron}] \times 100 \quad [1]$$

Iron bioavailability was determined by the Hemoglobin Regeneration Efficiency (HRE) method (13,17).

Statistical analysis. The data obtained were analyzed by using a general linear model, with diet as the independent variable and the blood and tissue parameters as the dependent variables. The least square means multiple comparison procedure was used to determine significant differences between means within a group.

RESULTS AND DISCUSSION

Mean food intake and body weights were not significantly different among the treatment groups (Table 2). Animals in the H30 and H120 groups had significantly higher ($p<0.0001$) fecal iron concentrations than those in the UH and H10 groups (64.4 and 61 mcg/g compared to 57.8 and 59.1 mcg/g, respectively). Apparent iron absorption ranged from 93 to 95%, which was indicative of the anemic status of the animals. The availability of dietary iron is affected by the chemical form of the ingested iron compound, amount ingested and the presence of enhancers or inhibitors of iron absorption (9). It has been reported that raw soybeans and their products have poor mineral bioavailability, which is improved by heating (12). This could be due to the destruction of heat-labile factors that interfere with the utilization of protein and other nutrients (18). Picciano *et al.* (19) determined the relative iron bioavailability from soy flour, freeze-dried soy beverage and soy protein concentrate by the hemoglobin repletion bioassay in weanling anemic male rats. Iron bioavailability of 92, 81 and 66%, respectively, was reported for soy flour, freeze-dried soy beverage and soy protein concentrate (19). In the present study, apparent iron absorption was only slightly lower in the group fed the H30 diet and was not significantly different between the H10 and H120 diet groups.

Hemoglobin regeneration efficiency was slightly lower in the H10 and H120 groups than in the UH and H30 groups, although the differences were not significant (Table 2). The HRE values obtained in this study were much lower than the 40% HREs previously reported for

TABLE 2

Body Weight (BW), Bioavailability and Tissue Weights in Rats Fed Heated and Unheated Soy Flour*

Experimental group	Body weight (g)	Iron intake (mg)	Apparent iron absorption (%)	HRE[1] (%)	Tissue weights (g/kg BW) Liver	Spleen	Kidney	Heart	Gastrocnemius muscle
BL[2]	451.1±3.8[a]	—	—	—	29.3±1.1[a]	1.9±0.1[a]	6.5±0.2[a]	3.2±0.1[a]	11.3±0.2[a]
UH[3]	479.3±4.0[b]	22.0±0.4[a]	94.7±0.1[a]	17.6±0.5[a]	25.7±0.4[a]	1.7±0.1[a]	6.1±0.1[a]	2.9±0.1[a]	11.3±0.5[a]
H10[4]	467.5±3.8[b]	21.6±0.3[a]	94.3±0.1[b]	16.8±0.5[a]	27.0±0.6[a]	1.8±0.1[a]	6.7±0.1[a]	3.0±0.1[a]	11.6±0.4[a]
H30[5]	472.5±3.8[b]	21.8±0.5[a]	93.9±0.1[b]	17.7±0.5[a]	25.6±0.8[a]	1.7±0.1[a]	6.4±0.1[a]	3.0±0.1[a]	11.4±0.2[a]
H120[6]	473.8±3.8[b]	22.4±0.3[a]	94.3±0.1[b]	16.8±0.5[a]	25.6±0.8[a]	1.8±0.1[a]	6.4±0.2[a]	2.9±0.1[a]	11.1±0.8[a]

*Means ± SEM. Means within a column with different superscripts are significantly different (p<.0001).
[1]HRE = hemoglobin regeneration efficiency.
[2]BL = baseline.
[3]UH = unheated soy flour.
[4]H10 = soy flour heated for 10 min.
[5]H30 = soy flour heated for 30 min.
[6]H120 = soy flour heated for 120 min.

TABLE 3

Hematological Indices of Iron Status in the Different Treatment Groups*

Experimental group	Hemoglobin (g/dl)	Hematocrit (%)	Serum iron (mcg/dl)	TIBC[1] (mcg/dl)
BL[2]	12.9 ± 0.3[a]	30.9 ± 0.6[a]	164.7 ± 2.7[a]	619.8 ± 4.2[a]
UH[3]	15.5 ± 0.4[b]	46.5 ± 0.4[b]	127.6 ± 2.1[b]	435.5 ± 3.1[b]
H10[4]	16.7 ± 0.5[b]	46.0 ± 0.5[c]	142.0 ± 4.0[d]	431.7 ± 4.7[b]
H30[5]	16.7 ± 0.5[b]	45.8 ± 0.8[c]	151.6 ± 3.4[c]	489.3 ± 4.0[c]
H120[6]	16.1 ± 0.4[c]	48.3 ± 0.5[d]	141.6 ± 3.4[d]	516.6 ± 4.1[c]

*Means ± SEM. Means within a column with different superscripts are significantly different (p<.0001) as determined by ANOVA and least square means.
[1]TIBC = total iron binding capacity.
[2]BL = baseline.
[3]UH = unheated soy flour.
[4]H10 = soy flour heated for 10 min.
[5]H30 = soy flour heated for 30 min.
[6]H120 = soy flour heated for 120 min.

TABLE 4

Least Square Means for Serum Iron in Rats Fed Heated and Unheated Soy Flour

Diet	Serum iron BL[2]	H10[3]	H30[4]	H120[5]	TIBC[1] BL[2]	H10[3]	H30[4]	H120[5]
BL[2]	—				—			
H10[3]	.0001	—			.0001	—		
H30[4]	.0062	.0431	—		.0001	.0001	—	
H120[5]	.0001	.9255	.0349	—	.0001	.0001	.0001	—
UH[6]	.0001	.0037	.0001	.0047	.0001	.5197	.0001	.0001

[1]TIBC = total iron binding capacity.
[2]BL = baseline.
[3]H10 = soy flour heated for 10 min.
[4]H30 = soy flour heated for 30 min.
[5]H120 = soy flour heated for 120 min.
[6]UH = unheated soy flour.

soybean diets providing 20 mg iron/kg, when fed to weanling anemic male rats (20). In the present study, the adult male rats were moderately anemic compared to the severely anemic weanling rats used by Ifon (20). Additionally, Ifon (20) used boiled soybeans while in the present study, the soy flour was dry heated. The present study suggests that dry heating of soy flour may lower iron bioavailability.

Although the hemoglobin levels in the different groups were not significantly different, the hematocrit values of the UH and H120 groups were significantly higher (p<0.0001) than those of the H10 and H30 groups (Table 3). Serum iron was significantly lower in the UH group, suggesting that unheated soy flour may result in reduced iron transport (Tables 3 and 4). Animals in the H120 group exhibited lower serum iron and higher TIBC values

TABLE 5

Tissue Iron Concentration in the Different Treatment Groups*

Experimental group	Liver (mcg/g)	Spleen (mcg/g)	Heart (mcg/g)	Kidney (mcg/g)	Gastrocnemius muscle (mcg/g)
BL[1]	67.4 ± 0.9[a]	197.0 ± 1.1[a]	81.7 ± 0.8[a]	35.6 ± 0.6[a]	17.3 ± 0.7[a]
UH[2]	75.5 ± 1.2[b]	197.0 ± 1.4[a]	83.6 ± 0.9[b]	36.3 ± 0.7[a]	15.8 ± 0.7[a]
H10[3]	79.7 ± 1.2[c]	196.3 ± 1.8[a]	83.6 ± 0.9[b]	36.8 ± 1.0[a]	15.9 ± 0.9[a]
H30[4]	65.2 ± 1.2[a]	192.5 ± 1.5[b]	83.9 ± 1.0[b]	31.8 ± 0.9[b]	15.2 ± 0.8[a]
H120[5]	67.6 ± 0.8[a]	189.4 ± 1.2[b]	79.9 ± 0.9[c]	34.4 ± 1.0[c]	15.4 ± 0.8[a]

*Means ± SE. Means within a column with different superscripts are significantly different as determined by ANOVA and LSM; liver ($p<0.0001$); spleen and kidney ($p<0.003$); heart ($p<0.035$).
[1]BL = baseline.
[2]UH = unheated soy flour.
[3]H10 = soy flour heated for 10 min.
[4]H30 = soy flour heated for 30 min.
[5]H120 = soy flour heated for 120 min.

TABLE 6

Least Square Means for Iron Concentration in Liver and Spleen of Rats Fed Heated and Unheated Soy Flour

Diet	Liver				Spleen			
	BL[1]	H10[2]	H30[3]	H120[4]	BL[1]	H10[2]	H30[3]	H120[4]
BL[1]	—				—			
H10[2]	.0001	—			.7287	—		
H30[3]	.1761	.0001	—		.0349	.0744	—	
H120[4]	.9034	.0001	.1415	—	.0006	.0017	.1351	—
UH[5]	.0001	.0132	.0001	.0001	.9187	.7185	.0378	.0007

[1]BL = baseline.
[2]H10 = soy flour heated for 10 min.
[3]H30 = soy flour heated for 30 min.
[4]H120 = soy flour heated for 120 min.
[5]UH = unheated soy flour.

than the animals in the other heat treatment groups, suggesting a greater degree of iron deficiency and iron depletion (Tables 3 and 4).

The iron status of the animals and the stage of growth could influence the ability to produce more hemoglobin. The information available in this area is inconsistent due to differences in the heat treatments and processing methods used to manufacture soy flour. Shricker *et al.* (21) reported that in rats, the iron bioavailabilities from soy flour and soy protein isolate were similar, while Cook *et al.* (9) reported that, in humans, iron bioavailability was better from soy flour than from soy protein isolate. Limited heating may beneficially affect iron bioavailability from soy protein isolates due to the inactivation of trypsin inhibitors and unfolding of protein molecules, which, in turn, increase protein digestion and release of iron from protein-iron-phytate complexes, thus improving iron bioavailability (10). It appears from the data in Table 3 that heating soy flour results in better hematological indices. However, heating soy flour for 120 min may have some adverse effects on iron hemostasis such as high TIBC and low serum iron.

The iron concentrations in the liver, spleen and the kidney were significantly lower in the H30 and H120 than in the UH and H10 groups (Table 5). Low tissue iron levels are indicative of iron depletion that results from an attempt to maintain serum iron levels and to utilize the iron for transport and for hemoglobin synthesis. Iron concentrations in the gastrocnemius muscle were not significantly different among the groups. Iron concentrations in tissues such as the liver and spleen were more sensitive to the effect of prolonged heat treatment than in the gastrocnemius muscle (Table 6).

The results of this study indicate that heating soy flour for 30 or 120 min reduces iron concentrations in the liver, spleen and kidney, elevates TIBC, and reduces serum iron. The need for further research to determine the optimal heating time for maximizing iron bioavailability in soy products is evident. Research is also needed to elucidate the nature and extent of interaction(s) of iron with soy protein and other compounds during dry heating.

REFERENCES

1. United States Department of Agriculture, *Agricultural Statistics 1989*, Washington, DC, 1989, pp. 124-130.
2. Wolf, W.F., and J.C. Cowan, *Soybeans as a Food Source*, CRC Press, Cleveland, OH, 1975, pp. 1-101.
3. Wang, H.L., G.C. Mustakas, W.J. Wolf, L.C. Wang, C.W. Hesseltine and E.B. Bagley, *Soybeans as Human Food: Unprocessed and Simply Processed*, Department of Agriculture, Science and Education Administration, Washington, DC, 1979, pp. 1-54.
4. Ellis, R., and E.R. Morris, *Nut. Rep. Int. 20*:739 (1979).
5. Shizuko, A., A. Fumatoshi and N. Tadeshi, *Anal. Chem. 330*:146 (1987).
6. Erdman, J.W., and R.M. Forbes, *J. Am. Oil Chem. Soc. 58*:489 (1981).
7. Miles, C.W., *Ibid. 66*:924 (1989).

8. Fontaine, T.D., W.A. Pons and G.W. Irving, *J. Biol. Chem. 164*:487 (1946).
9. Cook, J.D., T.A. Morck and S.R. Lynch, *Am. J. Clin. Nutr. 34*:2622 (1981).
10. Rodriguez, C.J., C.V. Morr, and M.E. Kinkel, *J. Food Sci. 50*:1072 (1985).
11. Jansuittivechakul, O., A.W. Mahoney, D.P. Cornforth and D.G. Hendricks, *Ibid. 50*:407 (1985).
12. Rackis, J.J., J.E. McGee and A.N. Booth, *Cereal Chem. 52*:85 (1975).
13. Mahoney, A.W., and D.G. Hendricks, *Am. Chem. Soc. Sym. Ser. 203*:1 (1982).
14. Bowering, J., and G.F. Norton, *J. Nutr. 111*:1648 (1981).
15. Timm, K.J., *Lab. Anim. Sci. 29*:636 (1979).
16. Crosby, W.H., J.I. Munn and F.W. Furth, *U.S. Armed Forces Med. J. 51*:693 (1954).
17. Forbes, A.L., C.E. Adams, M.J. Arnaud, C.O. Chichester, J.D. Cook, B.N. Harrison, R.F. Hurell, S.J. Kahn, E.R. Morris, J.T. Tanner and P. Whittaker, *Am. J. Clin. Nutr. 49*:225 (1989).
18. Liener, I.E., *J. Am. Oil Chem. Soc. 58*:406 (1981).
19. Picciano, M.F., K.E. Weingarter and J.W. Erdman, *J. Food Sci. 49*:1558 (1984).
20. Ifon, E.T., *Nutr. Rep. Int. 24*:25 (1981).
21. Schricker, B.R., D.D. Miller and D. Van Campen, *J. Nutr. 113*:996 (1983).

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