Total and Soluble Iron Content and Effect of Certain Inhibitors Present in Selected Varieties of Tepary Bean (*Phaseolus acutifolius*)

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Seven varieties of tepary bean (*Phaseolus acutifolius*) were evaluated for their total iron content, soluble iron content, and content of absorption inhibitors, such as tannins, phosphates, oxalates, and crude fiber. The mean total iron content of tepary bean determined by atomic absorption spectrophotometry was 7.59 ± 0.5 mg/100 g (dry weight basis) and was higher than the mean total iron content of pinto bean (*Phaseolus vulgaris*) (5.83 mg/100 g). The mean soluble iron content of tepary beans estimated *in vitro* was 0.7 mg/100 g and was higher compared to that of pinto beans (0.23 mg/100 g). Tannins, oxalates, and phosphates affected the iron solubility in tepary beans. Iron solubility was not affected by crude fiber.

INTRODUCTION

Legume seeds are high in protein and energy and can be used to improve diets in low-income countries. In several Latin American countries, particularly in Mexico, pinto beans (*Phaseolus vulgaris*) are the most commonly consumed as human food. Although a good source of iron, its absorption seems to be very low and the presence of strong inhibitors has been reported (Reddy et al., 1987).

The early residents of the southwestern United States and northern Mexico incorporated grain legumes, either wild or domesticated, into their daily diets (Bouscaren et al., 1983). One such legume, the tepary bean (*Phaseolus acutifolius*), which contributed substantially to the nutritional well-being of early settlers of both Spanish and Indian cultures, has remained an important food source for the people of Sonora. The tepary bean is a potentially good protein source and in combination with cereals is nutritionally adequate and superior to the common bean (Tinsley et al., 1985). Two types of domesticated tepary beans, based on their color, are used in stews, broths, and soups. This bean has an intrinsic value as an underdeveloped crop, is adapted to hot, arid climates, and is a potential donor of desirable traits to the common bean (*P. vulgaris*) (González de Mejía et al., 1988). The physiological and morphological components of heat and drought adaptation in tepary beans are still under study.

Seven varieties, of 70 lines, of tepary beans with the best physicochemical, sensory, and culinary characteristics were selected for the study (Gonzalez et al., 1990). The present study was directed to evaluate the total and soluble iron contents and the presence of inhibitory factors such as tannins, phosphates, oxalates, and crude fiber in the selected varieties of tepary beans.

MATERIALS AND METHODS

Materials. Table 1 shows the seven varieties of tepary beans used in this study, donated by Dr. Giles Waines of the University of California in Riverside. Pinto beans (*P. vulgaris*), available in Sonora supermarkets, were used for comparison.

Reagents. Ferrozine [3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine], Hepes [*N*-2-(hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid], and the pancreatin and pepsin used for digestion were obtained from Sigma Chemical Co.

Sample Preparations. Beans were soaked for 14 h at 20 °C with 1:2 parts of water and cooked in the conventional form with 1:5 volumes (w/v) of water. The cooked beans were blended in a commercial blender (Waring, Model 34BL22) with 1:2 volumes (w/v) of bean broth, dried at 50 °C by 9-10 h, and ground in a Thomas–Wiley laboratory mill (Thomas Scientific, Model 4) to a powder using a 100-mesh sieve. The powders thus obtained were stored in polyethylene bags at 5 °C and used for all of the determinations.

Table 1. Tepary Bean (*P. acutifolius*) Varieties Used in This Study

line	color	location
PI319-443	white	Sonora, Mexico
BUFF	ginger	Arizona
PI-321-637	maroon	Beltsville, MD
L-242-25	light brown	Sonora, Mexico
L-246-123	dark brown	Sonora, Mexico
L-242-43	black	Sonora, Mexico
L-578	pinto	Puerto Rico

Chemical Analysis. Moisture was determined in an oven (100–105 °C) to constant weight (AOAC, 1984, Method 14.004) and protein by the Kjeldahl procedure using the factor 6.25 to convert total percent nitrogen into percent protein (AOAC, 1984, Method 14.067). Crude fat (AOAC, 1984, Method 7.062) and total ash were determined by incineration in a muffle furnace (Thermolyne) at 550–600 °C according to Method 7.009 of the AOAC (1984).

Total Iron. Duplicates samples (1.00 ± 0.05 g) were digested by the wet ashing method [AOAC, 1984, Section 3.014(b)]. Iron content was determined by atomic absorption spectrophotometry (Varian Associates Inc., Walnut Creek, CA, Model 1475) using NIST/SRM bovine liver 1577b (Gaithersburg, MD) as a reference.

Iron solubility was determined according to the *in vitro* procedure described by Reddy et al. (1987). Samples of bean flour (3.5 g) were sequentially incubated with pepsin–HCl at pH 3 for 1 h at 37 °C and pancreatin at pH 5. After digestion, the samples were centrifuged and soluble iron was determined using sodium dithionite and Ferrozine. The visible absorption of Fe^{2+}–Ferrozine complex was measured at 563 nm using a Spectronic 21 (Milton Roy Co.). The supernatant blanks (without Ferrozine) and the reagent blanks (without supernatants) were subtracted from each absorbance value. Fe^{2+} concentrations were determined using the above method with sodium dithionite omitted. Fe^{3+} concentrations were estimated by subtracting Fe^{2+} from the total iron.

Estimated Absorbable Iron. Absorbable iron was calcultated by assuming that 40% of ferrous iron (Hallberg, 1981) and 13% of ferric iron (Brise and Hallberg, 1962) are absorbed, and the following equation was used for calculating estimated absorbable iron (EAI):

$$EAI = 0.40[Fe^{2+}] + 0.13[Fe^{3+}] \quad (1)$$

Fe^{2+} and Fe^{3+} are the amounts of iron solubilized from the beans.

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Table 2. Chemical Composition of Tepary Beans[a]

bean	protein[b] (N × 6.25)	ash[b]	fat[b]	crude fiber[b]
tepary bean				
white	25.23	4.66	1.04	2.23
ginger	21.50	4.50	1.00	3.14
maroon	24.20	3.66	0.88	2.61
light brown	24.63	3.88	0.97	2.14
dark brown	25.31	4.49	1.10	2.81
black	24.22	3.94	0.92	2.53
pinto	24.94	4.21	1.76	2.78
mean ± SD[c]	24.28[a] ± 1.30	4.19[a] ± 0.37	1.09[a] ± 0.3	2.59[a] ± 0.43
pinto (*P. vulgaris*)[c]	23.99[a]	3.73[b]	1.21[a]	3.90[b]

[a] All results are expressed on dry basis (g/100 g). [b] Mean ± standard deviation, average of three determinations. [c] Means followed by different superscripts in columns are significantly different (Scheffé, $p < 0.05$).

Tannins were determined according to the method of Buttler et al. (1982). **Phosphates** (Method 2.020), **oxalates** (Method 32.040), and **crude fiber** (Method 7.066) were determined according to AOAC (1984) procedures.

Statistics. A completely random design was used in this experiment with seven bean varieties and two replicates. The statistical analysis to compare varieties consisted of an analysis of variance, multiple-range Tukey and Scheffe tests. All comparisons were made at a 5% level of significance. Soluble iron and the potential inhibitors were modeled through a multiple regression using a stepwise procedure. The model was in the form

$$\text{soluble iron} = \beta_0 + \beta_1[\text{tannins}] + \beta_2[\text{oxalates}] + \beta_3[\text{phosphates}] + \beta_4[\text{crude fiber}] + \text{error} \quad (2)$$

All data were analyzed, using the Statistical Analysis System, 6.04 version (SAS, 1991).

RESULTS AND DISCUSSION

The chemical compositions for the tepary and pinto beans (*P. vulgaris*) are shown in Table 2. The protein and fat contents of tepary and pinto beans were not significant ($p < 0.05$). Tepary beans are a good source of protein, containing 21.5–25.3 g/100 g, and these values are in concurrence with the values reported by Nabhan et al. (1980).

The mean total ash content (4.2 g/100 g) in tepary beans was significantly higher than that of pinto beans ($p < 0.05$) and similar to the values reported by Nabhan et al. (1980) (4.4 g/100 g) and Idouraine et al. (1989) (4.6 g/100 g). The crude fiber content of tepary beans was significantly lower than that of pinto beans ($p < 0.05$).

Iron Content. The total and soluble iron contents of tepary beans were higher compared to those of pinto beans ($p < 0.05$, Table 3); however, the total iron content of white and pinto varieties of tepary bean were higher compared to that of the other varieties examined ($p < 0.05$, Table 3). The soluble iron contents of white and ginger varieties of tepary beans were higher compared to that of the other varieties examined ($p < 0.05$; Table 3). The soluble iron content of pinto beans obtained in this study was similar to those reported by Reddy et al. (1987). The greater proportion of soluble iron is in the ferric form (Table 3); this ionic form is known to be unstable, because it has high reactivity with other compounds present in the diet, and it can form insoluble compounds that are not available for absorption (Clydesdale, 1983). Assuming that the beans would be consumed alone, only approximately 9% of the iron becomes soluble.

Table 3. Total and Soluble Iron Content of Tepary (*P. acutifolius*) and Pinto Beans (*P. vulgaris*)[a]

bean varieties	total iron, mg/100 g	soluble iron (mg) in 100 g: Fe^{2+} (*A*)	Fe^{3+} (*B*)	total sol Fe (*A* + *B*)
tepary bean				
white	8.32[d]	0.076	0.889	0.965[a]
ginger	7.11[b]	0.017	0.866	0.883[a]
marron	7.57[bc]	0.017	0.607	0.624[b]
light brown	7.12[cd]	0.053	0.594	0.647[b]
dark brown	7.49[b]	0.025	0.750	0.775[b]
black	7.22[b]	0.006	0.313	0.319[c]
pinto	8.25[cd]	0.000	0.664	0.664[b]
mean ± SD	7.58 ± 0.5	0.019 ± 0.01	0.669 ± 0.19	0.696 ± 0.20
pinto (*P. vulgaris*)	5.83[a]	0.000	0.326	0.326[c]

[a] All results are expressed on dry basis, mg/100 g, and all values are means of three determinations. Values followed by different superscripts are significantly different (Tukey, $p < 0.05$).

Table 4. Estimated Absorbable Iron (EAI) in Selected Varieties of Tepary Beans[a]

bean variety	EAI, mg/100 g: Fe^{2+} (*A* × 0.4)	Fe^{3+} (*B* × 0.13)	EAI (*A* + *B*)	% of RDA[b]
P. acutifolius				
white	0.030	0.115	0.145[a]	9.66
ginger	0.006	0.112	0.118[ab]	7.86
maroon	0.006	0.079	0.085[b]	5.66
light brown	0.021	0.077	0.098[b]	6.53
dark brown	0.010	0.097	0.107[b]	7.13
black	0.002	0.040	0.042[c]	2.8
pinto	0.000	0.086	0.086[b]	5.73
P. vulgaris				
pinto	0.000	0.042	0.042[c]	2.80

[a] All results are expressed on dry basis, and all values are means of three determinations. Values followed by different superscripts are significantly different (Tukey, $p < 0.05$). [b] Based on the assumption that 10% of the total absorbable iron is available. *A* = soluble Fe^{2+}; *B* = soluble Fe^{3+}.

EAI when 100 g of tepary beans is consumed is shown in Table 4. The deduced EAI values for all varieties of tepary beans in general are almost twice that of pinto beans and the black variety of tepary beans ($p < 0.05$; Table 4). The black variety of tepary beans and pinto beans had similar EAI values (Table 4). We calculated the percentage of RDA met by the EAI in tepary beans. The RDA is 15 mg of iron for women of 11–50 years of age (NAS, 1989), and the recent WHO recommendations suggest that about 10–15% of iron is absorbed from diets which are relatively high in available iron (FAO, 1988). Tepary beans were found to provide less than 10% of the available iron.

Inhibitors. The contents of some compounds considered to be inhibitors of iron absorption were evaluated, and the results are shown in Table 5. Tepary beans had lower levels of crude fiber, phosphates, and tannins than pinto beans (*P. vulgaris*), and among the tepary beans, the white beans had the lowest values of these inhibitors.

As determined by regresion analysis, tannins had the highest partial correlation coefficient ($r^2 = 0.65$), while oxalates and phosphates had lesser influence. The *F* value for this model was 16.32 ($p < 0.0002$) and $r^2 = 0.80$. Crude fiber did not have a significant effect over the concentrations of soluble iron.

Torrence et al. (1982) have suggested that high molecular weight polyphenolic compounds may be important inhibitors of food iron absorption in diets high in vegetables, particularly in legumes, because the seed coats have a high

Table 5. Inhibitor Contents of Iron Absorption in Tepary Beans (*P. acutifolius*) and Pinto Bean (*P. vulgaris*)[a]

bean variety	crude fiber, g	phosphates, mg	oxalates, mg	tannins, mg equiv of catechin
tepary bean				
white	2.16	179.16	1.135	ND[b]
ginger	3.14	254.97	1.370	7.41
maroon	2.61	236.67	1.245	7.34
light brown	2.14	296.98	0.865	6.5
dark brown	2.81	206.71	1.325	7.84
black	2.53	228.09	1.175	8.12
pinto	2.78	245.43	1.310	6.86
mean	2.59[a]	235.43[a]	1.20[a]	6.30[a]
pinto (*P. vulgaris*)	3.90[b] ± 0.02	300.40[b] ±0.87	1.135[a] ±0.035	13.10[b] ±0.21

[a] All results are expressed on dry basis, and all values are means of three determinations. Values followed by different superscripts are significantly different ($p < 0.05$). [b] ND, not detected.

tannin content. Their blocking effect in absorption has been attributed to the formation of highly insoluble iron tannates. Results of the present study agree with the observations made by Kojima et al. (1981) that tannins in tea markedly decreased the amounts of soluble iron in cooked pinto beans. Fiber has long been assumed to have a negative influence on iron absorption; however, conflicting results had been obtained from differents studies depending on the quantity, type, and source of the fiber (Garcia et al., 1985). On the other hand, the majority of the studies had been made using a model system with a single binding component, and they cannot be extrapolated to foods in which more than one binding component exists. These results also illustrate that the interactive effects of binding components are critical in terms of iron solubility and thus may affect iron bioavailability.

We conclude that the iron availability can be affected depending on the combination of the compounds present intrinsically in the beans and that tannins are one of the major inhibitor factors of iron solubilization in tepary beans.

In general, the different varieties of tepary beans contain a higher quantity of total iron content and soluble iron and indicate they will have a higher iron bioavailability than the common bean (*P. vulgaris*). The white beans had the highest value.

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