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Effect of Varieties and Processing Methods on the Total and Ionizable Iron Contents of Grain Legumes[†]

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A comparison of the commonly consumed Indian pulses with soybean was made to test the effect that genetic variations have on total and ionizable iron contents and how ionizable iron was influenced by processing methods. Mean total iron content was the highest in soybean, followed by chickpea, urd bean, mung bean and pigeon pea. Mean values for available iron were the highest in chickpea and the lowest in urd bean. Germination and fermentation resulted in significant increases ($P < 0.01$) in the available iron of chickpea, urd bean, and soybean. Except for mung bean, no significant changes in ionizable iron content were observed by either autoclaving or roasting these legumes. The beneficial effect of germination on iron availability in all legumes was found to be more pronounced than the fermentation process.

Keywords: *Legumes; pulses; ionizable iron; germination; fermentation; autoclaving; roasting*

INTRODUCTION

Micronutrient malnutrition is a serious problem in many developing countries. Many millions of people, particularly the pregnant women and children, suffer from iron deficiency-anemia, a chronic nutritional problem (Annapurani and Murthy, 1985). Minerals from cereals, legumes, and other plant foods, in contrast to minerals from animal sources, are generally poorly utilized by man and other monogastric animals. Diets in developing countries contain mostly non-heme iron, derived mainly from cereals, legumes, fruits, and vegetables (Hallberg, 1981). Some of the iron present in the diet may be in a chemical form that is either poorly or not at all absorbable, therefore data on the availability of iron in vegetable foods are of great value.

Although Indian diets contained sufficient iron (2−3 mg/100 g), only a limited proportion of it was absorbed by the gastrointestinal mucosa in humans. Poor iron assimilation suggests that the bioavailability of dietary iron is a major determinant of the iron status of the body rather than the total iron intake through the diet (Narasinga Rao and Rao, 1980). The low bioavailability of iron from cereals and pulses has been attributed to the presence of different inhibitors of which phytate and tannins are of major importance (Hallberg et al., 1987).

Grain legumes, called pulses in India, are the major sources of both protein, and minerals, particularly iron in the habitual diets of the people. Chickpea, the most important grain legume in India, is present in two basic types, desi and kabuli. Desi seeds, generally, yellow to black in color, are smaller and have a rougher surface. Kabuli seeds are usually large and light colored. Other important legumes grown extensively in India include pigeon pea, mung bean, and urd bean. The major portion of these legumes is consumed in the form of dhal, decorticated dry split cotyledons (Singh, 1995). Traditional methods of using legumes in India include soaking, germination, fermentation, boiling, and roasting. These processes influence the availability of iron in food crops (Annapurani and Murthy, 1985; Gahlawat and Sehgal, 1994). The objective of our study was to determine the extent of variability in the total and available iron among the commonly consumed pulses in India as compared with a reference legume—soybean. Secondly to evaluate the effect of processing practices, namely germination, fermentation, autoclaving, and roasting, on the content of ionizable iron in these pulses.

MATERIALS AND METHODS

Seed Material. Sixteen genotypes each of pigeon pea (*Cajanus cajan* L.) and chickpea (*Cicer arietinum* L.), three genotypes of mung bean (*Phaseolus aureus*), four genotypes of urd bean (*Phaseolus mungo*), and six genotypes of soybean (*Glycine max*) were used in this study. Seed samples of pigeon pea and chickpea grown in the 1992/93 post-rainy season at ICRISAT Asia Center, Patancheru, India, were provided by the breeding units of the International Crops Research Institute for the Semi Arid Tropics (ICRISAT). Mung bean and urd bean seed samples grown at Vijayawada, Andhra Pradesh, India, during the post-rainy season of 1992/93 were obtained from the Andhra Pradesh State Seeds Development Corporation, Hyderabad, India. The University of Agricultural Sciences, Dharwad, Karnataka, India, supplied the seed samples of soybean grown during the rainly season of 1992/93. All the grain samples were cleaned and stored in a cold room at 5 °C before analysis.

Dehulling. The decortication of whole-seed samples of these legumes was carried out by using a tangential abrasive dehulling device (TADD). Seeds were moistened, dried in an oven at 55 °C overnight, and dehulled in a TADD. For chemical analysis dhal samples (dry split cotyledons) were ground in a Udy cyclone mill using a 0.4 mm screen. Samples were then defatted in a Soxhlet apparatus using *n*-hexane. Finely ground raw dhal samples were used as control.

Processing. To study the effect of different processing methods on ionizable iron, one genotype each of pigeon pea (ICP 8094), chickpea (ICCV 10), mung bean (ML 267), urd bean (LBG 611), and soybean (MACS 124) was subjected to the following four different processing treatments: germina-

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tion, fermentation, autoclaving (also called wet-heating), and roasting (also called dry-heating).

Germination. Seed samples of all legumes were germinated in sterile Petri dishes lined with wet filter paper after an initial soaking treatment in distilled water for 12 h at room temperature (25 ± 1 °C). To obtain a sprout measuring about 1.5 cm, the seeds of pigeon pea and soybean were germinated for 72 h and those of chickpea, mung bean, and urd bean were germinated for 48 h. Seed coats were removed manually from the sprouted samples and the cotyledons and radicles were freeze-dried. Freeze-dried material was ground to a fine powder in a Waring blender.

Fermentation. Pigeon pea, chickpea, and soybean dhal samples were initially soaked in distilled water at 25 °C, for 16 h, and those of mung bean and urd bean for 2 h in a seed to water ratio of 1:2 (w/v). The soaked dhal samples were then ground to a batter in a Waring blender. Inoculum of a natural curd sample containing lactic acid bacteria 1.5% (w/v) was then added. The samples were stirred well and allowed to ferment for 24 h in an incubator at 30 °C. The fermented batter was freeze-dried and ground to a fine powder.

Autoclaving. Dhal samples were autoclaved at 15 psi pressure for 15 min for pigeon pea, chickpea, and soybean and 10 min for mung bean and urd bean, in a dhal to water ratio of 1:2 (w/v). The whole cooked broth was freeze-dried and ground to a fine powder as above.

Roasting. Whole seed samples of all these legumes were roasted separately in a sand bath at 200 °C for 2 min. The roasted samples were then separated from the sand by sieving and dehulled by using TADD mill. The roasted and dehulled samples were ground to a fine powder in a Waring blender.

Determination of Total Iron. For determination of total iron content, defatted dhal samples (0.5 g) were weighed, transferred to glass tubes, and digested in a block digestor using 10 mL of tri-acid mixture containing nitric acid, perchloric acid, and sulfuric acid in the ratio of 20:4:1 (Piper, 1966). The contents were digested first at 70 °C for 30 min, then at 180 °C for 30 min, and finally at 220 °C for 30 min. After digestion the mixture was cooled and dissolved in glass-distilled water, and the volume was made up to 50 mL with glass-distilled water. Suitable aliquots were analyzed for iron in an atomic absorption spectrophotometer (Varian Tectron model 1200).

Determination of Ionizable Iron. The ionizable iron was estimated by using a simulated *in vitro* gastrointestinal digestion procedure (Miller et al., 1981). The ionizable iron was extracted by pepsin-HCl solution. A 2 g finely ground sample was incubated with 25 mL of pepsin-HCl solution at 37 °C for 90 min. Then pH was adjusted to 7.5 with NaOH solution and further incubated at 37 °C for 90 min in a shaker water bath. After incubation, the content was centrifuged at 3000 rpm for 45 min. The supernatant was filtered and used. For estimation of ionizable iron, bathophenanthroline reactive iron was measured in the filtrate. The protein precipitate solution was added to the filtrate at a ratio of 1:1 and thoroughly mixed in a vortex mixer. The mixture was centrifuged at 5000 rpm for 10 min. An aliquot of the clear supernatant was transferred to a test tube and chromagen solution added. After 10 min, the absorbance was read at 533 nm. The ionizable iron present in the extract was calculated from a standard curve of absorbance against the standard iron concentration. The ionizable iron content in the sample was expressed as mg/100 g.

Statistical Analysis. For total iron and ionizable iron assays, two replicates were used for the determination of each constituent. Standard errors (SE) were determined by one-way analysis of variance (Snedecor and Cochran, 1967). Variance was used to determine the impact of various processing practices of commonly consumed Indian pulses and soybeans. Significance was accepted at the $P < 0.05$ and $P < 0.01$ levels, depending on the standard error.

Table 1. Total and Ionizable Iron in Chickpea and Pigeon Pea[a]

legume	genotype	total iron, mg/100 g	ionizable iron, as % of total
chickpea	ICCV 89211 (Desi)	6.4	25.0
	ICCV 89214 (Desi)	6.1	24.6
	ICCV 89217 (Desi)	6.7	23.9
	ICCV 89405 (Desi)	6.2	24.2
	ICCV 88202 (Desi)	7.1	22.5
	ICCC 37 (Desi)	6.5	26.2
	ICCV 10 (Desi)	5.6	25.0
	ICCV 6 (Kabuli)	5.6	26.8
	ICCV 3 (Kabuli)	6.3	31.7
	ICCV 2 (Kabuli)	5.1	27.5
	mean	6.1	25.7
	±SE	0.32	0.58
pigeon pea	ICPL 87051	4.0	22.5
	ICPL 87119	2.9	27.6
	ICP 8094	4.5	22.2
	ICP 8863	4.3	23.3
	ICPL 88046	3.8	26.3
	ICPL 85012	4.1	22.0
	UPAS 120	3.8	21.1
	ICPL 85010	3.1	19.4
	ICPL 4	4.7	19.1
	ICPL 366	3.0	23.3
	mean	3.8	22.7
	±SE	0.11	0.96

[a] Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

Table 2. Total and Ionizable Iron in Mung Bean, Urd Bean, and Soybean[a]

legume	genotype	total iron, mg/100 g	ionizable iron, as % of total
mung bean	PS 16	4.5	22.2
	ML 267	4.7	19.1
	LGG 407	3.7	24.3
	mean	4.3	21.9
	±SE	0.09	0.51
urd bean	T 9	4.1	12.2
	LBG 611	4.2	16.7
	LBG 22	4.0	20.0
	LBG 17	5.5	18.2
	mean	4.4	16.8
	±SE	0.14	1.20
soybean	MONETTA	6.6	22.7
	MACS 58	7.7	16.9
	MACS 124	6.2	17.7
	JS 335	5.6	21.4
	PK 472	6.3	22.2
	KhSB 2	6.5	18.5
	mean	6.4	19.9
	±SE	0.13	1.45

[a] Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

RESULTS AND DISCUSSION

Considerable genotypic differences were observed in the total and ionizable iron content of the legumes tested (Tables 1 and 2). Mean total iron content was the highest (6.4 mg/100 g) in soybean, followed by chickpea, urd bean, mung bean, and pigeon pea. Williams and Singh (1987) reported that the total iron content of chickpea seeds of several cultivars ranged between 3.9 and 9.8 mg/100 g with mean being 6.6 mg/100 g. In the present study, total iron content of chickpea cultivars ranged between 5.1 and 7.2 with the mean being 6.1 mg/100 g.

Mean values for ionizable iron were the highest in chickpea (Table 1) and the lowest in urd bean (Table 2). Annapurani and Murthy (1985) in their study on bioavailability of iron from commonly consumed Indian pulses reported that Bengal gram had the highest value

Table 3. Effect of Processing on the Content of Ionizable Iron in Chickpea (ICCV 10) and Pigeon Pea (ICP 8094)[a]

	ionizable iron in chickpea		ionizable iron in pigeon pea	
treatment	mg/100 g	% of total	mg/100 g	% of total
control	1.4	25.0	1.0	22.2
germination	1.7	30.4	1.3	29.5
fermentation	1.6	30.2	1.1	24.4
autoclaving	1.4	25.4	1.0	22.2
roasting	1.3	23.2	0.9	20.5
mean	1.5	27.3	1.1	24.4
±SE	0.05	0.47	0.08	1.13

[a] Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

Table 4. Effect of Processing on the Content of Ionizable Iron in Mung Bean (ML 67), Urd Bean (LBG 611), and Soybean (MACS 124)[a]

	ionizable iron in mung bean		ionizable iron in urd bean		ionizable iron in soybean	
treatment	mg/100 g	% of total	mg/100 g	% of total	mg/100 g	% of total
control	0.9	19.1	0.7	16.7	1.1	17.7
germination	1.2	25.5	1.0	25.0	1.6	26.7
fermentation	1.0	21.7	0.9	22.0	1.3	21.0
autoclaving	1.0	21.3	0.7	16.7	1.1	17.7
roasting	1.1	23.9	0.7	17.0	1.2	19.6
mean	1.0	21.3	0.8	19.5	1.3	21.3
±SE	0.02	0.49	0.05	1.08	0.06	1.07

[a] Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

of available iron content followed by soybean, green gram, and black gram. The amount of ionizable iron in pigeon pea ranged from 19.1% to 27.6% of the total iron, showing significant differences ($P < 0.01$) among the genotypes (Table 1). Snehalata (1984) reported that only 20.8% of the total iron is ionizable in pigeon pea. In chickpea, nearly 26% of the total iron in the grain was the ionizable iron (Table 1). Results of this study indicate that chickpea not only contains the highest amount of total iron except for slightly higher total iron in soybeans but that its ionizable iron content is also the highest among the commonly consumed pulse crops in India (Table 1). There were no clear-cut differences in the total iron and ionizable iron contents of desi and kabuli genotypes of chickpea (Table 1). However, the mean value for available iron as percent of the total was noticeably higher for kabuli than desi genotypes (Table 1). This indicated that bioavailability of iron may be more in kabuli than in desi genotypes of chickpea. ICCV 3, a kabuli genotype of chickpea, showed the highest value for the ionizable iron.

Pigeon pea genotypes showed larger variations in total iron and ionizable iron as compared to other legumes. The lower iron availability from legumes was attributed to their high polyphenol (Rao and Prabhavati, 1982) and phytate contents (Hazell, 1988). The absorption of iron from the commonly consumed legumes (lentils, split peas, mung beans, and black beans) was considerably lower than soybean (Lynch et al., 1984).

The total and ionizable iron in the control and processed samples of the legumes studied are given in Tables 3 and 4. Germination resulted in a significant increase ($P < 0.01$) in the ionizable iron content of all these legumes. The increase in ionizable iron as a result of germination can be attributed to a decrease in the phytic acid due to an increase in the activity of endogenous phytase and probably an increase in ascorbic acid content (Craig, 1994). Hallberg et al. (1989) reported that the inhibitory effect of phytate on iron absorption was markedly counteracted by ascorbic acid, it being an enhancer of iron availability. The effect of ascorbic acid on iron absorption may be due to its ability to form complexes with ferric ions and to its reducing action on the iron, which produces more soluble ferrous iron (Craig, 1994). Ferrous iron was better absorbed than the ferric iron (Hallberg, 1981). Bau and Debry (1979), apart from showing the losses of phytate during germination, also demonstrated increased levels of ascorbic acid in soybeans.

No significant changes in the levels of total and ionizable iron content due to autoclaving and roasting were observed in chickpea, pigeon pea, and urd bean, or soybean. However, these processes significantly ($P < 0.01$) increased the ionizable iron content in mung bean (Table 4). Heating may have a direct effect on protein matrix through denaturation, and this may result in a different proteolytic pattern with release of peptides with altered iron-binding properties. Both fermentation and germination processes resulted in a considerable increase in the ionizable iron content of chickpea and pigeon pea. However, no striking differences between these two processes were observed with respect to improvement in ionizable iron. The roasting process slightly increased the levels of ionizable iron in mung bean and soybean (Table 4), whereas the levels decreased in chickpea and pigeon pea (Table 3). Gahlawat and Sehgal (1994) reported a 16−17% increase in iron availability of weaning foods containing roasted mung bean. The improved availability of iron in roasted weaning foods was attributed to a decrease in phytic acid content, possibly through its destruction on roasting. Rodriguez et al. (1985) reported that the beneficial effects of heat treatment upon iron bioavailability from soy protein were probably due to inactivation of trypsin inhibitors and unfolding of the protein molecular structures to increase the susceptibility of the proteins and phytate to digestion by proteolytic enzymes and phytase, respectively. These latter processes would then facilitate release of iron from the protein−Fe−phytate complex with concomitant improvement in bioavailability.

The ionizable iron significantly ($P \leq 0.01$) increased in all legumes after germination (Tables 3 and 4). However, the effect was more pronounced in soybean which recorded the highest increase in ionizable iron after germination (Table 4). Increases in available iron in pulses after germination have been attributed to increase in phytase activity and a decrease in phytate content (Annapurani and Murthy, 1985). Fermentation increased ionizable iron in chickpea and soybean but not in pigeon pea, urd bean, or mung bean (Tables 3 and 4). Moeljopawiro et al. (1987) reported that fermentation by either lactic acid producing bacteria or *Rhizopus oligosporus* increased the relative biological value of iron in soybeans. The increase in the relative biological value of iron by lactic acid fermentation was attributed either to the release of iron from protein−mineral complexes by enzymes, such as proteases and phytases, produced by lactic acid microorganisms, or to the fact that lactic acid produced by microorganisms acts as a chelator for iron. The present results indicate that fermentation of legumes could also increase ionizable iron. However, the beneficial effect of germination on ionizable iron was found to be more pronounced than the other processing methods in the present study.

CONCLUSION

In conclusion, results of our study suggested that a large variability exists in the iron content of these legumes. Chickpea, particularly the kabuli genotypes, contained the highest amount of ionizable iron. Some processing methods considerably improved the ionizable iron. Germination and fermentation were more effective in increasing ionizable iron in chickpea and soybean. Additional efforts are needed to identify and develop legume genotypes with higher available iron content, and these efforts will help alleviate iron deficiency prevailing in many developing countries.

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