Mössbauer Study of Iron in Soybean Hulls and Cotyledons†

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Oxidation states of iron in hulls and cotyledons separated from immature and mature soybean seeds were studied by Mössbauer spectroscopy. It was found that iron in immature and mature hulls was exclusively in a trivalent high-spin state and that iron in immature and mature cotyledons was in both trivalent high-spin and divalent high-spin states, the former being dominant. Upon treatment of the hulls and cotyledons with 2 mol dm^{-3} hydrochloric acid, ferric ions were found to be gradually reduced to ferrous ions, giving an identical isomer shift and quadrupole splitting. These characteristics are close to those of ferrous ions coordinated octahedrally with six water molecules.

Needless to say, the bioavailability of iron from plant foods to human beings is important from the viewpoint of nutrition. The poor bioavailability of iron from plant food sources is generally attributed to insolubilization of iron by binding to phytic acid, tannin, and dietary fiber as well as by formation of ferric hydroxides in the gastrointestinal tract (Rao and Prabhavathi, 1978; Fairweather-Tait and Wright, 1985; Mugula, 1992; Hurrell et al., 1992).

Soybeans are rich in not only protein and fat but also iron. Iron in soybeans is concentrated in the seed coats (hulls). As much as 20% of the iron of the whole seed is in the hull, whereas its mass is less than 10% of the whole seed mass (Levine et al., 1982). The bioavailability of iron from the hulls is reported to be equivalent to that of ferrous sulfate (Johnson et al., 1985). The reason for the high availability of iron from this source has not yet been clarified.

Mössbauer spectroscopy, a nondestructive analytical method, can provide useful information concerning the chemical states of iron without disturbing the chemical environment of the iron. Our previous Mössbauer study on whole soybean seeds demonstrated that most of the iron in mature seeds is trivalent but that it exists in both bi- and trivalent states in immature seeds (Ambe et al., 1987). However, it was reported later that most of the iron extracted from soybean hulls with 2 mol dm^{-3} hydrochloric acid is in a ferrous state and these ferrous ions are stable against chemical oxidants (Laszlo, 1988). This discrepancy motivated the present work, in which the chemical states of iron in soybean hulls as well as cotyledons have been separately studied by Mössbauer spectroscopy. The chemical change of iron in the hulls induced by treatment with water or hydrochloric acid was followed over time. A similar experiment was also carried out on heat-treated hulls and cotyledons.

EXPERIMENTAL PROCEDURES

Culture of Soybean Plants. Soybean (*Glycine max* L. Merrill cv. Okuharawase) seeds were obtained commercially. Soybean plants were cultivated hydroponically with an enriched stable isotope, $^{57}Fe^{3+}$, without soil in a greenhouse in the absence of root nodule bacteria. The constituent of the nutrient solution was the same as in the previous work (Ambe et al., 1987). After 3 months of cultivation, immature seeds were taken from some of the pods while both the pods and the seeds were still green. They were stored in a deep-freezer until Mössbauer measurement. After 4 months of cultivation in total, the ripe seeds were harvested. The hulls were carefully separated from the seeds by hand.

Treatment of Samples. Part of the hulls and cotyledons were heated at 140 °C for 1 h, and the former at 210 °C as well, for 15 min in air. Distilled water or 2 mol dm^{-3} hydrochloric acid (200–400 μL) was added to 100–200 mg of the hulls and to 1000–2000 mg of cotyledons both in a raw state and after heating. The samples treated with hydrochloric acid were stored in a nitrogen gas stream at room temperature.

Measurement of Mössbauer Spectra. The samples containing ^{57}Fe were subjected to Mössbauer measurement at room and liquid nitrogen temperatures by a conventional Mössbauer spectrometer (Ranger 700 series) against a 740 MBq $^{57}Co/Rh$ source. The 14.4-keV γ-rays were detected with a proportional counter filled with Kr + 3% CO_2 (Reuter-Stokes Inc.).

The spectra were least-squares-fitted to lines of Lorentzian shape with a FACOM M1800 computer at our institute, considering a background absorption in the case of the measurement at liquid nitrogen temperature. In the analysis the position and line width of the background absorption were fixed to the values 0.33 and 0.61 mm/s, respectively, which were determined by the independent background absorption measurement. The isomer shifts are presented relative to metallic iron at room temperature.

RESULTS

Mössbauer Spectra of ^{57}Fe in Soybean Hulls and Cotyledons. The curves in Figure 1 are the results of least-squares fitting of the data points, assuming one or two doublets of Lorentzian shape. In the spectra obtained at liquid nitrogen temperature, an absorption due to background is shown as a dotted line. As can be seen in Figure 1, the ferric doublet is apparently more asymmetric at liquid nitrogen temperature than at room temperature. However, it turned out by computer analysis that the asymmetry was due to superposition of a background absorption on the one peak of the ferric doublet at liquid nitrogen temperature. At both room and liquid nitrogen temperatures, the Mössbauer spectra of ^{57}Fe in the hulls separated from ripe seeds showed only a doublet ascribable to high-spin trivalent iron, as shown in Figure 1A,B. The hulls separated from immature seeds measured at liquid nitrogen temperature also gave a spectrum quite similar to that of the mature hulls, indicating the absence of divalent iron (Figure 1C). On the other hand, the spectrum of ^{57}Fe in the ripe cotyledons represented a distinct absorption of high-spin divalent iron in addition to a major doublet due to high-spin trivalent iron at room temperature (Figure 1D). A similar spectrum was observed for immature cotyledons at liquid nitrogen temperature (Figure 1E). Their Mössbauer parameters are summarized in

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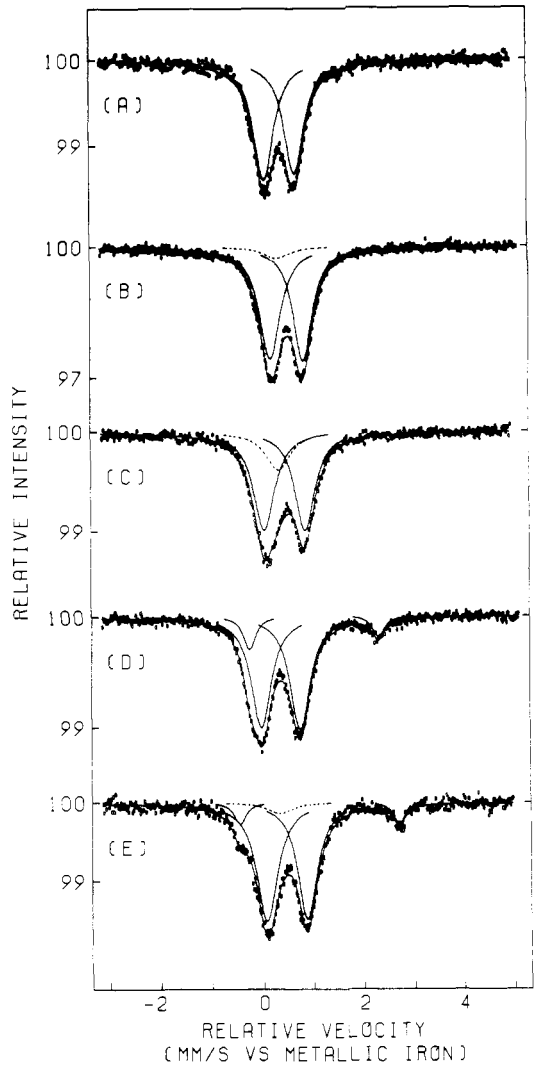

Figure 1. Mössbauer spectra of ^{57}Fe in (A) mature soybean hulls at room temperature, (B) mature soybean hulls at liquid nitrogen temperature, (C) immature soybean hulls at liquid nitrogen temperature, (D) mature cotyledons at room temperature, and (E) immature cotyledons at liquid nitrogen temperature. Dotted lines show background absorptions.

Table 1. The isomer shift of the ferric doublet for the cotyledons is essentially the same as that for the hulls within the experimental uncertainties.

Effect of Distilled Water on Hulls. In Mössbauer spectra of ^{57}Fe in hulls treated with distilled water no Mössbauer absorption was observed at room temperature after the hulls imbibed water. When they were subjected to measurement at liquid nitrogen temperature, they showed a spectrum consisting only of a doublet due to high-spin trivalent iron. The isomer shift is the same as that of the dry hulls measured at liquid nitrogen temperature, though the quadrupole splitting is a little smaller (Table 1).

Effect of Hydrochloric Acid on Hulls. Figure 2 shows Mössbauer spectra of the hulls treated with hydrochloric acid. Spectra A–E were decomposed into four peaks without any constraint and a background peak. In the fitting of spectrum F, it was necessary to fix the position of the ferric doublet because of low intensity. Therefore, the positions of the doublet were fixed to the values obtained for spectrum E. Since application of the similar fitting to spectrum G was unsuccessful, it was analyzed by assuming a doublet and singlet, the peak position and half-width of the latter being fixed to the values 0.33 and 0.61 mm/s, respectively.

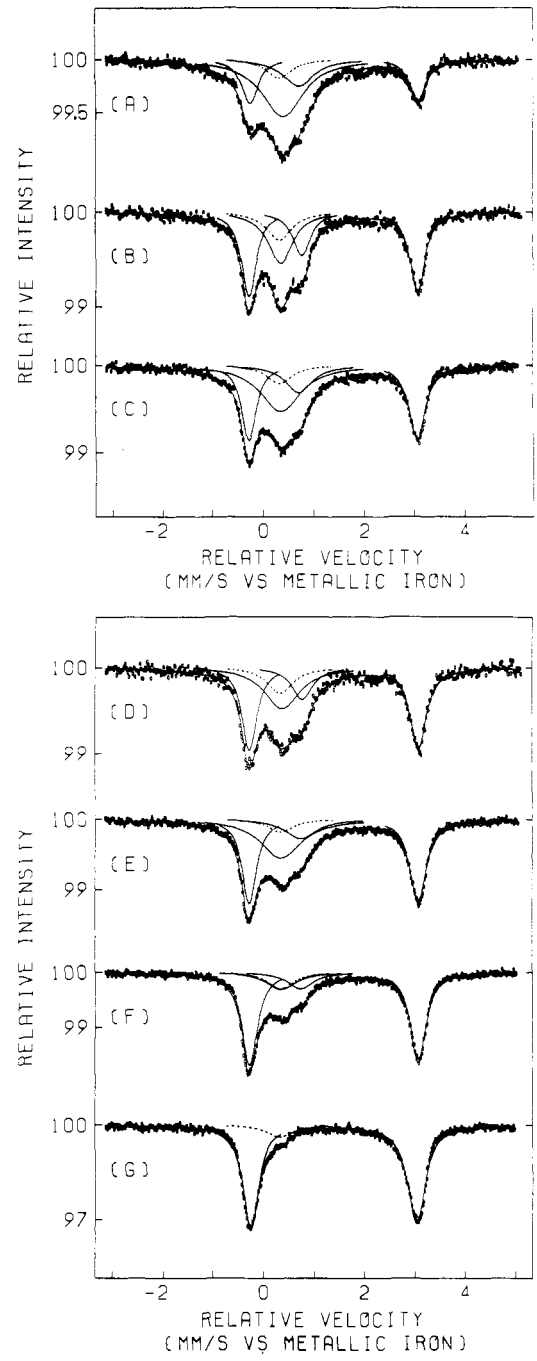

Figure 2. Mössbauer spectra of ^{57}Fe in mature soybean hulls treated with hydrochloric acid and stored in nitrogen gas at room temperature for (A) 0.3, (B) 1.3, (C) 2.0, (D) 3, (E) 6, (F) 14, and (G) 91 h (measurement at liquid nitrogen temperature). Dotted lines show background absorptions.

Although the effect of water is not significant at all, a drastic change in the spectrum was observed when the hulls were treated with 2 mol dm^{-3} hydrochloric acid: a doublet due to high-spin divalent iron appeared in the spectrum. The relative intensity of the doublet increased with the storage duration of the acid-treated samples in nitrogen gas atmosphere at room temperature. The small peak at the position of around 0.3 mm/s in Figure 2G has a depth corresponding to the background absorption, showing complete reduction of ferric ions. The relative areas of the ferric doublets are plotted against the storage time in Figure 3. This shows that the reduction of ferric to ferrous species proceeds very quickly. It is estimated by extrapolating the curve that reduction is completed within 2 days.

Table 1. Mössbauer Parameters of Soybean Samples and Related Iron Compounds

sample	temp (K)	isomer shift[a] (mm/s)	quadrupole splitting (mm/s)	rel area	assignment	half-width,[o] −/+ (mm/s)
mature hulls	RT[b]	0.39 ± 0.03	0.64 ± 0.03	100	Fe(III)	
mature hulls	LN[c]	0.51 ± 0.03	0.65 ± 0.03	100	Fe(III)	
immature hulls	LN	0.46 ± 0.05	0.82 ± 0.05	100	Fe(III)	
mature hulls + H_2O	LN	0.51 ± 0.03	0.56 ± 0.03	100	Fe(III)	
mature hulls + HCl	LN	0.54 ± 0.05	0.33 ± 0.05	70	Fe(III)	1.00/0.75
storage duration 0.3 h		1.40 ± 0.03	3.32 ± 0.03	30	Fe(II)	0.39/0.38
mature hulls + HCl	LN	0.56 ± 0.05	0.42 ± 0.05	45	Fe(III)	0.59/0.43
storage duration 1.3 h		1.39 ± 0.03	3.34 ± 0.03	55	Fe(II)	0.35/0.36
mature hulls + HCl	LN	0.53 ± 0.05	0.36 ± 0.05	55	Fe(III)	0.97/0.64
storage duration 2.0 h		1.39 ± 0.03	3.32 ± 0.03	45	Fe(II)	0.34/0.38
mature hulls + HCl	LN	0.56 ± 0.05	0.39 ± 0.05	45	Fe(III)	0.84/0.49
storage duration 3 h		1.39 ± 0.03	3.33 ± 0.03	55	Fe(II)	0.35/0.38
mature hulls + HCl	LN	0.53 ± 0.05	0.38 ± 0.05	45	Fe(III)	0.97/0.74
storage duration 6 h		1.39 ± 0.03	3.34 ± 0.03	55	Fe(II)	0.34/0.39
mature hulls + HCl	LN	0.53 ± 0.05	0.38 ± 0.05	25	Fe(III)	0.71/0.62
storage duration 14 h		1.39 ± 0.03	3.33 ± 0.03	75	Fe(II)	0.36/0.36
mature hulls + HCl	LN	1.38 ± 0.03	3.28 ± 0.03	100	Fe(II)	0.37/0.41
storage duration 91 h						
[mature hulls + HCl, 91 h] + air at 0 °C	LN	0.54 ± 0.05	0.48 ± 0.05	10	Fe(III)	0.74/0.21
		1.39 ± 0.05	3.28 ± 0.05	90	Fe(II)	0.39/0.39
[mature hulls + HCl, 91 h] + air at RT	LN	0.54 ± 0.05	0.48 ± 0.05	20	Fe(III)	0.74/0.21
		1.39 ± 0.05	3.30 ± 0.05	80	Fe(II)	0.36/0.36
mature hulls heated at 140 °C	LN	0.51 ± 0.03	0.59 ± 0.03	100	Fe(III)	
mature hulls heated at 140 °C + HCl	LN	1.38 ± 0.03	3.29 ± 0.03	100	Fe(II)	
mature hulls heated at 210 °C	LN	0.51 ± 0.05	0.68 ± 0.05	80	Fe(III)	
		1.32 ± 0.05	2.94 ± 0.05	20	Fe(II)	
mature cotyledons	RT	0.36 ± 0.03	0.79 ± 0.03	90	Fe(III)	
		1.06 ± 0.05	2.49 ± 0.05	10	Fe(II)	
immature cotyledons	LN	0.47 ± 0.03	0.81 ± 0.03	90	Fe(III)	
		1.12 ± 0.05	3.15 ± 0.05	10	Fe(II)	
mature cotyledons heated at 140 °C	LN	0.50 ± 0.05	0.72 ± 0.05	70	Fe(III)	
		1.27 ± 0.05	2.82 ± 0.05	30	Fe(II)	
mature cotyledons heated at 140 °C + HCl	LN	1.40 ± 0.03	3.28 ± 0.03	100	Fe(II)	
horse spleen ferritin[d]	80	0.50 ± 0.03	0.66 ± 0.05	25	Fe(III)	
		1.29 ± 0.03	2.78 ± 0.05	75	Fe(II)	
horse spleen ferritin[e]	293	0.35 ± 0.05[k]	0.68 ± 0.05		Fe(III)	
	86	0.45 ± 0.05[k]	0.70 ± 0.05		Fe(III)	
monoferric phytate[f]	RT	0.43 ± 0.03	0.51 ± 0.03		Fe(III)	
	LN	0.53 ± 0.03	0.62 ± 0.03		Fe(III)	
monoferric phytate[g]	80	0.51 ± 0.03[k]	0.55 ± 0.03		Fe(III)	
ferric citrate[h]	77	0.50 ± 0.03[k]	0.54 ± 0.05		Fe(III)	
ferrous citrate[h]	77	1.38 ± 0.05[k]	3.38 ± 0.05		Fe(II)	
$[Fe(H_2O)_6]^{2+}$ [i,j]	80	1.38	3.35		Fe(II)	
$[Fe(H_2O)_6]^{2+}$ [l,j]	77	1.31 ± 0.03[k]	3.25 ± 0.04		Fe(II)	
$FeCl_2 \cdot 4H_2O$[l]	77	1.31 ± 0.03[k]	3.08 ± 0.04		Fe(II)	
$[Fe(H_2O)_6]^{3+}$ [m,j]	77		~0		Fe(III)	
$[Fe(H_2O)_5Cl]^{2+}$ [m,j]	77	0.42 ± 0.04[k]	0.30		Fe(III)	
$FeCl_3 \cdot 6H_2O$[m]	77	0.46 ± 0.05[k]	0.85 ± 0.10		Fe(III)	
$FeCl_3 \cdot 6H_2O$[n]	78	0.60[k]	0.91		Fe(III)	

[a] Relative to metallic iron at room temperature. [b] Room temperature. [c] Liquid nitrogen temperature. [d] Frankel et al. (1987). [e] Fishbach et al. (1971). [f] Ambe et al. (1987). [g] May et al. (1980). [h] Baggio-Saitovitch et al. (1972). [i] Ruby et al. (1971). [j] Frozen solution. [k] Converted values relative to metallic iron. [l] Nozik and Kaplan (1967). [m] Nozik and Kaplan (1968). [n] Thrane and Trumpy (1970). [o] Left/right peaks of the doublet.

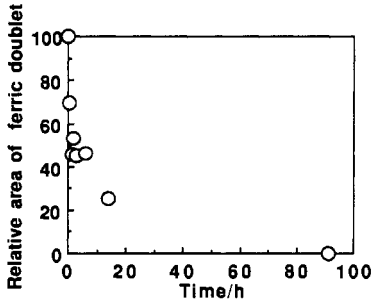

Figure 3. Relative area of ferric doublet in the soybean hulls against storage time in nitrogen gas after treatment with hydrochloric acid (measurement at liquid nitrogen temperature).

Addition of hydrochloric acid to the hulls also induced a slight increase in the isomer shift and a decrease in the quadrupole splitting of the ferric doublet, which was accompanied by transformation of the symmetrical doublet into an asymmetrical and broad one. The half-widths of the ferric doublet as well as those of the ferrous doublet are listed in Table 1. This observation suggests formation of new ferric species along with the original one in the untreated hulls. Both the ferric and ferrous doublets observed in the acid-treated hulls showed little change in the isomer shifts and quadrupole splittings throughout all stages of the reduction reaction. The isomer shifts and quadrupole splittings are 0.54 and 0.38 mm/s on the average for the ferric species and 1.39 and 3.33 mm/s for the ferrous one, respectively. The Mössbauer parameters of the ferric doublet are close to those for $[Fe(H_2O)_5Cl]^{2+}$ in a frozen solution (Nozik and Kaplan, 1968). The isomer shift and quadrupole splitting of the ferrous doublet correspond to those of ferrous ions coordinated octahedrally with water molecules (Ruby et al., 1987; Nozik and Kaplan, 1967). This suggests that the ferrous ion exists in water in the hulls but is not bound. This would be consistent with the results obtained by Laszlo that 2 mol dm^{-3} hydrochloric

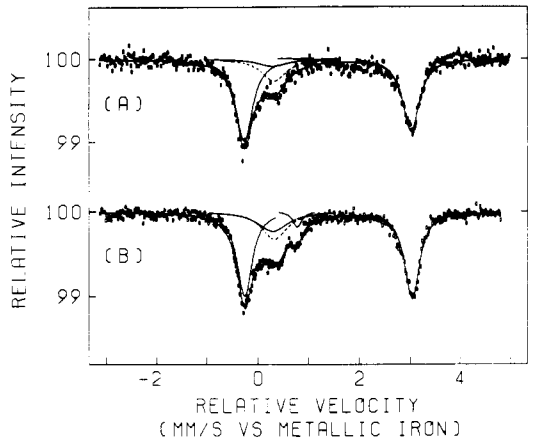

Figure 4. Oxidation of ferrous species formed in the acid-treated hulls (the sample of Figure 2G) by air after storage at (A) 0 °C for 17 days and (B) room temperature for 6 days (measurement at liquid nitrogen temperature). Dotted lines show background absorptions.

acid extracts almost all of the iron from the soybean hulls (Laszlo, 1988, 1991).

Oxidation of Ferrous Species in Acid-Treated Hulls by Air. Figure 4 shows the Mössbauer spectra of oxidation of ferrous ion in the acid-treated hulls by air. Spectrum B was analyzed in a similar way to spectra A–E in Figure 2, but spectrum A was fitted by fixing the positions and line widths of the ferric doublet to the values obtained for spectrum B. The hull sample whose ferric ions were completely reduced to ferrous ions by the acid treatment (the sample of Figure 2G) was stored in air at 0 °C for 17 days. A small absorption peak due to ferric ions was observed after this storage. The intensity of the ferric doublet increased in the sample further stored in air at room temperature for 6 days. These results show that oxidation of ferrous ions in the hulls is very slow compared with the reduction of ferric ions by hydrochloric acid. The ferric species produced by air oxidation showed the isomer shift and quadupole splitting close to those of ferric species in the hull treated with hydrochloric acid (Table 1).

Effect of Heating on Hulls and Cotyledons. The results described above were obtained for raw samples. Since soybeans are always taken as food after heat treatment, the effect of hydrochloric acid was also studied on heated soybean hulls and cotyledons. The hulls heated at 140 °C for 1 h showed a spectrum similar to that of the unheated hulls, consisting of a ferric doublet (Figure 5A).

The hulls heated at 140 °C were treated with 2 mol dm^{-3} hydrochloric acid. After 8 days of storage in nitrogen gas at room temperature, their Mössbauer spectrum was measured at liquid nitrogen temperature. The spectrum of the sample (Figure 5B) was quite similar to that of the unheated hulls treated with hydrochloric acid (Figure 2G), consisting of a doublet due to high-spin divalent iron and a background absorption. It is notable that reduction of ferric ions to ferrous ions by hydrochloric acid treatment was observed even for the heated sample. The hulls heated at 210 °C for 15 min in air gave a doublet due to newborn ferrous ions in addition to that of the original ferric ions (Figure 5C). This suggests that a reducing agent may have been released from the solid components and/or produced during the heating of the sample.

Mössbauer spectra of the mature cotyledons heated at 140 °C and then treated with 2 mol dm^{-3} hydrochloric acid are shown in Figure 6. In the spectrum of the cotyledons heated at 140 °C, a ferrous doublet appeared with Mössbauer parameters different from those of the

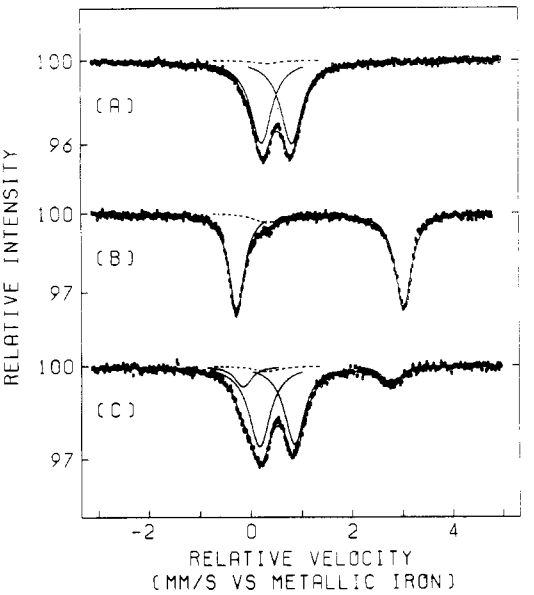

Figure 5. Mössbauer spectra of ^{57}Fe in mature soybean hulls (A) heated at 140 °C, (B) heated at 140 °C followed by addition of hydrochloric acid and storage in nitrogen gas at room temperature for 8 days, and (C) heated at 210 °C (measurement at liquid nitrogen temperature). Dotted lines show background absorptions.

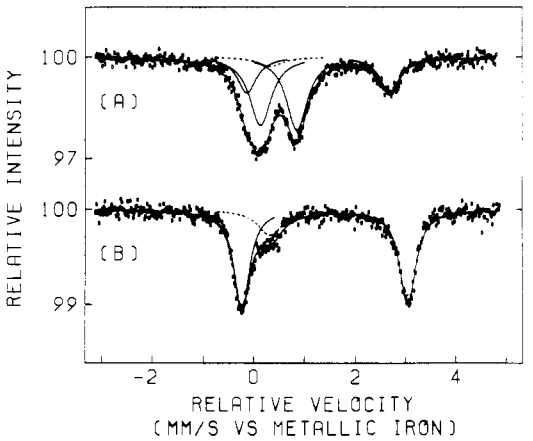

Figure 6. Mössbauer spectra of ^{57}Fe in mature soybean cotyledons (A) heated at 140 °C and (B) heated at 140 °C followed by addition of hydrochloric acid and storage in nitrogen gas at room temperature for 1.6 days (measurement at liquid nitrogen temperature). Dotted lines show background absorptions.

ferrous doublet of endogenous iron. The cotyledons heated at 140 °C followed by addition of hydrochloric acid and storage in nitrogen gas at room temperature for 1.6 days showed a ferrous doublet having essentially the same Mössbauer parameters as those of ferrous ions produced in the acid-treated hulls, suggesting formation of almost identical ferrous species in both hulls and cotyledons as a result of acid treatment. The small peak at the position of around 0.3 mm/s in Figure 6B has a depth comparable to that of the background absorption, suggesting complete reduction of ferric ions to ferrous ions.

DISCUSSION

The present work demonstrated that the endogenous iron in immature and mature soybean hulls is exclusively in a ferric state but that in immature and mature cotyledons both ferric and ferrous ions are present, the former being dominant. We previously reported that the relative area of Mössbauer absorption attributed to ferrous ions was much larger in immature whole seeds than in

ripe ones (Ambe et al., 1987). Because ferrous ions were found in immature seeds more than in mature ones, it was postulated that ferrous ions should be found in immature hulls in relation to their relatively high metabolic activities compared to those of mature ones. However, ferrous ions were not found in immature hulls.

The chemical forms of iron in soybean seeds are not yet known. Study on the distribution of the molecular weight of intrinsic iron compounds in soybean seeds showed that most of the extracted iron is in a protein fraction (Yoshida, 1989). A number of lipoxygenase isoenzymes containing iron were identified in developing soybean seeds (Funk et al., 1986). The content of iron in the enzymes was estimated from Funk's work to be 10^{-6}–10^{-7} g of Fe/g of seed (dry wt). The iron content is too low to give an appreciable Mössbauer absorption, even in the enzyme samples enriched in ^{57}Fe.

Ferritin, an iron storage protein, was isolated from seeds of lentil and soybean (Crichton et al., 1978; Van der Mark et al., 1983; Sczekan and Joshi, 1987). The iron storage protein consists of a shell of protein subunits surrounding a core of ferric hydroxyphosphate $(FeOOH)_8{\cdot}(FeOOPO_3H_2)$ (Crichton, 1973). Evidence for the reduction of ferric ions and their retention as ferrous ions in ferritin was obtained by means of Mössbauer spectroscopy (Watt et al., 1985; Frankel et al., 1987).

The isomer shifts of ferric doublets of the soybean cotyledons and hulls are in the range of the values for ferric ions octahedrally coordinated with six oxygen atoms. However, coordination of other elements such as chlorine atoms to iron is not ruled out. The isomer shift of the ferrous doublet observed for the cotyledons is lower than that for the ferrous ions coordinated with six oxygen atoms. The Mössbauer parameters of the observed ferric species are in good agreement with those of ferritin, but the parameters of the ferrous doublet are smaller than those of reduced ferritin. Ferric phytates (Ambe et al., 1987; May et al., 1980) and ferric citrate (Baggio-Saitovitch et al., 1972) also have values similar to those of the ferric species.

Disappearance of an absorption peak in the spectrum of hulls imbibing water at room temperature suggests that the ferric species is in solution. The isomer shift for the hulls imbibing water obtained at liquid nitrogen temperature is essentially the same as that for the dry hulls obtained at the same temperature, though the quadrupole splitting of the former is smaller than that of the latter. This indicates that imbibing of water causes little change in the chemical states of the iron but diminishes markedly the recoilless fraction at room temperature.

Heating of the hulls at 140 °C caused little change in the isomer shift, but a slight decrease in the quadrupole splitting was observed. However, heating of the hulls at 210 °C induced reduction of part of the ferric ions to ferrous ions (Figure 5C). The hulls heated at 210 °C were brown. Ferrous ions were also produced in the cotyledons by heating at 140 °C. A similar phenomenon was observed for green laver heated at 360 °C in air: carbonization of the sample accompanied an increase in the relative area of ferrous components in the Mössbauer spectra (Ambe et al., 1986). This suggests that reducing agents may be released from the solid components of the hulls by heating or produced in plant samples during thermal decomposition of their organic constituents.

The above finding that hydrochloric acid induced the reduction reaction of ferric ions in the hulls explains why ferrous ions were found in the hydrochloric acid extract from soybean hulls (Laszlo, 1988, 1991). It is estimated from Figure 3 that all of the ferric ions in the hulls would be reduced within 2 days under our experimental conditions. On the other hand, for oxidation of the ferrous ions in the hulls by exposure to air, a longer time was required compared to the reduction of ferric ions by addition of hydrochloric acid. This implies that an ample quantity of reducing agent is present in the hulls. Laszlo pointed out that acid extract from soybean hulls possesses the capacity to reduce not only endogenous ferric ions but also added ones (Laszlo, 1988, 1991).

In general, enzymes mediate catalytic reactions under mild conditions and their activities are irreversibly lost at high temperatures. The fact that reduction of ferric ions after acid treatment was observed for not only raw hulls but also hulls and cotyledons heated at 140 °C excludes the possibility of enzymes as reducing agents in the observed reaction. It is considered that chemical reducing agents are involved in the reduction of ferric species. In tissues the reaction of the reductants with iron is hindered since the iron is tightly bonded with ligands.

The effect of the reducing power of food on iron absorption has been demonstrated (Forth and Rummel, 1973). Plant tissues contain reductants such as ascorbic acid. Mature soybean seeds contain 200–700 ppm of ascorbic acid (Reddy and Kumari, 1988; Islam and Lea, 1979), though the content of the ascorbic acid varies depending on varieties and stages of maturity of soybeans. The content is nearly equivalent in molar ratio to that of iron in the seed. Kapsokefalou and Miller (1991) found that ascorbic acid, glutathione, and cysteine have the capacity to reduce nonheme ferric ions to ferrous forms during in-vitro simulated gastrointestinal digestion. This is in fairly good agreement with the experimental results obtained in our present work.

Treatment of hulls with hydrochloric acid gave rise to a change in the isomer shift and transformation from a symmetric to an asymmetrical ferric doublet followed by broadening of the doublet, which probably reflect rearrangement of ligands of the iron, that is, dissociation of organic ligands followed by hydration. The resultant ferric ions are considered to be attacked by reductants. A possible mechanism of formation for ferrous ions is

$$\text{Fe(III)–ligands} \xrightarrow[\text{dissociation}]{\text{HCl}} [Fe(OH_2)_nCl_{6-n}]^{(n-3)+} + \text{free ligands}$$

$$[Fe(OH_2)_nCl_{6-n}]^{(n-3)+} + \text{reductant} \rightarrow [Fe(OH_2)_nCl_{6-n}]^{(n-4)+}$$

where n is estimated to be 5 for the hydrated ferric species from the Mössbauer parameters. However, the possibility of coexistence of other species with $n = 4$ and 6 cannot be ruled out because of the line broadening. The isomer shifts for the hydrated ferric species with $n = 4$–6 are reported to be constant, while the quadrupole splittings decrease with n values (Nozik and Kaplan, 1968). The Mössbauer parameters suggest $n = 6$ for the hydrated ferrous species. No data on Mössbauer parameters for hydrated ferrous ion with $n = 5$ are available so far. Similar chemical reactions are expected to occur in the stomach, where gastric acid is secreted, as was demonstrated by an in-vitro experiment (Kapsokefalou and Miller, 1991).

Mössbauer studies demonstrated that most of the iron in plants is in the ferric state (Ambe, 1989, 1990; Ambe et al., 1985, 1987; Goodman and DeKock, 1982; Goodman et al., 1982; May et al., 1980), though the number of studies on plant samples is still limited. The bioavailability of

iron in soybean hulls is high and equivalent to that of ferrous sulfate (Johnson et al., 1985). By contrast, iron in the soybean seeds or iron administered with soy protein isolates shows low absorption (Fairweather-Tait and Wright, 1985; Migita, 1988; Hurrell et al., 1992). The difference in the bioavailability of iron between the hulls and cotyledons indicates that, in addition to the reducing capability found in the present work, another factor operates in the bioavailability of iron, since hulls and cotyledons showed almost equivalent reducing capability after treatment with hydrochloric acid in this work. It is reported that ascorbic acid plays an important role in the bioavailability of iron, not only as a reducing agent but also as a chelating agent (Rao and Prabhavathi, 1978; Rao and Rao, 1992; Migita, 1988). Phytic acid combines with solubilized iron, yielding insoluble iron phytate. Cotyledons contain phytic acid, while hulls do not (Weaver et al., 1984; Laszlo, 1988). Iron absorption increases upon reduction of phytate content in diet (Mugula, 1992; Hurrell et al., 1992).

Bioavailability of iron in soybean seeds is considered to have a close relation with formation of ferrous ions, found in the present work, under an acid condition comparable to gastric juice from the stomach.

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