Table III. Fatty Acid Analysis of Neutral and Polar Lipid Fractions of SC and SM[a]

	Neutral lipids		Polar lipids	
	SC	SM	SC	SM
12:0[b]	tr	tr	1.0	1.0
14:0	2.7	1.1	1.0	1.2
15:0	0.3	tr	tr	tr
i-16:0	tr	tr	2.2	3.8
16:0	48.7	43.8	25.9	27.7
16:1	1.9	0.3	1.1	0.7
17:0	tr	0.4	0.3	0.8
i-18:0	tr	tr	1.5	0.9
18:0	16.0	13.9	12.4	12.5
18:1	14.9	16.4	15.3	15.2
18:2	5.3	13.0	4.4	3.5
20:0	0.6	1.2	2.3	1.0
20:4	1.3	3.2	13.4	13.2
22:0	0.5	tr	12.3	12.2
23:0	1.1	0.3	1.5	0.8
24:0	1.3	1.3	4.8	1.9
26:0	tr	tr	1.0	0.4

[a] Percent of total fatty acid. [b] Shorthand designation of fatty acids; carbon chain length:number of double bonds; tr = trace, less than 0.2%.

The patterns of fatty acid compositions of polar lipid fractions of SC and SM were similar. Palmitic, stearic, oleic, eicosanoic, docosanoic, tetracosanoic, and arachidonic acids were present in the highest concentrations. Peaks for unknown fatty acids were minor quantities as illustrated in Figure 5.

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Localization of Iron in *Vigna sinensis* L. and *Zea mays* L.

Jacqueline W. Jacobs*[1] and Richard B. Walker

With the aid of histo- and cytochemical examinations, and elemental x-ray analyses of maize, *Zea mays* L. var. Idahybrid 216, iron was found to be highly concentrated in the outer cell layers of the scutellum and in the aleurone layer. It is associated with roughly spherical structures identified as protein bodies. Analyses of the blackeyed pea, *Vigna sinensis* L., seeds indicate that iron is distributed throughout the cotyledon; however, the most intense concentration is at the periphery where the protein bodies are quite numerous. The histo- and cytochemical tests were made with the aid of the Prussian Blue reaction (acidified potassium ferrocyanide) and the Ferrozine reagent. The x-ray analyses were made with the aid of the elemental x-ray analysis method (EXAM) using a Model 707A energy dispersive x-ray analyzer(EDAX), and a scanning electron microscope (SEM).

The study of iron absorption and incorporation into food plants has in itself intrinsic interest, and as well can contribute substantially in the area of iron nutrition of man and animals. The plant may serve as the link between soil (the source) and man (the consumer).

Some studies by Layrisse (1970) and Layrisse et al. (1969) have suggested that iron from animal sources is more available than iron from vegetable sources. When values of food iron absorption were compared to those of absorption from iron salts, the iron from foods proved to be less well absorbed.

The literature contains few data on the localization and/or form(s) of iron in seeds and grain. This problem has seldom been studied, even though knowledge concerning the storage forms of iron in seeds and grain should help to explain the difficulties which the human organism encounters in utilizing iron from nutritional products derived from plants.

Therefore, this study deals primarily with locating the highly concentrated iron sites in seeds and grain. This knowledge should aid in future studies dealing with identification and isolation or iron-containing compounds.

BACKGROUND

Protein bodies, or aleurone grains, were discovered by Hartig (1865) and have since then been studied in great detail by other investigators. There is general agreement that protein bodies occur widely in the cotyledons and endosperm of both starch-bearing and oil-bearing seeds, that some of the protein bodies contain crystalline inclusions of inorganic salts, that they are probably surrounded by membranes, that they contain most of the cellular protein but none of the oil, that their formation commences during the later stages of ripening of the seed

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Table I. Culture Solution Nutrient Levels

Salt	Solution A[a] 1.0 mM P	Solution B[a] 0.2 mM P
$NH_4H_2PO_4$	1.0 mM	0.2 mM
KNO_3	6.0 mM	6.0 mM
$Ca(NO_3)_2$	4.0 mM	4.0 mM
$MgSO_4$	2.0 mM	2.0 mM
NaCl	0.2 mM	0.2 mM
NH_4Cl		0.8 mM
A-5[b]	1.0 mL/L	1.0 mL/L

[a] Modified in this laboratory from solutions of D. R. Hoagland and D. I. Aron (California Experiment Station Circular No. 347). [b] A-5 micronutrient supplement; use 1.0 mL of the following stock solution for each liter of nutrient solution: mg/L of Fe (in NaFeEDTA), 5.0; Mn, 0.5; Zn, 0.05; B, 0.5; Cu, 0.02; and Mo, 0.05.

and that they swell, coalesce, and disappear early in germination. Since these early observations, many other investigators have studied protein bodies and there has been a recent resurgence of interest in them.

Jacobson et al. (1971) described spherical bodies in barley aleurone as protein–carbohydrate bodies. In 1972 he revealed that most of the iron in the aleuron layer of barley is associated with the protein–carbohydrate bodies and that only membrane "bound" iron could be observed.

Varner and Schidlovsky (1963) reported that in addition to all the structures normally present in plant cells their examination of the pea cotyledon by electron microscopy revealed that a major fraction of the cell volume was occupied by relatively large roughly spherical bodies, with no internal structure. The presence of similar protein bodies in peanut cotyledons has also been reported by Dieckert et al. (1962). Hyde et al. (1963) observed that the peripheral cells of pea cotyledons contain relatively little stored starch.

MATERIALS AND METHODS

Growing and Harvesting Plants. All of the plant material used in this study was grown in the greenhouse in solution cultures or soil. Field maize, *Zea mays* L. var. Idahybrid 216 (Crookham Co., Caldwell, Idaho), and blackeyed peas, *Vigna sinensis* L. (Giles Brothers, Montgomery, Ala.), were used.

The seeds were germinated in greenhouse flats containing washed silica sand. All seeds were soaked in distilled water for 1 h before planting. On the 8th day after sowing, the seedlings were transferred to either 23-L stainless steel tanks or 2-qt or 1-gal Mason jars. The tanks and covers had been coated with two coats of black asphaltum varnish. The jars were acid washed before use to remove any extractable Fe in them.

The composition of the experimental nutrient solution used is listed in Table I. Usually, stock solutions were made up ten times stronger than listed in the table and were diluted with distilled water at the time of use. Reagent grade chemicals were used throughout. Fe was added to the diluted solution as ethylenediaminetetraacetic acid (EDTA) ferric complex at a level of 2.5 ppm of Fe.

A special lot of seeds was grown under three different regimes: field-grown Idahybrid 216 (grown in Idaho), greenhouse (hydroponic) grown Idahybrid 216 with iron-59 present throughout the growing period, and greenhouse (hydroponic) grown Idahybrid 216 with iron-59 present only from flowering onward.

Iron Analyses. Total Fe was determined photometrically with Ferrozine according to a modification of the method of Stookey (1970). The Ferrozine reagent (purchased from the Hach Chemical Co., Ames, Iowa) provides a quick and efficient method for assaying iron levels of solution or tissue digests. The Ferrozine iron colored complex (magenta) forms rapidly (within 10 min) throughout a broad pH range (4.0–10.0) and is stable for days. The amount of iron was determined by measuring the absorbance of the colored solution at 562 nm on a Gilford-2400 spectrophometer against a reagent blank and comparing these absorbance values with a standard curve.

Histo- and Cytochemical Tests. *Tissue Preparation.* The dry seeds and grains were prepared for staining and/or radioautography by one of several methods: (1) by chemically fixing it (Carnoy's fluid) and embedding it in paraffin, using a modification of the method of Johansen (1962), (2) by soaking the material in 70% EtOH from 3 to 24 h, or (3) by soaking material in distilled water from 3 to 24 h. The specimens were prepared for SEM by prefixing the material in distilled water with a mixture of glutaraldehyde, paraformaldehyde, and acrolein, following this with a postfixation in OsO_4, and embedding the material in araldite plastic according to a modification of the method of Mollenhauer and Totten (1971).

Only the germ and section of the endosperm containing the aleurone layer of maize were used. The grain was soaked in distilled water just enough to soften it so the germ could be manually separated from the seed coat. The blackeyed peas were also soaked in distilled water long enough to allow removal of the seed coat.

Sections were cut with a razor blade and a Porter-Blum Model MT-1 microtome.

Staining. The Prussian Blue test was used for ferric iron, and the formation of Turnbull's Blue for ferrous iron as described by Glick (1949). Sections prepared as described under tissue preparation were treated with the conversion reagent for 24–36 h at 35 °C and then washed in 90% alcohol followed by distilled water and placed in the organic reagent for 5 min. Sections were examined for inorganic iron by preparing them as described earlier and placing them in Prussian or Turnbull's Blue reagent for 3–15 min. They were counterstained with 1% neutral red. Sections were also stained with an aqueous solution of Ferrozine reagent.

Elemental X-Ray Analysis of Materials. Sections of the dried material were analyzed using a Model 707A energy dispersive x-ray analyzer (EDAX) and a scanning electron microscope (SEM). These analyses were made at the Material Analysis Center at the University of Washington.

This analyzer can detect all elements above the atomic number 8 in the periodic table, in amounts ranging down to less than 1 ppm in solids, liquids, and powders. It is nondestructive and gives results directly in elemental composition. It has an efficiency of 100% within the x-ray energy range of 5–15 keV.

RESULTS

Localization of Iron-Containing Tissue. *Experiments with Maize: Histo- and Cytochemical Examinations.* Localization of high concentrations with both the Ferrozine reagent and the Prussian Blue reaction procedures was done with chemically fixed maize (Carnoy's fluid), sections of water-soaked grain, and sections of grain embedded in araldite.

Both of these staining procedures demonstrated that iron was concentrated in the outer region of the scutellum and the aleurone.

Plants grown by each of the three culture methods all showed a concentration of iron in the outer region of the scutellum and in the aleurone layer. The reaction was more intense in the greenhouse-grown material, presumably because of higher Fe levels.

Table II. Iron Contents of Maize Germ and Endosperm

Treatment	Fe, μg/g	
	Germ	Endosperm
^{59}Fe vegetative and flowering periods; 1.0 mM level maintained	97.9	21.7
^{59}Fe flowering period only; P changed from 1.0 to 0.2 mM	107.4	20.7
^{59}Fe vegetative and flowering periods; P changed from 1.0 to 0.2 mM		
^{59}Fe flowering period only; 1.0 mM P level maintained	69.7	19.2
No ^{59}Fe; P changed from 1.0 to 0.2 mM	84.2	24.7
^{59}Fe injected in stem; seedlings were grown in soil	48.2	19.4

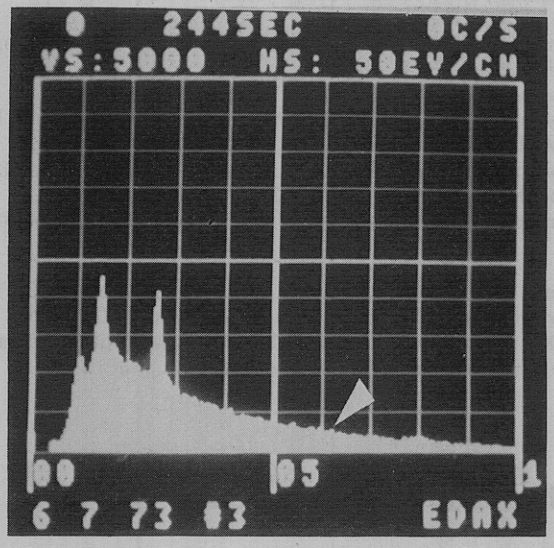

Figure 1. EDAX control. Key for identification of elements. Each vertical line represents 1 keV: Mg = 1.25, Na = 1.04, P = 2.01, Cl = 2.62, Pd = 2.84, Ca = 3.69, Al = 1.50, Si = 1.74, S = 2.31, Au = 2.14, K = 3.31, and, *Fe = 6.40 (arrow) keV.

To further verify the localization of iron in the maize kernel, the kernel was separated into germ and endosperm. Comparisons of iron in germ and endosperm are shown in Table II.

It was necessary to use water in the histochemical analyses which might have caused some transfer of Fe; therefore, dried materials were used in another technique to show localization of iron in the maize kernel. Analyses of the dried material were made using the elemental x-ray analysis of materials method (EXAM). The elements present are identified by peaks along the vertical axis of the analyzer (EDAX). Each of the vertical lines represents 1 keV as shown in Figure 1. Figure 2 shows the results of the analyses of maize germ.

Experiments with Blackeyed Peas. Histochemical staining by means of the Prussian Blue reaction was used to indicate possible sites of iron concentration. The entire cotyledon showed a positive reaction of iron; however, the color was more intense at the periphery.

An EDAX analysis of the blackeyed pea revealed the data as shown in Figures 3 and 4. Figure 3 of the blackeyed pea cotyledon shows a significant Fe peak. The analysis was made at the periphery of the cotyledon. Figure 4 shows a highly magnified section of the peripheral area of the cotyledon, where protein bodies can be observed.

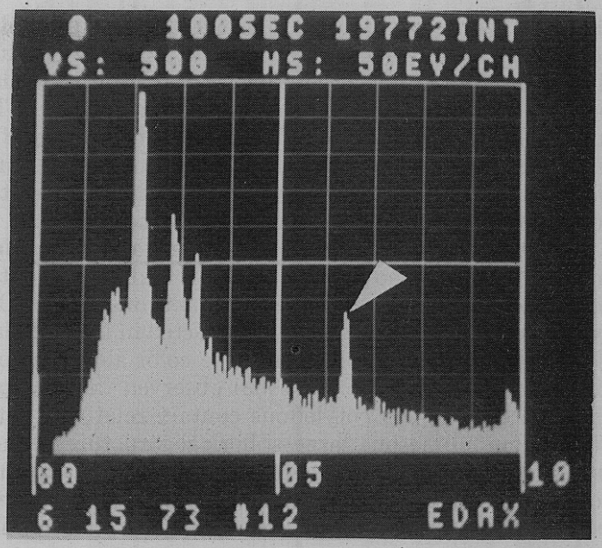

Figure 2. EDAX of maize germ. Fe (6.4 keV) shows a significant peak.

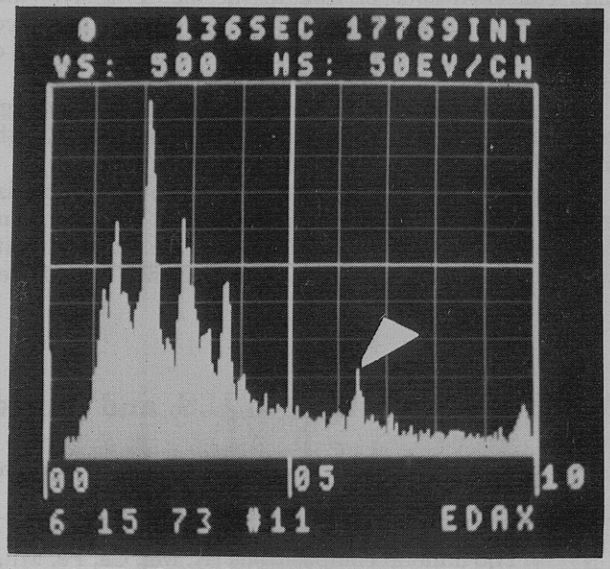

Figure 3. EDAX of a blackeyed pea cotyledon. Fe peak is quite significant.

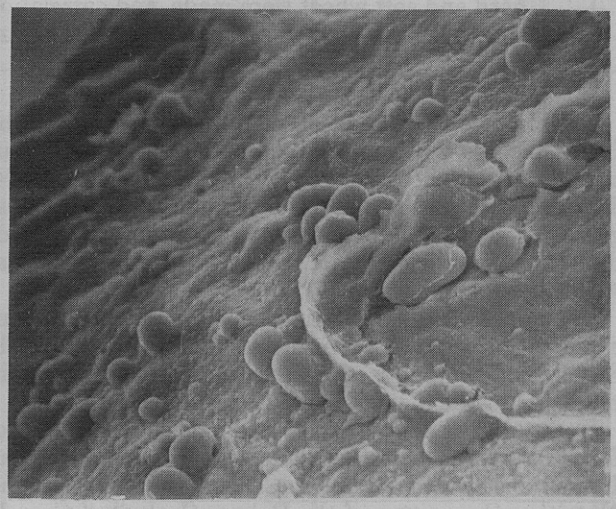

Figure 4. SEM of a small section of the blackeyed pea cotyledon showing large spherical protein bodies.

SUMMARY

The ultimate objective of this study is to chemically characterize the form of iron in seeds and grain. Knowing

that iron is or is not associated with a specific subcellular component or biochemical is valuable, not only because it tells us something about the form of iron, but also because it will facilitate future isolation efforts. The investigator can then use appropriate techniques for isolation of either organelles, particles, or compounds, and perhaps more importantly it will enable the investigator to maintain the materials' integrity during isolation.

The maize sections showed a positive staining reaction to the Prussian Blue test for ferric iron along the outer region of the scutellum and in the aleurone layer. Cotyledons of the blackeyed peas also reacted in the Prussian Blue test, showing the most intense color along the peripheral area. Hyde et al. (1963) observed that the peripheral cells of pea cotyledons contain relatively little starch, but numerous large spherical structures which probably represent stored proteins. He also noted that toward the center of the cotyledon the number of protein bodies per cell decreases, while the number of starch grains increases.

The histo- and cytochemical examinations indicating the high concentrations of iron in the aleurone layer and scutellum of maize as well as at the periphery of the cotyledon of blackeyed peas were confirmed with the aid of the x-ray analysis (EDAX and SEM).

The maize germ analysis gives evidence of a high concentration of Fe. Maize endosperm shows only a slight indication of the presence of Fe.

Further study has been done to chemically characterize iron in seeds and grains. This work dealt with the iron–protein complex, phytoferritin, and the iron–phytate complex, ferric phytate. These findings will be reported in a future paper.

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Mineral Composition of U.S. and Canadian Wheats and Wheat Blends

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The mineral compositions (K, Mg, Ca, Fe, Mn, Zn, Na, and Cu) of 65 samples of hard and soft wheats, collected from mills in the U.S. and Canada after the 1975 harvest, were determined. Average values of each mineral in the different wheat classes are presented. Comparisons in mineral content between hard and soft wheat were made, and correlation coefficients were calculated between percent protein and percent ash in wheat, and each of the mineral elements determined.

An expansion of the cereal fortification program in the United States has been proposed by the Food and Nutrition Board of the National Academy of Sciences in 1974. The proposal suggests additional fortification of cereal grain products with nutrients for which there is a risk of deficiency within certain population groups (National Academy of Sciences, 1974).

Canada is also considering an expanded cereal enrichment program after results of the Nutrition Canada Survey indicated less than adequate levels of certain nutrients in the diets of some Canadians.

To study the feasibility of expanded cereal product fortification, the American Bakers Association formed an Inter-Industry Committee. In Canada, the Technical and Nutrition Committee of the Bakery Council of Canada accepted the task (Ranum and Kulp, 1976).

Before some definite recommendations for changes in cereal product fortification can be made, however, it was felt that analysis of the nutrient composition of wheat varieties, grown presently in different parts of the U.S. and Canada, and their milling fractions are needed. It is realized that the mineral composition of various wheat varieties has been reported by many cereal researchers. However, with the introduction of new wheat varieties and modifications in agronomic practices, changes in mineral composition are possible.

This paper presents the mineral composition of hard and soft wheats from different areas of the U.S. and Canada and of wheat mixes blended by various mills for milling into flours for specific bakery applications.

MATERIALS AND METHODS

a. Sample Identification and Chemical Analyses. Sixty-five wheats and wheat mixes were collected after the 1975 harvest by representatives of the Pennwalt Corporation and the Research Products Company. The number

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