Distribution of Elements in the Rice Kernel Determined by X-Ray Analysis and Atomic Absorption Spectroscopy[1]

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Cereal Chem. 58(2):148-152

ABSTRACT

Elemental composition of rice cultivars Caloro and S6 were determined by atomic absorption spectroscopy for hand-dissected kernel fractions. Elemental composition was also conducted by the combined techniques of scanning electron microscopy and X-ray analysis on selected tissues of Caloro rice. The rice husk was rich in total ash, had large amounts of Si and small amounts of P, and was relatively high in K, Ca, Mn, and Fe and relatively low in Mg, Zn, and Cu. The aleurone-pericarp fraction was relatively rich in protein and most mineral components, including P, K, Mg, Zn, Fe, and Mn. The aleurone and germ contained a large amount of K, Mg, and P but relatively little Ca. The major mineral components of the low-ash starchy endosperm were P and K.

Knowledge of the distribution of mineral components in cereal grains at the cellular level is of interest for several reasons. It contributes to a better understanding of the physiological roles of mineral elements in quiescent and germinated grains; it provides information about the composition and nutritive values of various grain tissues; and it can be used as basis for the development of assays to evaluate changes resulting from processing, ie, effectiveness and degree of milling. In the last 25 years, atomic absorption spectroscopy (AAS) has become a powerful and convenient method to quantitatively determine mineral elements in whole or ground cereals, in milled fractions, and in hand-separated sections or tissues. The main limitation of AAS in mineral distribution studies is that cereal grains cannot be easily separated into morphological and botanical entities. The combined techniques of scanning electron microscopy (SEM) and X-ray microanalysis can allow for the precise location of individual mineral components, especially as they occur in biological tissues, and can help in the interpretation of mineral assays obtained by AAS.

The use of AAS and SEM in combination with X-ray microanalysis in studies of barley, malt, and malt sprouts has been the subject of several publications (Liu et al 1974; Liu and Pomeranz 1975a, 1975b, 1976a, 1976b; Pomeranz 1973).

Numerous studies of rice kernel structure have been made with light microscopy (del Rosario et al 1968, Little and Dawson 1960, Mitsuda et al 1969), transmission electron microscopy (Bechtel and Pomeranz 1977, 1978a, 1978b, 1980), and SEM (Ando and Ichikawa 1974, Evers and Juliano 1976, Maeda 1972, Watson and Dikeman 1977, Watson et al 1975). Several reports have concerned studies of mineral components determined chemically and/or instrumentally, ie, by AAS (Houston 1972, Kennedy and Schelstraete 1975, Kim and Cheigh 1979, McCall et al 1953, Primo et al 1970, Resurreccion et al 1979, Wildman and Brandon 1969). Several studies of SEM X-ray microanalyses have been conducted on the rice caryopsis. Tanaka et al (1974a, 1976, 1977) analyzed and compared the scutellum to the aleurone layer. Ogawa et al (1979a, 1979b, 1979c) used X-ray analysis to observe changes in the composition of globoids in aleurone particles of developing rice grains. Gross and mineral (P, K, Mg, Ca) composition was confirmed by analyses of particles isolated from rice scutellum (Ogawa et al 1977). The chemical composition of electron-dense inclusions was established by energy dispersive X-ray analysis (EDXA), indicating that the inclusions in the rice scutellum were deposits of magnesium and potassium salts of phytic acid (Tanaka et al 1977).

Ogawa et al (1975) isolated particles containing high levels of phytin from rice bran and used a combination of AAS and SEM to study the mineral content of the particles. Ogawa et al (1979a) confirmed by EDXA of phytin globoids in aleurone particles of

[1]Cooperative investigations, Agricultural Research Service, U.S. Department of Agriculture, and Department of Grain Science and Industry, Kansas State University. Contribution No. 925-J, Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Manhattan 66506.

Mention of firm names or trade products does not constitute endorsement by the USDA over others of a similar nature not mentioned.

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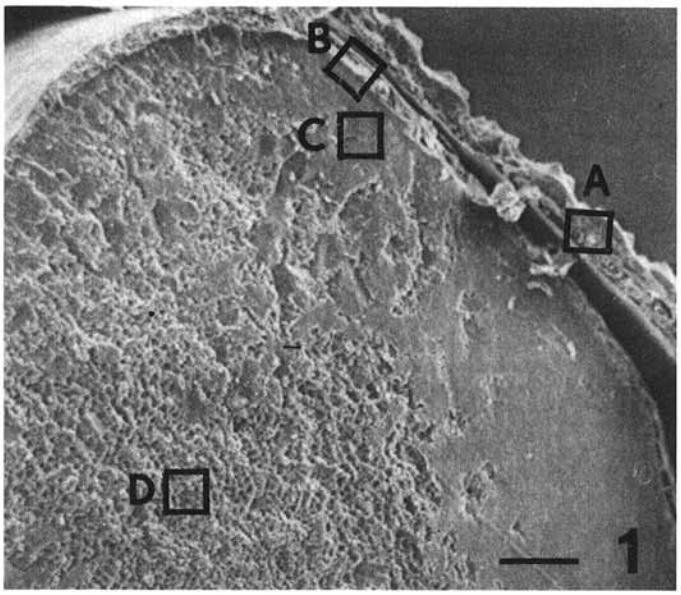

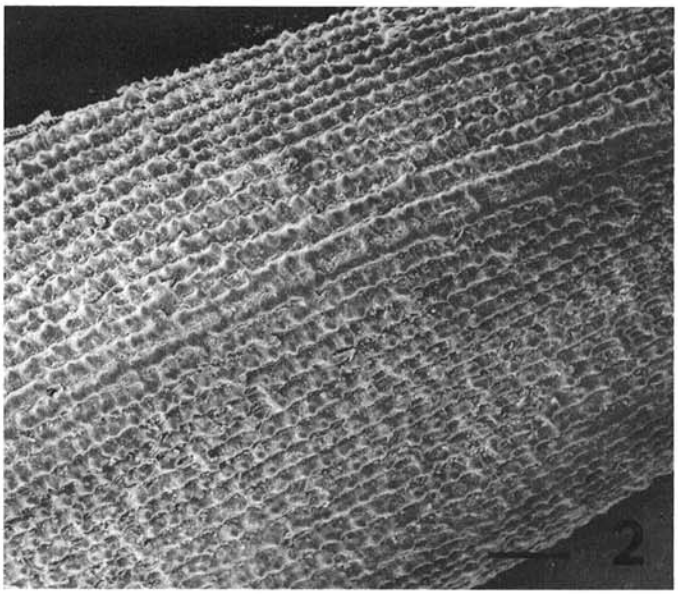

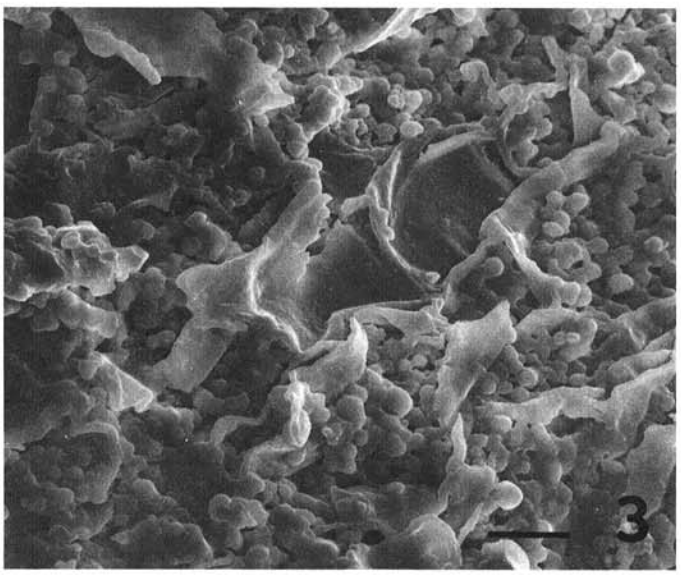

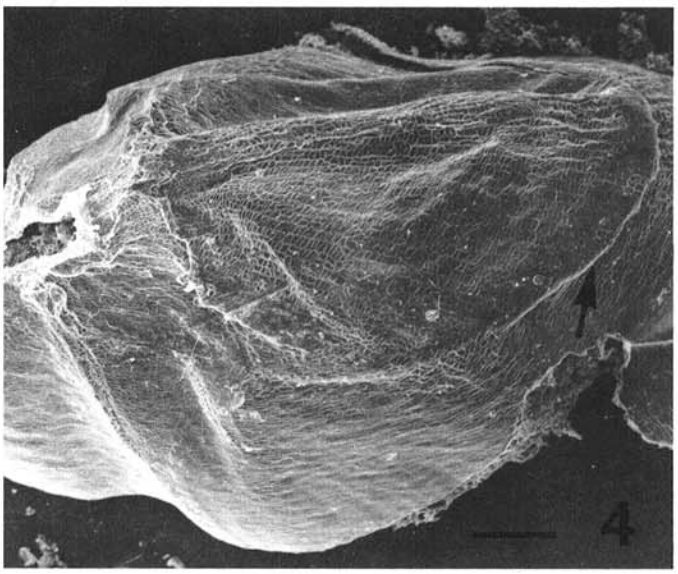

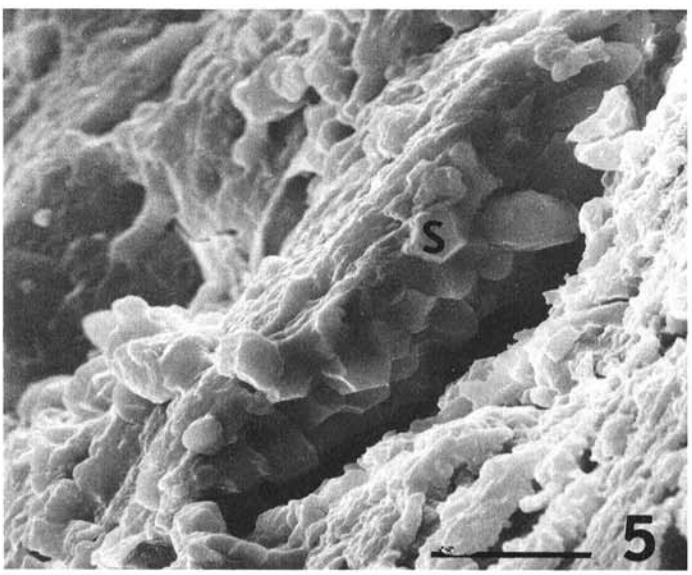

Fig. 1. Scanning electron microscopy of a cross section through a rice kernel showing areas scanned for enery dispersive X-ray analysis. Scale bar = 100 μm. **A** = Inner husk tissues, **B** = aleurone and pericarp, **C** = subaleurone, **D** = starchy endosperm. **Fig. 2.** Scanning electron microscopy of the outer dentate surface of the husk. Scale bar = 100 μm. **Fig. 3.** Scanning electron microscopy of the dissected aleurone tissue showing aleurone grains. Scale bar = 10 μm. **Fig. 4.** Scanning electron microscopy of the exterior of a rice germ removed from kernel. Scale bar = 100 μm. Arrow points to ridge, which indicates area of attachment of the embryo to the scutellum. **Fig. 5.** Scanning electron microscopy of the surface of a scraped rice kernel showing compound starch granule(s) of the endosperm. Scale bar = 10 μm.

developing rice that the accumulation of P, Mg, and K in rice grains was closely related to the formation of phytin globoids. P and Mg began to concentrate in the aleurone layer about the 12th day after flowering (Ogawa et al 1979b). The accumulation and distribution patterns of P and Mg were similar. In contrast, K was not concentrated in the aleurone layer till the 19th day and was present in relatively high concentrations in the starchy endosperm. A subsequent study (Ogawa et al 1979c) showed that biosynthesis of phytic acid in the developing rice grain occurred in the aleurone layer.

We determined the mineral elements in rice tissues as part of an ongoing program for characterization, processing, and grading milled rice.

MATERIALS AND METHODS

Rice and Rice Fractions

Two short grain rice cultivars, Caloro and S6, were dehusked by hand and then degermed with a stainless steel blade. The dehusked degermed kernels were scraped with a stainless steel blade under a dissecting microscope until the pericarp and aleurone layers were removed. The rice kernels were then rubbed with a rough cloth, which removed any remaining aleurone material. Four fractions of the dissected rice kernels were obtained: the husks, germ, pericarp-aleurone, and endosperm. Composition of the kernels by weight is given in Table I. Approximately 1,000 kernels of each variety were dissected to yield enough material for analysis. Whole kernels were ground on a Krup coffee mill, and all fractions used for AAS were dried over phosphorous pentoxide for four days. Only cultivar Caloro was used for SEM scanning.

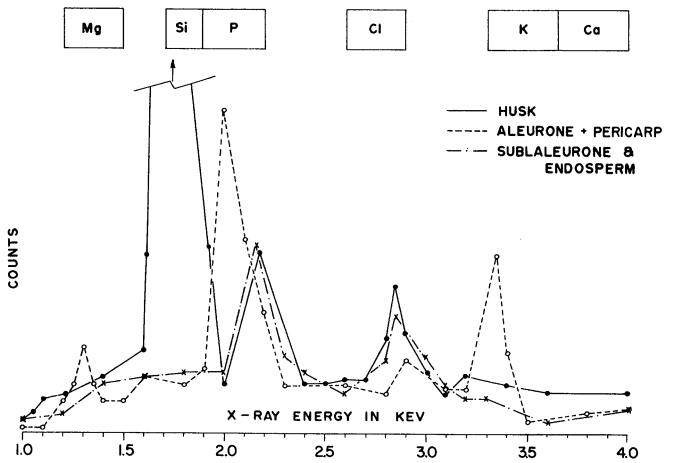

Fig. 6. Energy dispersive analysis of X-rays of microregions (A–D in Fig. 1) of husk, aleurone, and pericarp, and starchy endosperm in rice. Areas magnified 11,000 times and identified by scanning electron microscopy were scanned for 60 sec at 20 kV. Areas under peaks represent counts per minute. Elements in relatively high concentrations are identified.

Microscopy and X-Ray Analysis

For X-ray analysis, transverse cross sections, about 2 mm thick, were cut by a razor blade from about the midpoint of a kernel. The sections were mounted on specimen stubs and coated with 10 nm of carbon and 20 nm of gold. A JEOL-SEM 35 scanning electron microscope coupled with a Kevex X-ray analyzer was employed for EDXA. Selected areas were magnified 11,000 times and scanned for 60 sec at 20 kV for the highest possible elemental resolution.

In addition, an ETEC Autoscan scanning electron microscope operated at 5 kV was used to characterize structures of hand-separated fractions to be analyzed by AAS.

Atomic Absorption Spectroscopy Analysis

A Perkin-Elmer model 306 instrument was used for AAS measurements. Controls, standards, and operating parameters used were those recommended by the manufacturer. An air-acetylene flame was employed for analysis of K, Mg, Fe, Zn, Mn, and Cu, and an acetylene-nitrous oxide flame was used for analysis of Ca. Distilled, deionized water was aspirated between each sample, and standards and blanks were aspirated after every 7–10 samples. Sample size varied with tissue to give optimum elemental determination. All samples were analyzed in duplicate, and two 10-sec integrated readings were recorded for each duplicate. Solutions of 1,000 ppm Na and 1,500 ppm La, respectively, were used to dilute samples for K and Ca determinations.

Microash and micronitrogen determinations were conducted (AOAC 1970). Samples for mineral analyses were wet-ashed in nitric, perchloric, and sulfuric acids by the procedure of Liu et al (1974) adapted to a microwave oven. Phosphorus was analyzed colorimetrically by the molybdenum phosphate blue procedure (AOAC 1970), and absorbance was read at 625 nm on a Bausch and Lomb Spectronic 20 spectrophotometer.

RESULTS AND DISCUSSION

Description of Hand-Dissected Fractions

X-ray microanalysis in the scanning electron microscope was conducted on a cross-fractured kernel of Caloro (Fig. 1). The kernel, surrounded by a husk, included the pericarp and aleurone covering the subaleurone endosperm and starchy endosperm.

Hand-dissected fractions of rice used for AAS elemental analysis were characterized by SEM to determine which tissue(s) were present in the fractions. Figure 2 shows the outer dentate surface of the husk. Pericarp-aleurone fractions clearly showed spherical structures identical to aleurone grains found in intact aleurone

TABLE I
Protein, Ash, and Individual Elements in Hand-Dissected Fractions of Rough Rice[a]

	Whole Kernel		Husk		Degermed Dehusked Kernel		Germ		Aleurone and Pericarp		Starchy Endosperm	
Component	S6	Caloro	S6	Caloro	S6	Caloro	S6	Caloro	S6	Caloro	S6	Caloro
Rough rice or fraction weight (mg)	28.6	29.1	5.1	5.0	22.6	23.3	0.9	0.8	2.4	1.6	20.1	21.7
Protein[b] (%)	6.7	6.0	...[c]	...[c]	6.4	5.6	19.0	16.4	11.2	11.9	4.9	4.6
Total ash (%)	6.6	5.2	24.6	23.4	1.1	1.1	7.9	5.6	11.4	10.8	0.5	0.5
P (%)	0.41	0.37	0.03	0.05	0.45	0.37	2.1	1.7	2.7	2.1	0.15	0.15
K (%)	0.43	0.39	0.77	0.87	0.29	0.28	1.6	1.4	1.6	1.9	0.12	0.12
Mg (%)	0.12	0.12	0.04	0.04	0.13	0.12	0.489	0.422	0.84	0.98	0.03	0.03
Ca (ppm)	219	246	702	1,028	76	97	418	488	425	563	58	77
Zn (ppm)	15.8	12.8	17.3	19.6	13.8	11.5	81.0	66.1	116	105	12.8	11.0
Fe (ppm)	26.4	21.2	82.1	102	15.5	8.45	82.6	69.5	118	225	2.64	10.1
Mn (ppm)	109	75.5	256	293	40.4	32.0	254	221	262	250	14.8	12.2
Cu (ppm)	2.72	2.10	1.67	2.16	2.32	2.14	6.83	6.34	1.08	1.11	1.67	1.77

[a] Moisture free basis; minerals determined by atomic absorption spectroscopy.
[b] N × 5.95.
[c] Not detectable.

layers (Fig. 3). The germ usually fractured between the scutellum and the starchy endosperm during dissection. When dissected germs were viewed in the scanning electron microscope, the exterior surface showed a ridge that outlined the attachment area between the embryo and the scutellum (Fig. 4). The procedure used to remove the aleurone layer from the starchy endosperm proved to be effective. Figure 5 shows the surface of a clean rice kernel with no remnants of aleurone tissue. The fracture into its interior shows polygonal compound starch granules.

Elemental Analysis

A practical standard for quantifying the EDXA for biological material was not available, and consequently AAS of hand-dissected fractions was used to compare the EDXA results. X-ray microanalysis of the husk revealed a substantial amount of Si and some Cl (Fig. 6). A peak at 2.2 keV was not due to P because the amount of P as determined by AAS (Table I) and by the data of Houston (1972) was not large enough to be seen by EDXA; therefore, the EDXA peak at 2.2 keV was assumed to be an artifact of unknown cause. The rice husk has previously been shown to contain a large amount of Si (Cote 1974, Houston 1972, Thomas and Jones 1970). The Si is apparently associated with the husk epidermis (Kaufman et al 1973, Kunoh and Akai 1979, Soni et al 1972). The Cl peak is real because the Cl content of rice husk ash has been determined to be 0.42% (McCall et al 1953).

Elements in the husk not detected by EDXA but quantified by AAS included Ca, in the highest concentration of all rice parts, and Mn, in concentrations equal to those found in the aleurone-pericarp fractions. These two elements were found at substantially higher concentrations than had been found previously by McCall et al (1953).

EDXA of the aleurone-pericarp layers revealed peaks corresponding to Mg, P, Cl, and K, but no peak for Ca (the concentration of which was too low for detection by EDXA). AAS showed that the aleurone-pericarp fraction contained the largest amount of P, K, and Mg but was relatively low in Ca. Pomeranz (1973) obtained similar results for barley aleurone grains and postulated the P was located in the aleurone grains and not in the cell walls or the starch granules. Low Ca content and high K concentration, as determined by AAS and EDXA, support the hypothesis that the phosphorus in the aleurone grains is primarily in the form of K-Mg phytate (Ogawa et al 1975; Tanaka et al 1974b, 1974c) and not Ca-Mg phytate. Primo et al (1970) showed similar results for analyses of rice bran and polish, which contain pericarp, aleurone, and some germ. The Zn concentration we obtained was high compared to that found by Primo et al (1970), indicating that we may have had some contamination from the scalpel used for scraping the rice. This fact was also substantiated by the inconsistently high Fe concentrations for the aleurone-pericarp fractions (Table I).

The germ had the highest concentration of protein of all the fractions. The elemental composition of the germ was similar to that of the aleurone except that the germ had a lower ash content, half the Mg content, and six times the Cu content. Tanaka et al (1974b, 1976) found that the P, K, and Mg distribution in the aleurone was similar to that in the scutellum of the germ and that the Ca was concentrated in the pericarp rather than in the aleurone layer.

EDXA distributions of elements in the subaleurone and starchy endosperm were identical and were therefore combined into one plot (Fig. 6). Only two peaks were observed for the subaleurone and starchy endosperm—one identical to the artifactual peak for P obtained for the husk and the other, a Cl peak. Chemical analysis revealed that P was only 0.15% and consequently could not be detected with EDXA. The ash content of the endosperm was the lowest one of the four rice fractions (Table I) and was consequently reflected in low elemental concentration. P, K, and Mg were the major components of the endosperm ash, but trace amounts of Ca, Zn, Fe, Mn, and Cu were present. Similar results were reported by McCall et al (1953).

The percent of Ca in the ash of each fraction varied between the two cultivars. Each dissected fraction of the Caloro kernel contained more Ca than did those of S6. Differences in the other mineral components were less consistent.

Both AAS and EDXA have advantages and disadvantages for elemental determination in cereals. The AAS requires tissue fractions and long times for sample preparation but gives reliable results, for many elements, in the parts per million range. EDXA does not require tissue fractionation and long preparative times; however, the accuracy and quantitation is very limited. For example: magnesium could only be detected at the 1% level, phosphorus at the 2% level, potassium at the 1.9% level, and EDXA was useful only for determining elements that were present in large quantities. Certainly, the lower limits of the EDXA detection could be improved via specimen preparation and computer analysis; however, the accuracy is not likely to approach that of AAS.

ACKNOWLEDGMENTS

We thank H. T. Horner, Jr., Department of Botany and Plant Pathology, Iowa State University, Ames, for use of the JEOL scanning electron microscope and X-ray analyzer, and C. W. Pitts, Jr., Department of Entomology, Kansas State University, Manhattan, for use of the ETEC scanning electron microscope.

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[Received July 21, 1980. Accepted October 9, 1980]