

## NOTE ON THE PHYTIN-CONTAINING PARTICLES ISOLATED FROM RICE SCUTELLUM

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It has been accepted that the scutellum, a part of the embryo of grass caryopses seed, plays an important part as the pathway along which reserve materials in the endosperm move to the growing axis during germination. Further, scutellum itself is important as a storage site of nutrients; Swift and O'Brien (1,2) showed that many proteinaceous particles and lipid droplets exist in the scutellar cells of wheat. Observations by Tanaka *et al.* (3), using SEM<sup>1</sup> and EMX analyses, established that the distributions of P, Mg, and K in the scutellar cell of rice are very similar to that in the aleurone cell. In this work, the nature of isolated subcellular particles from the rice scutellum is reported to determine the chemical composition of the particles more precisely.

### MATERIALS AND METHODS

#### Preparation of Embryos

Husked nonglutinous rice grains (*Oryza sativa* L. *Japonica* cv. Koshihikari) were cultured in the rice plot of this institute and polished at 3°C with a polishing machine (Marumasu Co. Ltd., Toyama, Japan). The initial bran fraction comprised 2% of the whole grains by weight and was discarded. The following

<sup>1</sup>The abbreviations used are: SEM, Scanning Electron Microscope; TEM, Transmission Electron Microscope; EMX, Electron Microprobe X-ray.

bran fraction collected comprised another 2% of the grain by weight. This fraction was abundant in embryos which had been detached during the polishing. Embryos were collected from the rice bran, using sieves as follows. The rice bran was first passed through a No. 16 mesh sieve and then twice through a No. 20 mesh sieve. The material retained on the No. 20 mesh sieve was rich in detached embryos. The embryos were arranged by size using a No. 26 mesh to remove broken embryos and aleurone cells contaminating into this fraction.

#### Isolation of Particles

One kilogram of collected embryos was powdered using a coffee mill. The resultant powder was fractionated in a nonaqueous medium, a mixture of cottonseed oil and carbon tetrachloride at various densities ranging from  $\rho = 1.25$  to  $\rho = 1.35$  (4).

Isolation of particles by an aqueous medium was by the procedure described by Ogawa *et al.* (5). From 1 kg of powdered embryos, 3 g of particles were obtained.

#### Electron Microscopical Observation

**SEM.** Isolated particles were dispersed onto the surface of a specimen stub. The surface was coated with gold in a vacuum evaporator. Samples were then examined in an SEM (JEM-U-3 type, Japan Electron Optics Laboratory Co., Tokyo).

**TEM.** Isolated particles were fixed with 5% glutaraldehyde in phosphate buffer at pH 7 for 2 hr at 0–4°C and subsequently stained for 1 hr with 1% osmium tetroxide in the same buffer. After dehydration in a graded ethyl alcohol series, the materials were embedded in epoxyresin (Epikote 812). Thin sections of embedded particles were examined in a JEM-7-type electron microscope.

#### Determination of Components

For mineral estimation, samples were ashed in a crucible and the ash was dissolved in 9*N* perchloric acid. Phosphorus was determined by the method of Allen (6). The phytic-acid content was calculated from the quantity of acid-soluble phosphorous (5). K, Mg, and Ca were estimated using an atomic absorption spectrometer (Hitachi-Perkin-Elmer 303). To estimate Ca, lanthanum oxide was added up to 5% to avoid disturbance by phosphorus. The metal contents were corrected by the internal standard method. Nitrogen was determined using a micro-Kjeldahl procedure after hydrolysis with sulfuric acid. Protein was calculated as  $N \times 6.0$ . Water-soluble carbohydrates were determined by the anthrone reaction (7). The suspension of isolated particles in water was boiled for 10 min, then the supernatant fluid was used for the determinations. Absorbance at 625 nm was measured with glucose as standard.

## RESULTS AND DISCUSSION

The particles isolated from embryos with a nonaqueous medium are spherical particles about 2–3  $\mu\text{m}$  in diameter (Fig. 1a). The surface of the particles has a golf ball-like appearance with many concavities, each about 0.4  $\mu\text{m}$  in diameter.

The frequency and the size of these concavities suggest they may have been the site where spherosomes were in contact with the particle.

The particles isolated with a nonaqueous medium contain electron-dense inclusions when viewed in the TEM (Fig. 1b). The electron-dense core was enveloped by an electron-transparent layer. The interparticular space contains reticulate structures (Fig. 1b). These structures may be derived from the outer envelope of the isolated particles (5). Staining by osmium tetroxide implies that these reticulate structures are proteinaceous material.

The surface of the particles isolated by an aqueous medium were smoother than that of the particles isolated by a nonaqueous medium with no concavities (Fig. 2a). Also, some of the particles were fractured. Sectioned particles, 1–2  $\mu\text{m}$  in diameter, lacked the electron-transparent layer, and the interparticular space lacked reticulate structures (Fig. 2b).

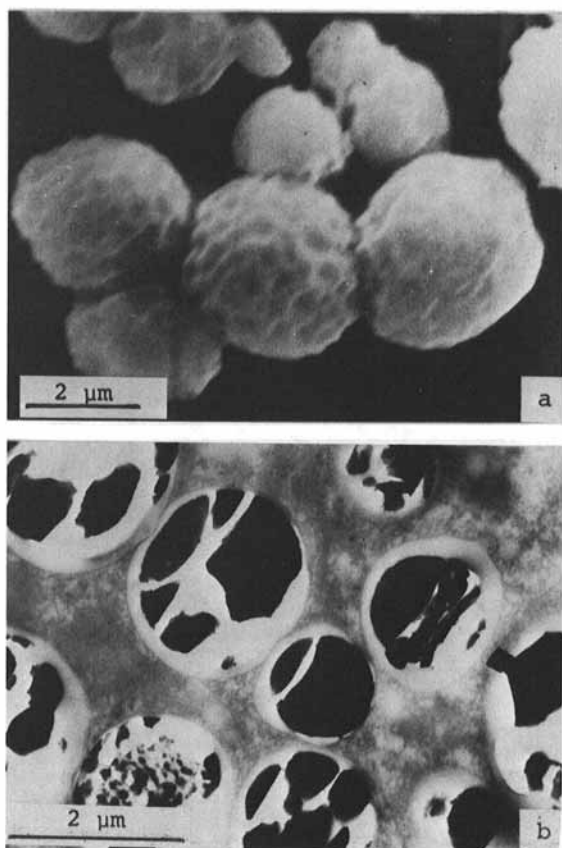


Fig. 1. Electron micrographs of phytin-containing particles isolated from rice embryo by a nonaqueous medium: a) SEM of the isolated particles, and b) TEM of the isolated particles.

**TABLE I**  
**Chemical Composition of Phytin-Containing Particles Isolated from the Embryos**

Component	Isolated Particles	
	Nonaqueous medium mg 100 mg	Aqueous medium mg/100 mg
Protein	20.2	0.8
Carbohydrate	10.7	0.7
Phytic acid	43.4	70.3
Metals		
K	18.9	22.8
Mg	5.3	9.5
Ca	0.5	0.7

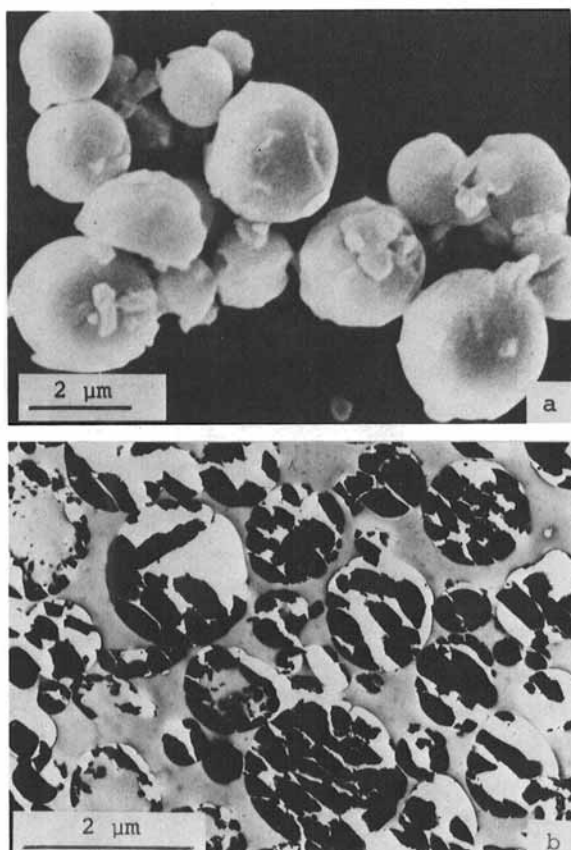


Fig. 2. Electron micrographs of phytin-containing particles isolated from rice embryo by an aqueous medium: a) SEM of the isolated particles, and b) TEM of the isolated particles.

The chemical composition of the particles isolated from the embryos is shown in Table I. The composition of the particles isolated was characterized by high phytic acid, K, and Mg content. Particles isolated by a nonaqueous medium were rich in protein and carbohydrates; particles isolated by an aqueous medium were low in protein. From the decrease of nitrogen and carbohydrates during the isolation by an aqueous medium, it may be inferred that protein and carbohydrates, associated with the isolated particles by a nonaqueous medium are soluble in water or miscible by hydration. This suggests that the protein, associated with the isolated particles by a nonaqueous medium, is rich in albumin.

The particles isolated here can be regarded as the particles existing in the scutellar cells (3). These particles are distributed in the scutellar cells; however, the size and the frequency are larger in the scutellar parenchyma cells than in the scutellar epithelium cells (8). The chemical composition of the isolated particles from the embryos by the aqueous media established that the electron-dense cores observed within the scutellar cells are mainly composed of Mg and K salts of phytic acid. The content of phytic acid in the isolated particles was calculated from the amounts of acid-soluble phosphorus in the isolated particles. This was based on the fact that in the acid-soluble organic phosphorus (0.5M perchloric acid-soluble and Ba-insoluble at pH 8.0) a phosphorus compound other than phytic acid was not detected when the acid-soluble phosphorous compound of the isolated particles was checked by paper chromatography (acetone:water 3:1) and paper electrophoresis (ammonium formate buffer, pH 3.2, at 70 V/cm, for 20 min). However, the presence of small amounts of nucleic acid in the isolated particles cannot be excluded (4).

In rice grain, protein bodies and aleurone particles are well known high-protein-containing particles. The former do not contain phytic acid (9), but the latter are characterized by a high content of phytic acid (4). Thus, particles in the scutellum of rice grains are to be classified as a type of aleurone particles, according to morphology and chemical composition. The difference between the scutellar particles and the aleurone particles is seen only in the amount of protein. The former are richer in protein than the latter. Whether the difference in protein is of a quantitative or qualitative nature is yet to be established.

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