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MICROSTRUCTURAL APPROACH TO LEGUME SEEDS FOR FOOD USES

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Abstract

This review summarizes the microstructures of several seed legumes based on previous work and some new findings. Fifteen species of tropically grown legumes, adzuki bean and soybeans (a leading variety and two local varieties) were examined by light and transmission electron microscopy in relation to food uses. Processing of adzuki beans to form *an* bean paste is discussed to illustrate the effects of processing on microstructure of starch grains. Differences in contents, shape and size of starch grains are emphasized in a comparison of soybeans with other legumes.

Introduction

The family Leguminosae includes numerous species and they are widely distributed from the tropic to the arctic regions. Especially in Asian countries, several legume seeds have been traditionally used as staple foods and have assumed a role of protein supplements in the inhabitants' diet. Most legume seeds are starch-rich but also richer in protein as compared with cereals. Some of them are starch-poor but protein-rich and/or oil-rich. Historically both types have been cooked and manufactured into sophisticated foods in various countries (1, 2).

Materials and Methods

Materials

Tropically grown legume seeds were collected by the Tropical Agricultural Research Center, Tsukuba. The kinds of legumes and countries of production are as follows: Benas (*Phaseolus vulgaris*, India), black gram (*Vigna mungo*, India), chickpea (*Cicer arietinum*, India), cowpea (*Vigna unguiculata*, Indonesia), clusterbean (*Cyamopsis tetragonoloba*, India), green gram (*Vigna radiata*, India), hyacinth bean (*Lablab purpureus*, Indonesia), khesari (*Lathyrus sativus*, India), lentil (*Lens culinaris*, India), pea (*Pisum sativum*, India), pigeonpea (*Cajanus cajan*, India), ricebean (*Vigna umbellata*, Thailand), swordbean (*Canavalia ensiformis*, Indonesia), winged bean (*Psophocarpus tetragonolobus*, Thailand), and yambean (*Pachyrhizus erosus*, Thailand).

Commercial adzuki beans (spelled azuki in Japanese, *Phaseolus radiatus*, Hokkaido, Japan) were used to prepare *an* (a Japanese bean paste) particles. Dried *an* was prepared by the laboratory of Kyoritsu Women's University, Tokyo. Fifty grams of adzuki beans were cooked carefully at 100°C for 90 minutes with 250 ml of water. The hulls were removed after cooling and separation of the cooking water. The cotyledons were ground, filtered and freeze-dried (10, 11).

Soybeans (*Glycine max*) were cultivated at the Agricultural Experiment Station of Hyogo prefecture, Wadayama, Japan.

Key Words: Legume, adzuki, *an*, soybean, light microscopy, transmission electron microscopy, starch.

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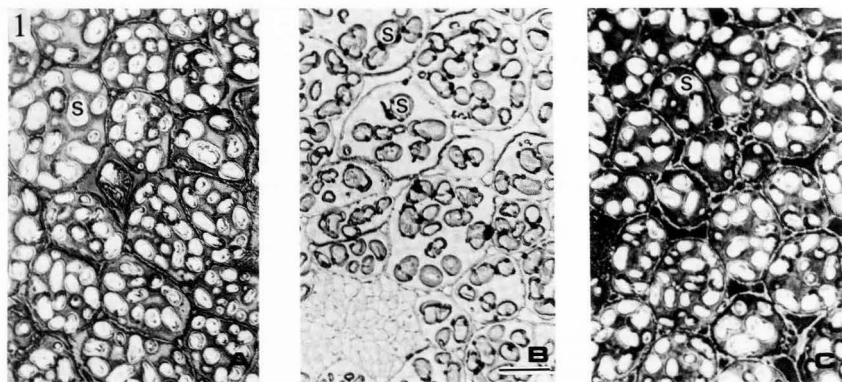


Figure 1 (above). Light micrographs (at same magnifications) of cowpea (*Vigna unguiculata*) stained by three methods. A: Coomassie Brilliant Blue (CBB), B: Periodic acid-Schiff (PAS), C: Sudan Black B (SB). Bar in B = 50 μ m.

Figure 2 (on the facing page). Light micrographs (at identical magnifications) of seed legumes (see Figure 1 legend for the identification of staining techniques given in parenthesis). A: Benas (PAS), B: Black gram (PAS), C: Chick pea (SB), D: Cowpea (PAS), E: Swordbean (SB), F: Green gram (CBB), G: Hyacinth bean (SB), H: Khesari (CBB), I: Lentil (CBB), J: Pea (CBB), K: Pigeonpea (SB), L: Ricebean (CBB), M: Clusterbean (SB), N: Winged bean (PAS), O: Yam bean (SB), P: Soybean (CBB). Bar in H = 50 μ m.

Table 1. Characteristics of soybean varieties used.

name	weight of 100 seeds (g)	total sugar (%)	crude protein (%)	crude fat (%)	ripening period (days)	variety status
Enrei	32	20.5 (0.6) ^a	45	20	75	leading
Tanbaguro	55	24 (3) ^a	45	23	109	local
Aomame	36	27	43	25	83	local

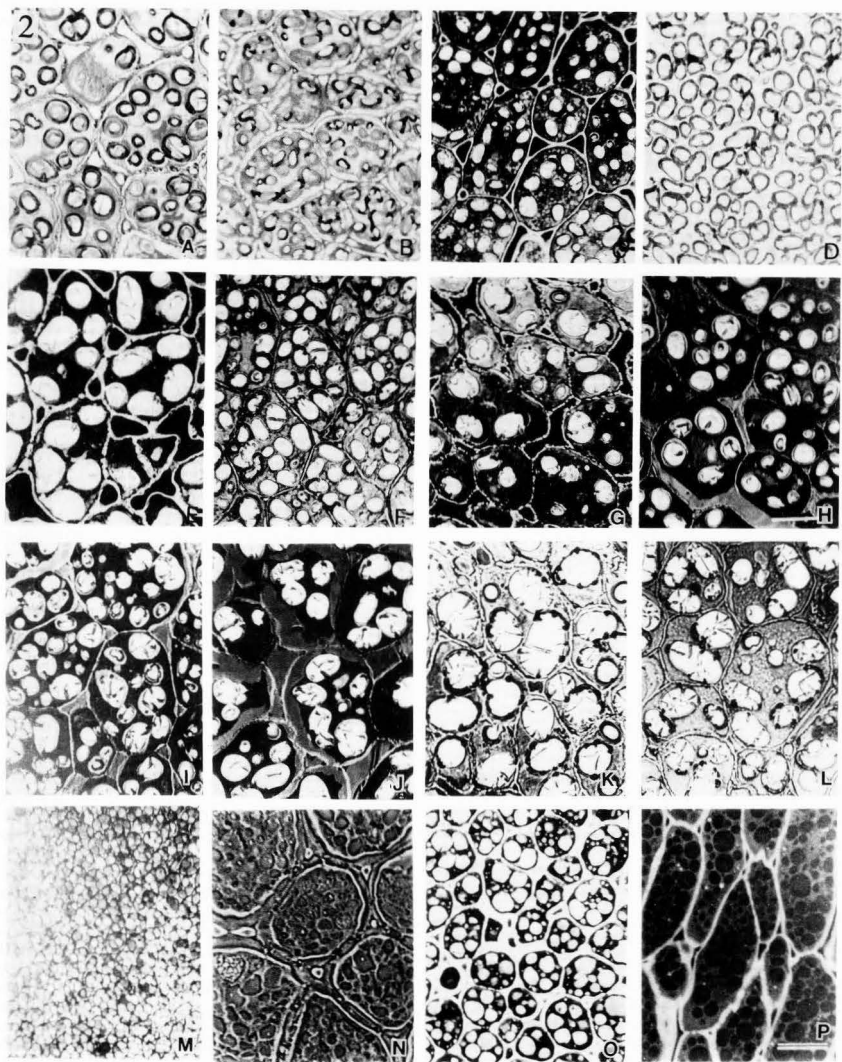
^aValues in parenthesis are starch content. Data were analyzed by the Hyogo Prefecture Experiment Station (1992).

Preparation of microscopic specimens

Small pieces (less than 1 x 1 x 3 mm) of cotyledonary tissue from each legume seed were cut with a razor blade, fixed with 0.5% glutaraldehyde and then 1% osmium tetroxide, dehydrated with a graded acetone series (40% to 100%) and embedded in Epon or Spurr's resin. For light microscopy (LM), the blocks, prepared as described above, were sliced with an Ultratome and affixed onto a glass slide. Three staining techniques were used: (a) specimens were stained for protein with 0.5% solution of Coomassie Brilliant Blue in 7% acetic acid-50% methanol overnight and decolorized with 7% acetic acid-50% methanol; (b) polysaccharides were stained with Schiff's reagent after oxidation with 0.5%

periodic acid solution (PAS); and (c) lipids were stained with a saturated solution of Sudan Black B in 50% ethanol (7). For transmission electron microscopy (TEM, JEOL SX-100 or EX-1200), the blocks used for the light microscope were sliced (about 0.2-0.3 μ m thick, to retain starch grains in the tissues) with a Reichert-Jung Ultratome E (the specimens shown in Figure 10 were ultrathin slices) and specimens were observed with or without double-staining with saturated uranyl acetate solution and saturated lead acetate.

Ag particles were suspended in melted agar at less than 40°C and cooled to gelatinize. The coagulated agar gel was cut into small pieces, which were then fixed, dehydrated and embedded in resin.



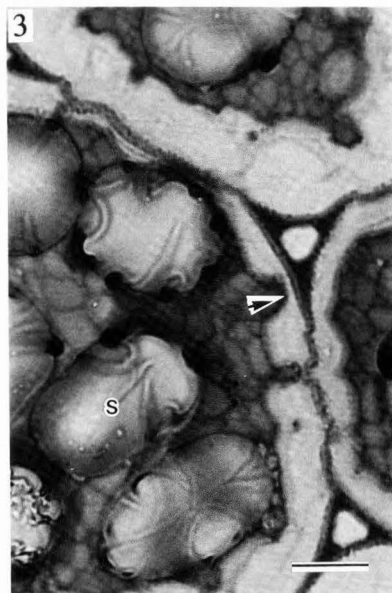


Figure 3. Light micrograph of green gram. Bar = 10 μ m. S: starch granule, arrow: pit-pair.

Results and Discussion

Tropically grown legume seeds

Figure 1 shows light micrographs of cotyledonary tissue in cowpea stained by the three methods. Starchy cells of cowpea could be clearly observed by all methods, where single starch grains ranging from 10–30 μ m in diameter were distributed. Starch and cell walls were stained with PAS. Cytoplasmic matrix was stained faintly with Sudan Black.

Since the principal features of legume seed structure were observed by any of the three staining methods, one of the clearest photographs was selected for each legume seed and they are shown in Figure 2.

Benas, black gram, chickpea, cowpea, swordbean, green gram, hyacinth bean, khesari, lentil, pea, pigeonpea and ricebean, all have a typical starchy cell structure (Figure 2). Although their starch grains are all single, instead of compound or aggregated, the shape, size and their numbers in a cell vary somewhat depending on the species. Black gram, cowpea, and green gram, which

Figure 4 (facing page, top). Transmission electron micrographs of cotyledonary cells of tropical legume seeds. A: Benas, B: Cowpea, C: Hyacinth bean, D: Pigeonpea. Bars = 1 μ m. S: starch granule, CW: cell wall, PB: protein body, arrow: pit-pair.

belong to the genus *Vigna*, show similar structures, having a number of round, ellipsoidal or kidney-shaped starch grains. Ricebean, however, was a little different, having bigger ellipsoidal starch granules with irregular swelling, in spite of being in the same genus. Rather, the shape of ricebean starch granules resembled those in pigeonpea and swordbean which were bigger and fewer in number per cell.

Clusterbean, winged bean, yambean and soybean are all protein-rich legumes. Globular materials in yambean and clusterbean appeared to be protein bodies, whereas winged bean and soybean definitely had protein bodies, but all four contained few, if any, starch grains, being different from the starch-rich legumes. Moreover, size and shape of starch grains, when present, in those four beans were quite different.

Some of the tropically grown legume seeds had very thick cell walls with pit-pairs, as reported previously in winged bean (8). As an example, a light micrograph of green gram is shown in Figure 3.

The results of TEM examination for four tropically grown legumes, benas, cowpea, hyacinth bean, and pigeonpea, are shown in Figure 4. The starch grains, which vary in shape and size, were observed. In the cytoplasmic matrix, for example in hyacinth bean and cowpea (Figures 5A and 5B), many cellular materials, such as vacuoles, protein bodies, and lipid bodies, were observed. Protein bodies were faint in electron density and lipid bodies rarely were found adjacent to cell walls, as compared with protein-rich legumes such as soybean and winged bean. The thick cell walls with pit-pairs described above were often found in tropically grown legume seeds. Pit-pairs with plasmodesmata, in benas, are shown in Figure 6. The microstructure of tropical legume seeds were reported previously also (3).

Tropically grown legumes are eaten in diverse food products prepared through processes such as soaking, cooking, roasting, mashing, milling, fermenting, frying, puffing, baking, gel-forming, germinating, etc., with or without decortication. These processing procedures have improved through history for effective use of the various legume characteristics. The thick cell walls often found in these seeds, are digested by fermentation, and some bioactive components, such as trypsin inhibitor and hemagglutinin, are inactivated by heating. The traditional use of *Phaseolus* beans for *an* (bean paste), described in the next paragraph, is an example of a highly developed processing method. The seeds of yambean are seldom utilized, although the fresh turnip-like roots are used as food; some legume seeds, pods, and leaves, are usually eaten as vegetables in their immature form. Clusterbeans are a source of galactomannan (guar gum).

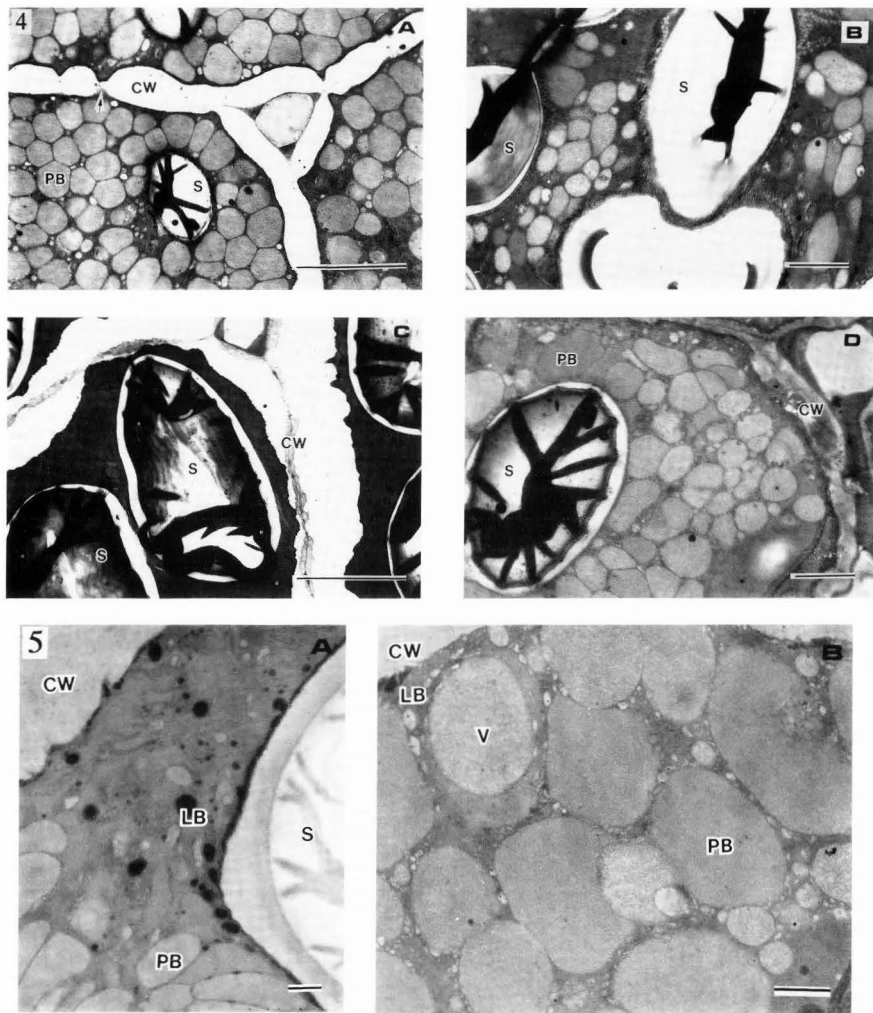


Figure 5. Transmission electron micrographs of cytoplasm in cotyledon of tropical legume seeds. Bars = 1 μ m. **A:** Hyacinth bean, **B:** Cowpea. **S:** starch granule, **PB:** protein body, **V:** vacuole, **LB:** lipid body, **CW:** cell wall.

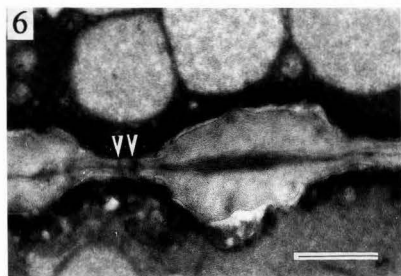


Figure 6. Transmission electron micrograph of cell wall in cotyledonary cell of benas. Bar = 1 μ m. Arrow: plasmodesmata.

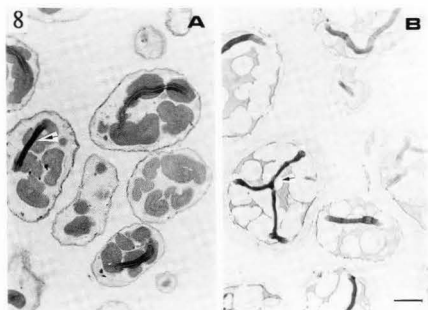


Figure 7. Flow sheet of *an* making.

Figure 8. Light micrographs (at identical magnifications) of *an* particles: A: PAS, B: CBB. Bar in B = 10 μ m. Arrow: artifact shown only in intact starch granule.

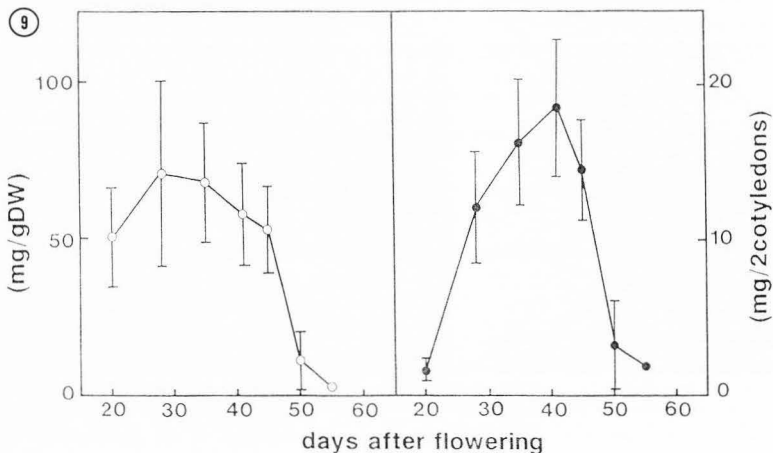
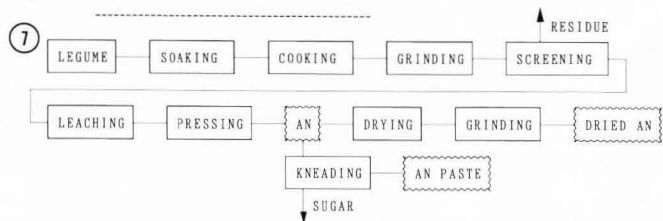


Figure 9. Starch content in developing soybean seeds (cv. Enrei).

Adzuki beans in *an* making

The traditional use of Phaseolea, such as adzuki bean, for *an* is popular in Japan. A brief procedure of *an* making is shown in Figure 7. Here, the cooking step is the most important and distinctly affects the quality of the final product. In order to obtain the characteristic texture of *an*, it is necessary to convert starch grains in the cell into the α -form without disruption of cell walls, that is, retaining the so called *an* particles. Figure 8 shows *an* particles, in which swollen starch grains are observed, being surrounded by intact cell walls. Insolubilized and heat-coagulated protein appears as linear aggregates that span several starch grains. According to Watanabe and Kozuma (10), such protein inhibits gelatinization of starch, in spite of being easily digestible by glucoamylase. Japanese are very familiar with traditional sweets which use *an*.

Local varieties of soybeans

More than four hundred varieties and strains of soybeans are said to be grown in Japan, and breeding has been systematically carried out since 1910. The main objectives of breeding for the leading varieties are high yield ability, resistance to diseases and insect pests, and chemical composition for food use. On the other hand, local varieties remain in cultivation on a small scale in each region, deeply rooted in Japanese regional and traditional dietary life, especially confectionaries and cooked beans served at festivals. Such local varieties often have large sized seeds, beautiful colors (green hulls and sometimes even cotyledons, brown, black and speckled) and are easy to cook.

Starch grains are widely distributed in soybean cotyledonary tissue during ripening, but rapidly decrease in number with maturation, as reported previously (4, 6, 9). A typical pattern of change in starch content is shown in Figure 9. EM changes of starch contents in developing seeds reported previously (9) are shown in Figure 10. Japanese eat immature soybean seeds as a vegetable in the periods when size and numbers of starch grains are almost maximum. In the present experiment, microstructures of cotyledonary tissue in the three varieties, Tanbaguro, Aomame, and Enrei, were observed with a TEM. The number of starch grains found in the cotyledonary cells (Figure 11) were in the order Aomame > Tanbaguro > Enrei. The data in Table 1 were collected in the Experiment Station where these varieties were produced (Minamide T, Fujimura K, Hata A, Hikino I: Stability of green color and processing qualities of green soybeans. Personal communication, 1992). It appears that the higher the amounts of total sugar, the greater the number of starch grains that were found in the cells. A few papers have reported that local varieties in Japan contained higher amounts of total sugars and/or starch (5, 13). Generally speaking, the local varieties have high water absorption capacities, are easy to soften by cooking, and have lower beany flavor with a sweet taste. As a result, they are used in many traditional Japanese dishes.

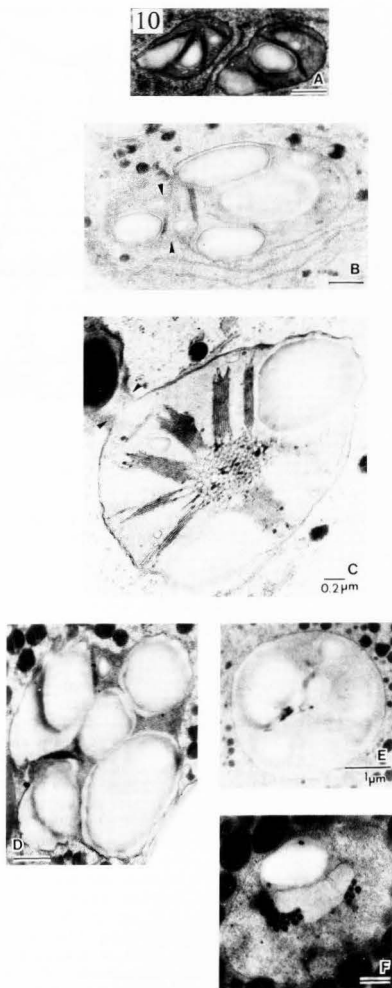


Figure 10. Microstructural changes of plastids (starch granule) in developing soybean (cv. Enrei). Bars in A, B, D, E, and F = 1 μ m. Arrow: dividing point of plastid. A: 15-20 days after flowering (DAF); B: 20-25 DAF; C: 25-30 DAF; D and E: 35-45 DAF; and F: 50-55 DAF.

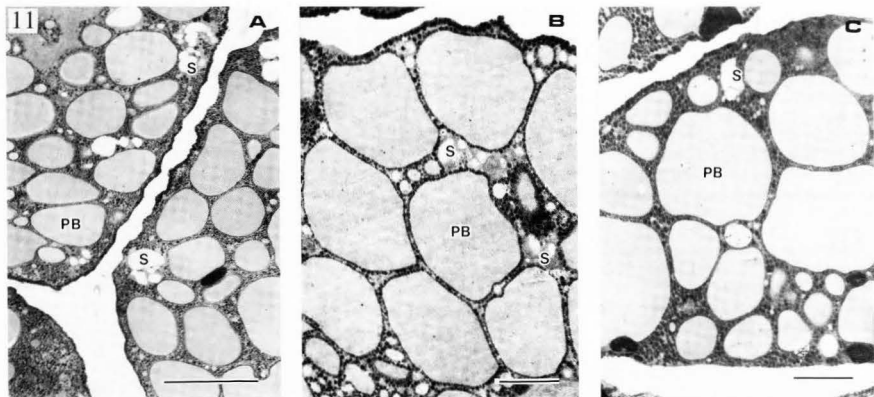


Figure 11. Transmission electron micrographs of cotyledonary cell of soybeans. A: Aomame, B: Tanbaguro, C: Enrei. Bars = 5 μ m. The magnification in A is smaller than B and C to show the frequent distribution of plastids. Lipid bodies are scattered in cell matrix as small and electron dense dots. PB: protein body, S: starch granule.

Table 2. Classification of individual starch grains According to Winton and Winton (12).

1	Globular (peanut, floury part of maize)
2	Lenticular (wheat, rye, barley)
3	Ellipsoidal (legumes)
4	Pear-shaped (potato, canna, banana)
5	Truncated (cassava, sago)
6	Polygonal (maize, rice, sorghum)
7	Bone-shaped (latex of euphorbia)

Comparison of starch grains between soybeans and the other legumes

Table 2 shows the classification of starch grains in seeds by Winton and Winton (12); they mentioned that the shape of legume starch grains was ellipsoidal, that those in Canna were the largest (170 μ m) and those in rice were the smallest (2-10 μ m) among the seeds which they examined. In relation to legumes, they also stated that starch grains in common beans reached up to 60 μ m and those in adzuki bean up to 80 μ m, but they mentioned that soybeans contained only traces of starch. In our experiments, starch grains in chickpea, green gram, black gram, cowpea, and beans ranged from 5-40 μ m, relatively smaller; on the other hand, those in hyacinth bean, pigeonpea, ricebean and swordbean were relatively larger, ranging from 15-80 μ m.

In contrast, the starch grains in soybeans, even the ones remaining after harvest, are considered to have a temporary storage function, being different from the ones in starch-rich legumes. Soybean starch grains are

smaller in size and localized one to a few in plastids. The starch grains in soybean, as in winged bean, can easily be detected with PAS staining with a short reduction-time with periodic acid.

Finally, legume seeds with higher nutritional quality than cereal grains, are grown on poorer land and given less attention, and they have been utilized domestically and industrially in a great variety of foods. However, most legumes, with the possible exception of soybeans, are still under investigation because of a wide genetic variation and confusion of the names by countries and regions.

Microstructural study of legume seeds by observation under light and electron microscopes can clarify relationships between approximate compositions and structures (e.g., high starch content and prevalence of starch grains) and reveal physical characteristics of importance in food applications (e.g., thickness of cell walls). Such studies are useful in considering how legumes are utilized in traditional foods and also how they may be applied to novel foods in the future.

Acknowledgements

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Discussion with Reviewers

Editor: Please explain the use of rather thick sections "about 0.2-0.3 μm thick, to retain starch grains in the tissues" for TEM examination.

Authors: If we prepare ultrathin slices, the big starch grains in the cytoplasmic matrix, which are quite difficult to fix with any reagent, drop out during cutting with Ultratome resulting in a section with many holes. This is why we prepared thicker sections, although they do not give beautiful micrographs under TEM.