Microstructural Differences Among Adzuki Bean (Vigna Angularis) Cultivars

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Abstract

Scanning electron microscopy (SEM) was used to study microstructural differences among five adzuki bean cultivars: Erimo, Express, Hatsune, Takara and VBSC. Seed coat surfaces showed different patterns of cracks, pits and deposits. Cross-sections of the seed coats revealed well organized layers of elongated palisade cells followed by many layers of amorphous parenchyma cells. Typical sub-epidermal layers of organized columnar, hour-glass cells were characteristically absent in the five cultivars of adzuki beans. The cross-sections of the cotyledons revealed intercellular materials between storage cells. Storage cells in Hatsune cultivar were loosely packed with many intercellular spaces as compared to the other four cultivars. Cross-sections of storage cells revealed starch granules embedded in protein matrices. The protein matrix appeared more granular in cultivars Erimo and VBSC and less granular in cultivars Express, Hatsune and Takara. The cross-sections of the cotyledons showed intercellular materials between storage cells.

Introduction

Adzuki beans are one of the oldest cultivated beans in the Orient, often used for human food, prepared as a bean paste used in soups and confections (Tjahjadi and Breene, 1984). The starch content of adzuki beans is about 50%, while the protein content ranges between 20%-25% (Tjahjadi and Breene, 1984).

Adzuki beans are a major crop consumed in Japan, China, and other oriental countries (Sacks, 1977). In Japan most adzuki beans are used in the form of sweetened paste called "An" in many confections, candies, cakes and ice cream (McClary et al., 1989). Between 1980 and 1989 about 27% of the adzuki beans consumed in Japan was provided by imports (McClary et al., 1989). Thus, a very promising future market exists in Japan for Washington grown adzuki beans.

Consumer acceptance is an important aspect of marketing adzuki beans. Japanese consumer acceptance for adzuki beans and substitutes depends upon the distinct color and shape of the bean and the end use in various foods (McClary et al., 1989). The size, shape, color and nutritional quality of adzuki beans vary among different cultivars of adzuki beans (Hsieh, 1991). Factors such as appearance, functional and textural properties, organoleptic and nutritional qualities, and the price of a particular cultivar of adzuki beans determine the potential as a marketable crop in the Japanese market.

Microstructural variations can be related to textural, chemical and physical variations among bean cultivars (Sefa-Dedeh and Stanley, 1979). Microstructural variations were used to identify different cultivars of soybeans (Wolf et al., 1981). Scanning electron microscopy (SEM) is a technique recently employed in various studies of food microstructure (Swanson et al., 1985). The objective of this study was to survey microstructural variations among five different cultivars of adzuki beans using SEM.
Table 1 - Microstructural Variations Among Adzuki Bean Cultivars

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Seed coat surface features</th>
<th>Storage cells</th>
<th>Starch granules</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>deposits</td>
<td>pits</td>
<td>cracks</td>
</tr>
<tr>
<td>Erimo</td>
<td>irregular shape, mostly clustered</td>
<td>few, small pits</td>
<td>no cracks</td>
</tr>
<tr>
<td></td>
<td>few scattered</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Express</td>
<td>compressed, flower-shaped, clustered or scattered</td>
<td>not common</td>
<td>distinct, * convoluted pattern of cracks</td>
</tr>
<tr>
<td>Hatsune</td>
<td>irregular shape, small, scattered, some very large</td>
<td>deep, long pits</td>
<td>no cracks</td>
</tr>
<tr>
<td>Takara</td>
<td>irregular shape, some small rod-shaped, scattered</td>
<td>not common</td>
<td>no cracks</td>
</tr>
<tr>
<td>VBSC</td>
<td>irregular shape, small, mostly scattered, some very large</td>
<td>deep, large pits</td>
<td>no cracks</td>
</tr>
</tbody>
</table>

* identifying feature

Materials and Methods

Five cultivars of adzuki beans (*Vigna angularis*), Erimo, Express, Hatsune, Takara and VBSC used in this study were obtained from Washington State University Irrigated Agriculture Research Extension Center, Prosser, WA.

Adzuki beans were surface cleaned with dry Kleenex® facial tissue to remove any dirt and other particles sticking to the surface. Cleaned beans were freeze fractured by freezing in liquid nitrogen and fracturing with a razor blade. Freeze fractured beans were attached to standard SEM mounts using double sided adhesive tape and dried overnight in a desiccator. To prepare adzuki bean flours 100 g of cleaned beans of each cultivar were ground to flour for 2 minutes in a Stein mill to pass through a sieve of 0.5 mm pore size. A tiny amount of flour for each bean cultivar was spread on double sided adhesive tape attached to a standard SEM mount and dried in a desiccator overnight. Dried specimens were sputter-coated with 300 A gold (Hummer Technics). The gold-coated specimens were examined and photographed with a Hitachi S570 SEM operating at 20 kV.

Results and Discussion

The significant microstructural differences among the adzuki bean cultivars observed in this study are summarized in Table 1.

Seed coat surface

Observations of seed coat surfaces of adzuki cultivars revealed distinct differences among the five cultivars. Seed coat surfaces of adzuki beans were characterized by the presence of deposits, pits and cracks. On the surface of the Erimo cultivar numerous deposits and few pits were observed (Figure 1). The surface deposits of Erimo cultivar were large and mostly clustered (Figure 2). The seed coat surface of the Express cultivar was characterized by convoluted pattern of cracks (Figure 3). The surface deposits of Express cultivars were large, compressed and flower-shaped (Figure 4). Pits and small scattered surface deposits were observed on the seed coat surfaces of Hatsune (Figure 5) and VBSC (Figure 6) cultivars. At greater magnification the largest surface deposits were observed on Hatsune (Figure 7) and VBSC (Figure 8) cultivars. Large surface deposits on Hatsune and VBSC cultivars exhibited shapes not observed in the other three cultivars examined. Surface cracks were not observed on surfaces of Hatsune and VBSC cultivars. In Takara cultivar, the surface of the seed coat was characterized by many small scattered irregular and rod shaped deposits (Figure 9). Rod-shaped deposits were the size of a
Figure 1. Adzuki bean cv. Erimo. Seed coat surface. SD - surface deposit.

Figure 2. Adzuki bean cv. Erimo. Seed coat surface deposits at higher magnification. SD - surface deposit.

Figure 3. Adzuki bean cv. Express. Seed coat surface pattern. C indicates convoluted pattern of cracks.

Figure 4. Adzuki bean cv. Express. Seed coat surface deposits at higher magnification. SD - flower shaped, compressed surface deposits.
Figure 5. Adzuki bean cv. Hatsune. Seed coat surface. P indicate surface pits.

Figure 6. Adzuki bean cv. VBSC. Seed coat surface. P indicates surface pit.

Figure 7. Adzuki bean cv. Hatsune. Seed coat surface deposits (SD) at higher magnification.

Figure 8. Adzuki bean cv. VBSC. Seed coat surface deposit (SD) at higher magnification.
Figure 9. Adzuki bean cv. Takara. Seed coat surface. SD - surface deposit.

Figure 10. Adzuki bean cv. Takara. Seed coat surface at higher magnification. Arrow head indicates small rod-shaped surface deposits. SD - irregular surface deposit.

Figure 11. Adzuki bean cv. Hatsune. Cross-section of the seed coat. PL - palisade cells, PR - parenchyma cells.

Figure 12. Adzuki bean cv. Express. Cross-section of the bean cotyledon showing arrangement of storage cells. Arrow head indicates intercellular materials.
Figure 13. Adzuki bean cv. Hatsune. Cross-section of bean cotyledon showing loosely packed storage cells. Arrow head indicates intercellular materials.

Figure 14. Adzuki bean cv. Erimo. Starch granules (S) embedded in protein matrix (PM).

Figure 15. Adzuki bean cv. VBSC. Starch granules (S) embedded in protein matrix (PM).

Figure 16. Adzuki bean cv. Erimo. Protein matrix (PM) formed aggregates of loose granular nature in flour sample.
Figure 17. Adzuki bean cv. Express. Protein matrix (PM) formed aggregates of loose granular nature in flour sample.

Figure 18. Adzuki bean cv. Express. Individual starch granule from flour sample. Spherical to irregular shape. S - starch granule, G - groove.

Figure 19. Adzuki bean cv. VBSC. Individual starch granule from flour sample. Oval shape. S - starch granule.

Figure 20. Adzuki bean cv. Takara. Individual starch granule from flour sample. Highly irregular to slightly spherical shape. S - starch granule, F - fissure.
bacteria, while the irregularly shaped deposits were larger (Figure 10). Pits and cracks were not observed on the seed-coat surface of Takara cultivar.

Seed coat surface characteristics are important in evaluating the differences among legume cultivars. Seeds of many plants have species-specific seed coat patterns (Hughes and Swanson, 1985). Obvious microstructural differences between lentils and other food legumes were observed by examining seed coat surfaces (Hughes and Swanson, 1986). Size, shape and frequency of papillae on lentil seed coats vary among lentil cultivars (Trivedi and Gupta, 1988). Numerous pits or pore-like indentations were observed on intact soybeans by Wolf et al. (1981). Observations of maturing seed coats of common bean (*Phaseolus vulgaris* L.) seeds revealed a characteristic pattern which developed as the seeds matured (Hughes and Swanson, 1985).

The significance of seed coat surface deposits to food processing is unknown, but large quantities of deposits on the surface can affect the luster and acceptability of the seed (Swanson et al., 1985). Pits and cracks on the seed coat may be naturally present or inflicted during harvesting and handling. Pits or cracks may influence the moisture retention capacity of the beans during storage, which in turn effects the market value of beans.

**Cross-sections of seed coats**

The cross-sections of seed coats of adzuki bean cultivars exhibited an organized layer of elongated palisade cells and multiple layers of amorphous parenchyma cells (Figure 11). A sub-epidermal layer of hour-glass cells was not observed in the adzuki bean cultivars examined in this study. The thickness of the palisade layer varied in five cultivars of the adzuki beans, from 40-44 µm in Hatsune, 50-56 µm in Erimo, 58-60 µm in Express. The differences in thickness of seed coat may account for differences in rate of water imbibition of the adzuki bean cultivars.

Cross-sections of seed coats of legumes are generally composed of three distinctive layers of cells: an epidermal layer of organized, elongated palisade cells, a sub-epidermal layer of shorter, columnar hour-glass cells supported by many layers of amorphous parenchyma cells (Corner, 1951). Three distinct layers of cells were observed in common beans (*Phaseolus vulgaris* L.) by Hughes and Swanson (1985). A sub-epidermal layer of hour-glass cells was observed in common black beans and Mexican red beans but not in adzuki beans (Chilukuri and Swanson, 1991). Sefa-Dedeh and Stanley (1979) evaluated the microstructure of the seed coats of adzuki, blackeye, soy, white and pinto beans and observed an initial decrease in water absorption rate with increasing seed coat thickness.

**Cross-sections of the cotyledons**

Cross-sections of adzuki bean cotyledons revealed storage cells containing starch granules and protein matrices. The arrangement of storage cells varied among the five cultivars of adzuki beans. Storage cells were compactly packed with few intercellular spaces in Erimo, Express, Takara and VBSC cultivars (Figure 12), with many intercellular materials. Contrary to the other four cultivars, Hatsune cultivar revealed loosely packed storage cells with large intercellular spaces and few intercellular materials (Figure 13). The intercellular materials between the cell walls of the adjacent cells may provide mechanical strength and firmness to the plant tissue.

Spherical starch granules were embedded in a granular protein matrix in the five cultivars of adzuki beans. Granules of the protein matrix were more distinct in Erimo (Figure 14) and VBSC (Figure 15) cultivars, than in Express Hatsune and Takara cultivars. Micrographs of the bean flour indicated that the starch granules remained intact upon milling, whereas the protein matrices formed aggregates of a loose granular nature (Figures 16, 17).

Bean cotyledons are the storehouses of common reserve starch, which is used for the nourishment of the growing embryo of the germinating seed. Common bean (*Phaseolus vulgaris* L.) cotyledons contain large (10-50 µm) spherical starch granules and small (5-10 µm) round protein bodies, embedded in a protein matrix (Hughes and Swanson, 1985).

**Starch granules**

The shapes of starch granules varied in the flours of five cultivars observed. Starch granules of Erimo cultivar were spherical, Express and Hatsune cultivars were spherical to irregular (Figure 18), Takara cultivar were highly irregular to slightly spherical and VBSC were oval or spherical to slightly irregular (Figure 19). The shape and size of the starch granules is dependent on the developmental stage of the granule, hence it is difficult to attribute a particular shape and size to a particular cultivar.

No grooves or fissures were observed on the surface of the starch granules of Erimo and VBSC cultivars. Shallow grooves were present on the surface of the starch granules of Express and Hatsune cultivars. The presence of a characteristic deep fissure was unique to Takara cultivar among adzuki beans (Figure 20). Grooves and fissures on the starch granules may be formed after progressive deposition of starch layers in a particular fashion around a nucleation point. Specific morphology and composition of starch granule may be unique to a particular cultivar, and assist in identification or differentiation of cultivars of adzuki beans.
The surfaces of the starch granules appeared smooth at low magnification, but at higher magnification, the surfaces were pitted with no noticeable cracks. Pitted surfaces on starch granules was also observed in faba beans by McEwen et al. (1974) and in Express cultivar by Chilukuri and Swanson (1991). Tjahjadi and Breene (1984), observed deep fissures on starch granules of Takara cultivar of adzuki beans and suggested that the shape and surface characteristics of the starch granules can be used to identify different cultivars of adzuki beans.

Conclusions

The five cultivars of adzuki beans examined in this study showed different patterns of cracks, pits and deposits on the seed coat surfaces. A convoluted pattern of cracks on the seed coat surface was unique to Express cultivar. Examination of cross-sections of the seed coat indicated the absence of typical sub-epidermal layer of columnar, hour-glass cell layer. Cross-section of the cotyledons in Hatsune cultivar revealed loosely packed storage cells with few intercellular materials and many intercellular spaces, which are in contrast to the cotyledon features observed in the other four cultivars. Observation of flours under SEM indicated that protein matrix formed aggregates of loose granular nature, during milling, while starch granules remained intact. The shape of the starch granules was characteristic to each cultivar. Irregularly shaped starch granules with a deep fissure on each granule, were unique to Takara cultivar.

Microstructural differences among adzuki beans are of significant economic importance because they affect grading, handling, storing and marketing conditions. Microstructural differences may also account for differences in functional, textural and physical properties. Seed coat surface characteristics such as deposits, pits and cracks, different cell layers and thickness of the seed coat, and starch granules account for important microstructural variations among adzuki bean cultivars. Thus a knowledge of microstructural variations among adzuki beans will enable us to determine conditions for optimum storage, and marketing conditions.

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References


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