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Microstructure of Peanut Seed: A Review

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Mature peanut seed microstructure of importance to the food industry is examined with regard to: (1) anatomy and cytology of peanut seed; (2) the effects of environment on peanut seed; and (3) the effects of various processing procedures on peanut seed. Current peanut seed microstructure research by the authors is directed toward evaluation of the quality of processed peanuts including using TEM, SEM and LM to evaluate the effects of different times of oven roasting at the same temperature, and a method for evaluating quality of homogenization of broken cell and tissue fragments, protein bodies and starch in stabilized peanut butter.

Peanut seed microstructure can be affected by such factors as: (1) peanut seed maturity; (2) environmental conditions under which the peanuts are grown; and (3) processing by the food industry. All three of these factors impact the microstructural characteristics that affect the quality of processed peanuts.

In this paper we review the literature of peanut seed microstructure of importance to the food industry. Although there are numerous microstructural investigations of the earlier stages of peanut seed development, here we examine only the mature peanut seed microstructure. References to investigations of earlier stages of development are summarized in the appendix and included in the bibliography.

**Anatomy and Cytology of Mature Peanut Seed**

According to Woodroof (1983), maturity of peanut is important because quality grades are dependent on factors related to maturity. Seed maturity is highly significant to the food industry because seed size, flavor, texture and color are influenced by it.

Woodroof and Leahy (1940) describe the mature peanut seed (Fig. 1) as having a seed coat which contains five kinds of cells: (1) outer epidermis; (2) spongy parenchyma; (3) vascular bundles; (4) inner epidermis and (5) perisperm. After the peanut seed matures, the seed coat (skin) has no cellular connection with the cotyledons. The seed coat is tougher and has a more heavily cutinized epidermis than the embryo and protects the embryo from mechanical damage.

The embryo or germ of the mature peanut seed consists of two cotyledons, a small radicle and a plumule (Fig. 2). Processors are chiefly concerned with the tissue of the peanut seed cotyledons which constitutes about 96% of the seed weight; the radicle and plumule (4% of seed weight) are removed in commercial blanching.

Each peanut seed cotyledon consists of epidermal, vascular and parenchyma tissue. The epidermal tissue is made up of a single layer of cells covering the surface of the cotyledon. The epidermal cells of the rounded outer surface are more or less rectangular in outline (Fig. 3). The epidermal cells of the inner surface are irregular in outline and additionally contain numerous guard cells and stomatal...
Figure 1. Mature peanut with seed coat. Bar = 1.5 mm.

Figure 2. Mature peanut with seed coat removed (left) and with seed separated (right) into one of its two cotyledons. Note the rounded outer surface (left) and the indented inner surface (right) which has the radicle and plumule (arrow) intact. Bar = 3 mm.

Figures 3 and 4. Scanning electron micrographs (SEM) of the epidermal cells of a peanut cotyledon. Figure 3: Outer rounded surface. Figure 4. Sunken areas (arrows) where guard cells and stomata occur on the inner surface. Bars = 20 \mu m.

openings (Fig. 4).

The vascular tissue of the peanut seed extends through each cotyledon of the embryo. Woodroof and Leahy (1940) described the vascular system as composed of two series, one series of six to eight bundles that follow the curvature of the outer surface, with another series of four to six centrally located bundles. This vascular tissue (Fig. 5) comprises only a small part of the total volume of each cotyledon.

The majority of the tissue of the cotyledon is made up of rather large, nearly isodiametric, parenchyma cells (Fig. 6). A cytoplasmic network (Fig. 7) extends throughout each parenchyma cell and surrounds the other subcellular organelles. The pitted walls of parenchyma cells from the resting seed have conspicuous depressions (Fig. 8). These pitted walls have been previously described (Woodroof and Leahy, 1940; Vaughan, 1970; Yatsu, 1981; Schadel et al., 1983).

The major subcellular organelles of the parenchyma cells are lipid bodies, protein bodies and starch grains. The
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Figure 5. SEM of cross-section of the vascular tissue (arrows) comprising a small part of each cotyledon. Bar = 50 μm.

Figure 6. Light micrograph of a cross-section of the parenchyma cells comprising the majority of the tissue of a peanut cotyledon. Note the protein bodies (p), starch grains (s) and cytoplasmic network (c) which delimits the spaces once occupied by lipid bodies (alcohol dehydration during specimen preparation removed the lipids). Bar = 20 μm.

Figure 7. SEM of a cross-section of the cotyledon network within a parenchyma cell in the mid-region of a peanut cotyledon. The cytoplasmic network (arrows) demarcates the spaces once occupied by lipid bodies. Bar = 2 μm.

Figure 8. Transmission electron micrograph (TEM) of cross-section of a cell-to-cell junction with pit regions (arrows) between parenchyma cells in the mid-region of a peanut cotyledon. Bar = 0.75 μm.

Transmission electron microscope has been used by Jacks et al. (1967) and Yatsu and Jacks (1972) to characterize the lipid bodies as particles about 1-2 micrometers in diameter bounded by a half unit-membrane (Fig. 9) which measures 2-3.5 nanometers in width.

The protein bodies (Fig. 10) of mature peanut seed range from 5-12 micrometers in diameter. Bagley et al. (1963) studied protein bodies during germination of peanut seed and concluded that there is an ordered series of events leading to the degradation of storage protein in cotyledonary cells during germination.

Starch grains (Fig. 11) of mature peanut seed range
from 4-15 micrometers in diameter and are characterized by a central hilum. Bagley et al. (1963), in a germination study, noted that by 15 days the parenchyma cells still contained numerous starch grains although all protein bodies had degraded by that time.

Effect of Environment on Peanut Seed Microstructure

Poor environmental condition can affect peanut seed microstructure by damaging the structural integrity of the tissue. Drought-induced damage to peanut seed cotyledons has been reported by Young and Schadel (1984, 1989).
Figure 13. SEM of the rounded outer surface of a drought-damaged peanut cotyledon with tissue damage (arrows) which appear as spotting. Bar = 1.0 mm.

Figure 14. SEM of the rounded outer surface of a drought-damaged peanut cotyledon with fissures from which cellular contents have extruded. Note the spherical thickened mass of extruded cellular contents (arrow). Bar = 50.0 μm.

Figure 15. Light micrograph of a cross-section of the outer surface of a drought-damaged peanut cotyledon with tissue damage. Note the extruded cellular content (arrow). Bar = 20.0 μm.

Figure 16. Electron probe microanalyzer linescan of calcium distribution (below the photograph, intensity being proportional to the amount of calcium) in a developing Florunner peanut inside its pod. The solid white line indicates the scanned area of the pod (pod) which surrounds the testa (T) which likewise surrounds the two developing cotyledons (cots). Note higher calcium concentration in the pod and testa. Bar = 1.0 mm. (Photo supplied by Craig Kvien).

Drought-induced damage includes: (1) tissue damage which appears as a narrow band along the interface of the rounded outer surface and the flattened edge of the inner surface (Fig. 12); and (2) tissue damage which appears macroscopically as spotting on the outer surface (Fig. 13) of the cotyledon. The spotting is a result of cracking and fissuring of parenchyma tissue (Figs. 14 and 15).

Poor environmental conditions can also seriously affect peanut seed structural integrity when peanut pods which contain the peanut seeds cannot develop properly. According
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to Smal et al. (1989), developing pods need to absorb adequate amounts of calcium from the soil in order to maintain healthy shells. Smal et al. (1989) studied solution calcium concentration and application date effects on pod calcium uptake and distribution (Fig. 16) in two peanut cultivars differing in shell and seed size. It was determined that large pods with thick, dense shells required higher calcium concentration in the soil to achieve the same seed calcium level as small, thin-shelled peanuts. Peanut cotyledon will not develop when the calcium level is too low.

Peanut pods are also in close contact with many different soil organisms which can destroy the structural integrity of the pods and seeds (i.e. physical decay). Garren and Jackson (1973) state that three potential pod rot fungi - Sclerotium rolfsii Sacc., Pythium myriotylum Drechs., and Rhizoctonia solani Kuhn - cause extensive pod rot damage in a majority of the peanut growing regions of the world. Another fungus, Aspergillus flavus Link, produces aflatoxin, which is a potent mycotoxin and carcinogen. Pettit et al. (1976) believe that one solution to the pod rot and mycotoxin problem is to develop disease resistant varieties. Initial microscope studies were directed towards discovering peanut seed coats which could resist fungal invasion (Dieckert et al., 1973; Taber et al., 1973). These studies revealed differences in the compactness of cell layers and type of wax deposition on the seed coats. Other studies have been conducted to identify features of peanut pods which could function as barriers to fungal invasion (Pettit et al., 1975; 1976; 1977). The most resistant pods contained compact sclerenchyma (scleroid) bands within the mesocarpic tissues.

Effect of Processing on Peanut Seed Microstructure

Woodroof and Leahy (1940) described peanut processing as the use and control of moisture, temperature, pressure, mechanical disintegration and solvents to increase the economic value of the peanuts. The application of such procedures involves process-specific conditions, whether peanuts are to be roasted, blanched, made into peanut butter, used in confection, pressed for oil, or treated for the recovery of proteins.

The most extensive light microscope studies on the effects these procedures have on peanut are by Woodroof and Leahy (1940). They studied the effects of numerous processes on peanut seed cells. In that study, they tabulate the changes in cells when peanut were processed by six different methods: (1) grinding or rolling almost to the fineness of peanut butter; (2) cooking at 250°F (121°C) in presence of high moisture; (3) dry roasting at 300°F (149°C); (4) roasting in oil at 280°F (138°C); (5) cold pressing at ordinary temperatures and a pressure of 5000 to 7000 pounds per square inch (psi); and (6) hot pressing at 250°F (121°C) or higher and a pressure of 5000 to 7000 psi. The results of their study are summarized in Table 1.

With the availability of TEM and SEM (transmission and scanning electron microscopes) other investigators were able to study the effects of processing on the ultrastructure of peanut products. Vix et al. (1972) used TEM to examine the ultrastructure of partially defatted peanuts after hydraulic pressing. They noted that lipid body membranes were broken, cell walls were compressed, and protein bodies were coalesced by pressure. This process for making partially defatted peanuts consists of: (1) hydraulically pressing definned (blanched) peanuts to remove oil; (2) expanding the peanuts in boiling water; (3) drying; and (4) roasting (Vix et al., 1967; Pominski et al., 1970a, b). Yatsu (1981) used SEM to study the cell walls of peanut cotyledons in peanut lots which required an unusual amount of pressure to express the oil. He concluded that cell wall material itself could not account for the extra pressure required by difficult-to-press peanuts. Schadel et al. (1983) investigated the cotyledon structure of peanut seed before and after hydraulic pressing and determined that escape of oil from the parenchyma cells (Fig. 17) was facilitated by surface fissures (Fig. 18) of the pressed cotyledons.

Young and Schadel (1990a) investigated the microstructure of oven-roasted peanuts (160°C, 16 min) using light microscopy (LM) and SEM. After roasting, the cytoplasmic network was disrupted, lipid bodies were burst and protein bodies were distended (Fig. 19 - compare with Fig. 6). Other thermal modifications of roasting including pitting and pock-marking of the epidermis of cotyledons and heat destruction of some middle lamellae of cell-to-cell junctions. Young and Schadel (1990b) also investigated the microstructure of oven-roasted peanuts (160°C, 16 min) using SEM and TEM. The better resolution of TEM enabled clearer documentation of thermal modifications of roasting on cell walls (Fig. 20).

The effects of oil cooking on peanut seed microstructure were evaluated by Young and Schadel (1990c) using TEM, SEM and LM. Many of the thermal modifications of oil cooking were determined to be similar to oven-roasting. For example, decreased electron stain affinity of starch grains (Fig. 21) was observed in oil-cooked as well as in oven-roasted peanuts.

Our current research (unpublished findings) includes using TEM, SEM and LM to evaluate the effects of different times of oven roasting at the same temperature. By examining peanut microstructure after timed intervals of roasting at 160°C, such as 7, 13 and 16 minutes (Figs. 22, 23 and 24, respectively), we are able to determine the extent of increase in thermal modifications (e.g., presence of cell wall separation; degree of protein body distension; range of cytoplasmic network disrupted) that occurred for each increased roasting time period.

Although we use primarily SEM and TEM to evaluate the effects of oil cooking and oven roasting on peanut seed microstructure, F.O. Flint (Univ. Leeds, personal communication) notes that the light microscope can be a useful tool in examining microstructural features, such as starch grains, after oil cooking or oven roasting. Flint has observed, for example, that the retention of birefringence in starch grains (Fig. 25) of oil-cooked peanuts indicated the absence of thermal damage. Young and Schadel’s work with the light microscope (unpublished findings) also indicated little thermal damage to starch grains after oil cooking at 160°C for 12 min (Fig. 26) or oven roasting at 160°C for 16 min (Fig. 27) since many of the starch grains retained a visible hilum.

Recently we (Young and Schadel, 1990d) have developed a method for evaluating the quality of homogenization
Figure 17. SEM of a cross-section of parenchyma cells of a hydraulically pressed peanut cotyledon. Note the absence of the distinct cytoplasmic network. Bar = 20.0 μm.

Figure 18. SEM of the rounded outer surface of a hydraulically pressed peanut cotyledon. Note the fissures (arrows) along the epidermal cell caused by hydraulic pressing. Bar = 20.0 μm.

Figure 19. Light micrograph of a cross-section of the parenchymal cells in a roasted peanut cotyledon. Note the distended protein bodies (P) and starch grains (S). Bar = 20.0 μm.

Figure 20. TEM of a cross-section of a cell-to-cell junction of parenchymal cells in a roasted peanut cotyledon. Observe that thermal modification has caused the disintegration (arrow) of the middle lamellum. Bar = 0.25 μm.

Future Research

Suggestions for additional research on the effects of processing on peanut seed microstructure include:

1) characterization of the surface structure of raw cotyledons as this relates to ease of blanching (skin removal);
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Figure 21. TEM of a cross-section of a starch grain within an oil-cooked peanut cotyledon. Note the electron transparent nature of some regions (arrows) of the starch grain. Bar = 0.5 μm.

Figures 22-24. SEM of cross-sections of parenchyma cells in the mid-region of a peanut cotyledon after 7 minutes (Figure 22), 13 minutes (Figure 23) and 16 minutes (Figure 24) of oven roasting at 160°C. Bars = 10.0 μm.

(2) determining advantages or disadvantages of different cooking methods such as microwave or infrared;
(3) evaluating optimum cooking rates that induce the least physical damage; and
(4) examining surface changes during cooking which may facilitate adhesion of coatings for peanut products.

Appendix - Additional References

For general peanut anatomy and cytology
Badami, 1935.

Bagley et al., 1963.
Banerji, 1938.
Conagin, 1957.
Dieckert and Snowden, 1960.
Jacks et al., 1967.
Mohammed et al., 1933.
Moss et al., 1988.
Figure 25. Polarized light micrograph of a cross-section of oil-cooked peanut showing distribution of starch which retains birefringence after roasting. Bar = 20 μm. (Photograph kindly provided by Dr. F. Olga Flint, Univ. Leeds, U.K.).

Figures 26-27. Light micrographs of parenchyma cells in the mid-region of peanut cotyledons after 12 minutes of oil cooking (Figure 26) and 16 minutes of oven roasting (Figure 27) at 160°C. Note the visible hilums of the starch grains (arrows). Bars = 20 μm (Figure 26) and 10 μm (Figure 27).

Figure 28. Light micrograph of a cross-section of stabilized peanut butter with complete homogenization of microstructural features. Note that broken cell wall fragments (purple), protein-bodies (red), and starch grains (white) are well-dispersed in a matrix of stabilized oil (grey). Bar = 10 μm.

Pettit, 1895.
Reed, 1924.
Shibuya, 1935.
Smith, 1950, 1956a, 1956b.

Thomas et al., 1983.
Vaughan, 1970.
Waldron, 1919.
Winton, 1904.
**Table 1: Changes in cells when peanuts were processed (from Woodroof and Leahy, 1940).**

<table>
<thead>
<tr>
<th>Processing Method</th>
<th>Cells and Cell Walls</th>
<th>Inter-Cellular Spaces</th>
<th>Nucleus</th>
<th>Aleurone Grains</th>
<th>Oil Droplets</th>
<th>Starch Grains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grinding or rolling almost to fineness of peanut butter.</td>
<td>About 50% of cells ruptured &amp; emptied of contents, many others were merely crushed, while from 10-25% were uninjured.</td>
<td>No change when cells were un-injured.</td>
<td>No change when cells were un-injured.</td>
<td>Relatively few were crushed. Most of them were crushed about independently even when the cell wall was severely crushed.</td>
<td>When the cells were crushed, the oil droplets ran together to form larger drops of free oil. If cells were not crushed, no change occurred in oil droplets.</td>
<td>Some were crushed, while most of them were not.</td>
</tr>
<tr>
<td>Cooking at 250°F (121°C) in presence of high moisture.</td>
<td>Turgidity destroyed; cell walls become slack, but only a few were broken.</td>
<td>Almost eliminated.</td>
<td>Coagulated, granular, centrally located but tended to break up.</td>
<td>Completely separated, scattered throughout cell and a few become lopsided. Total size and number unchanged.</td>
<td>Fusing of some droplets to form fewer and larger droplets within cells. A few drops of free oil appeared in each cell. Viscosity of oil greatly lowered.</td>
<td>Apparently destroyed or dissolved.</td>
</tr>
<tr>
<td>Dry roasting at 300°F (149°C).</td>
<td>Cell walls remained unchanged.</td>
<td>No change.</td>
<td>Coagulated into hard mass in center of cell.</td>
<td>Drawn away from cell walls into hard mass around nucleus. The number and size of grains was little changed. Proteins were precipitated and very granular.</td>
<td>Fused into larger and fewer droplets within the cells. Small amount of oil escaped into the cell as free oil. Cell walls and skins became oil-soaked.</td>
<td>Remained same in number and slightly reduced in size.</td>
</tr>
<tr>
<td>Roasting in oil at 280°F (138°C).</td>
<td>No apparent change.</td>
<td>No change.</td>
<td>Coagulated but almost indistinct due to crowding in cell.</td>
<td>Few grains were normal size, most of them were swollen 3 to 4 times original size due to absorption of oil. They were precipitated and very granular.</td>
<td>Due to absorption of oil from cooking bath and consequent swelling, the original oil droplets lost their identity.</td>
<td>No apparent change.</td>
</tr>
<tr>
<td>Cold pressing at ordinary temperature and 5000-7000 psi pressure.</td>
<td>Most of cells completely crushed.</td>
<td>None.</td>
<td>Crushed.</td>
<td>Mashed and distorted but many of them retained their original identity.</td>
<td>When the cells were crushed, the oil droplets ran together forming drops of free oil. If cells were not crushed, no change occurred in oil droplets.</td>
<td>Crushed.</td>
</tr>
<tr>
<td>Hot pressing at 250°F (121°C) or higher and 5000-7000 psi pressure.</td>
<td>Crushed.</td>
<td>None.</td>
<td>Crushed.</td>
<td>Crushed.</td>
<td>Viscosity of oil made very low. All droplets fused into drops of free oil.</td>
<td>Crushed.</td>
</tr>
</tbody>
</table>
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Woodroof, 1983.
Yarborough, 1949.
Yatsu and Jacks, 1972.

For effects of environment on peanut microstructure
Dieckert et al., 1973.
Smal et al., 1989.
Taber et al., 1973.

For effects of processing on peanut microstructure
Mikola et al., 1975.
Schadel et al., 1983.
Woodroof and Leahy, 1940.
Yatsu, 1981.
Young and Schadel, 1990a, 1990b, 1990c, 1990d.

References

Thomas RJ. Petit RE, Taber RA, Jones BD (1983). Peanut peg strength: Force required for pod detachment in...


Young CT, Schadel WE (1990c). The microstructure of peanut (Arachis hypogaea L. cv. Florigiant) cotyledons after oil cooking. J. Food Sci. accepted for publication.


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Discussion with Reviewers

Y.C. Hung: How much thermal modification is needed for peanuts during roasting in order to provide good eating quality?

Authors: Thermal modification of peanuts during roasting creates chemical as well as physical changes. When peanuts are roasted, amino acids combine with sugars to form flavor components of roasted peanuts. The production of these flavors combined with textural changes resulting from thermal modification of microstructure is optimal after roasting peanuts for 16 minutes at 160°C.

R.A. Taber: Can you, at this point in your research program, relate changes in cv. Florunner seed to changes in Spanish peanut cultivars after roasting?

Authors: Changes in peanut microstructure after roasting appear to be related to size and shape of cotyledons. As size and shape of cotyledons vary among cultivars, we believe rates of thermal modification also will vary. Roasting studies of cultivars that are smaller than cv. Florunner (e.g., Spanish peanuts) are best for testing this hypothesis.

R.A. Taber: Do the temperatures reached during roasting impact on the cuticular waxes such that they enter the seed coat?

Authors: Structural observations reveal that the epidermis which contains the cuticular waxes becomes pitted and pocked during roasting. We have not yet done histochemical tests for waxes in the seed coats to determine if the waxes enter the seed coat after roasting.

R.A. Taber: Could SEM X-ray microanalysis provide some helpful additional information concerning differences in element distribution in treated and untreated seeds?

Authors: Qualitative and quantitative elemental analysis using SEM should provide additional helpful information concerning the differences in treated and untreated seeds. However these methods have yet to be extensively applied in the studies of peanuts.

R.A. Taber: It would be helpful to have more detailed comparisons between effects on structures of roasted versus oil cooked peanuts.

Authors: We have recently completed an investigation comparing the effects on structures of roasted and oil cooked peanut seeds and are currently working on the manuscript for publication.