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TRANSMISSION AND SCANNING ELECTRON MICROSCOPY OF PEANUT (Arachis hypogaea L. CV. FLORIGIANT) COTYLEDON AFTER ROASTING

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Abstract
Changes in the microstructure of peanut (Arachis hypogaea L. cv. Florigiant) cotyledon after roasting at a temperature of 160°C for 16 minutes were investigated with transmission and scanning electron microscopy. Thermal modifications were documented with photomicrographs of the cytoplasmic network, protein bodies, starch grains and cell-to-cell junctions after oven roasting. These thermal modifications include disruption of the cytoplasmic network, distension of protein bodies, decreased stain affinity of starch grains, and disintegration of middle lamellae in some cell-to-cell junctions.

Introduction
Observation of the changes in the microstructure of peanut cotyledons after oven roasting enables investigators to understand thermal modifications that occur during roasting. Young and Schadel (1990) first noted thermal modifications of oven roasted peanut cotyledons microstructure using light and scanning electron microscopy (SEM). The purpose of the present study was to use transmission electron microscopy (TEM) in conjunction with SEM to achieve a greater resolution of the thermal modifications of peanut cotyledon microstructure after oven roasting.

Materials and Methods
Fixation Methodology
Cotyledons of peanuts (Arachis hypogaea L. cv. Florigiant) were obtained from the Tidewater Research Station, Suffolk, VA. Raw peanut cotyledons with intact skins were roasted in a hot air oven at 160°C for 16 minutes. Both raw and roasted peanut cotyledons were then prepared for TEM and SEM. Tissue blocks (1 mm³) of outer surface epidermis, mid-region parenchyma, and inner surface epidermis were cut from both the raw and roasted peanut cotyledons and fixed in a Karnovsky's (1965) fixative as modified by Young and Schadel (1989). Our modified fixative was prepared by mixing 25 mL of 8% formaldehyde, 3.6 mL of 70% glutaraldehyde and 28.6 mL of 0.1 M sodium phosphate buffer (hereinafter referred to as buffer). The pH of the mixture was adjusted to 7.0. The tissue blocks were fixed under vacuum for 30 minutes at 23°C and then fixed at atmospheric pressure for 48 hours at 4°C. Following a 24 hour wash in 6 changes of 0.1 M buffer (4°C, pH 7.0), the material was post-fixed for one hour in 1% osmium tetroxide in cold 0.1 M buffer. After post-fixation, the material was washed for 30 minutes in 0.1 M buffer (4°C pH 7.0) and dehydrated at room temperature in a graded series of aqueous ethanol (10, 25, 50, 75, and 95%) and then finally in absolute ethanol.

Preparation for TEM
Dehydrated tissue was embedded in Spurr's resin using the methodology of Spurr (1969) for long post-fixation electron microscopy, starch, protein, staining.

Key Words: Fixation, thermal modification, peanuts, scanning electron microscopy, transmission electron microscopy, starch, protein, staining.
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Figures 1 and 2. Scanning electron micrographs of cross-sections of parenchyma cells in the mid region of raw (Fig. 1) and oven roasted (Fig. 2) peanut cotyledons. Fig. 1 shows the cytoplasmic network (arrows) surrounding the storage reserve bodies of protein and starch, and the spaces once occupied by lipid bodies before removal of the lipid bodies by alcohol during specimen dehydration. Fig. 2 shows the loss of cellular organization and the absence of cytoplasm along the periphery of the cells (arrows).

Bars = 2 micrometers (Fig. 1) and 10 micrometers (Fig. 2).

Preparation for Scanning Electron Microscopy (SEM)
Dehydrated tissue was critical point dried in a Tousimis PVT-3B unit using liquid CO₂. Subsequently, the dried sections were mounted on aluminum specimen stubs with double-sided tape and silver conducting paint. Prepared stubs were coated with 30 nm gold-palladium alloy at room temperature in a Hummer V sputter coater fitted with a Technics Digital Thickness Monitor. Specimens were viewed with a Philips 505T SEM at a working distance of 15 mm and an accelerating voltage of 15 kV.

Results and Discussion
The majority of peanut cotyledonary tissue is made up of parenchymal cells. The major subcellular organelles of these parenchymal cells are lipid bodies, protein bodies and starch grains surrounded by a cytoplasmic network. With SEM, the cytoplasmic network can be observed to surround the almost spherical protein bodies, starch grains and spaces once occupied by lipid bodies before removal of the lipid bodies by alcohol dehydration during specimen preparation (Fig. 1). However, protein bodies and starch grains cannot be distinguished from one another with SEM since both of these organelles appear as indistinguishable smooth spheres. The use of SEM in observing the microstructure of parenchymal cells from oven roasted cotyledons (Fig. 2) has even more limited because oven roasting has disrupted the cytoplasmic network and distended protein bodies and starch grains. Therefore the use of TEM, in addition to SEM, is necessary to achieve a more thorough understanding of the thermal modifications of peanut cotyledonary microstructure after roasting.

When viewed with TEM, the cell-to-cell junctions are characterized by a distinct middle lamella (Fig. 3) existing between parenchymal cells of raw peanuts. After oven roasting some of these middle lamella (Fig. 4) separate as a result of thermal modifications which occur primarily within the first mm of tissue beneath the rounded outer cotyledon surface. In the raw cotyledon, the protein bodies are almost circular in outline and are surrounded by numerous lipid body membranes (Fig. 5). The electron dense protein bodies have a grainy appearance which is distinct from the smooth appearance of electron dense starch grains. After oven roasting the protein bodies are distended (Fig. 6). In the raw cotyledon, the starch grains appear compact and electron dense, and the cytoplasmic network is intact (Fig. 7). After oven roasting, the starch grains have some electron transparent regions and cytoplasmic network has been disrupted (Fig. 8). In the raw peanut cotyledon, the cytoplasmic network

Figures 3-8. Transmission electron micrographs of:
Fig. 3. A cross-section of a cell-to-cell junction of parenchymal cells in a raw peanut cotyledon. Note the distinct middle lamella (arrow).
Fig. 4. A cross-section of a cell-to-cell junction of parenchymal cells in an oven roasted peanut cotyledon. Observe that thermal modification has caused the cell walls to separate along the middle lamella (arrow).
Fig. 5. A protein body (P) surrounded by the cytoplasmic network within a raw peanut cotyledon. Note that the electron dense protein body is almost circular in outline and has a grainy appearance.
Fig. 6. A protein body (P) within an oven roasted peanut cotyledon. Note that the thermal modification has caused the protein body to become distended.
Fig. 7. A cross-section of a starch grain (ST) within a raw peanut cotyledon. Note the electron dense nature (arrow) of the starch grain and the continuous cytoplasmic network (points).
Fig. 8. A cross-section of a starch grain (ST) adjacent to disrupted cytoplasm (large arrows) within an oven roasted peanut cotyledon. Observe the electron transparent nature of some regions of the starch grains (small arrows).
Bars = 0.5 (Figs. 3, 7 and 8), 0.25 (Figs. 4 and 6) and 0.75 (Fig. 5) micrometers.
surrounding the lipid body membranes (Fig. 9) is continuous. After oven roasting, the heat has disrupted the cytoplasmic network (Fig. 10).

In summary, observations with TEM, in conjunction with SEM, reveal that oven roasted peanut cotyledonary parenchymal cells possess the following characteristics as a result of thermal modification:

1. some cell wall separations primarily within the first mm of tissue beneath the rounded outer cotyledon surface,
2. distended protein bodies,
3. starch grains with decreased stain affinity, and
4. disruption of the cytoplasmic network.

Figures 9 and 10. Transmission electron micrographs of:

Fig. 9. A cross-section of a parenchyma cell in a raw peanut cotyledon. Note that the cytoplasmic network is continuous (arrows). Bar = 0.75 micrometers.

Fig. 10. A cross-section of the disrupted cytoplasmic network (arrow) adjacent to a protein body (P) in an oven roasted peanut cotyledon. Bar = 0.75 micrometers.

References


