A Comparison of the Effects of Oven Roasting and Oil Cooking on the Microstructure of Peanut (Arachis Hypogaea L. cv. Florigiant) Cotyledon

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A COMPARISON OF THE EFFECTS OF OVEN ROASTING AND OIL COOKING ON THE MICROSTRUCTURE OF PEANUT (ARACHIS HYPOGAEA L. cv. FLORIGIANT) COTYLEDON

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Abstract

Peanut (Arachis hypogaea L. cv. Florigiant) cotyledon microstructure after oven roasting and oil cooking at 160°C was documented with scanning electron microscopy, light microscopy and transmission electron microscopy. Changes in peanut cotyledon microstructure were compared as thermal processing time at 160°C was increased for both oven roasting and oil cooking. The purpose was to evaluate thermal modifications in the cytoplasmic network, protein bodies and cell-to-cell junctions as thermal processing time increased for each heating method. Principal findings included differences in the times at which these modifications occurred during the two thermal processes. Oven roasting at 160°C consistently induced similar thermal modifications more slowly than oil cooking at 160°C.

Key Words: Light microscopy, scanning electron microscopy, transmission electron microscopy, oil cooked peanuts, oven roasted peanuts.

Introduction

Observation of the microstructural changes in peanut cotyledons during oven roasting and oil cooking enables processors to evaluate the extent of thermal modifications such as cytoplasmic network disruption, protein body distension, and cell wall separation that occur during these processes. Young and Schadel (1990a, 1990b) observed thermal modifications of oven roasted peanut cotyledon microstructure using light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Young and Schadel (1991) also documented these thermal modifications in oil cooked peanut cotyledon microstructure.

In the present study SEM, LM, and TEM of the changes in peanut cotyledon microstructure were compared as thermal processing time at 160°C was increased for oven roasting and oil cooking. The objective was to document thermal modifications in the cytoplasmic network, protein bodies and cell-to-cell junctions as thermal processing time increased for each heating method.

Materials and Methods

Fixation methodology

Cotyledons of peanuts (Arachis hypogaea L. cv. Florigiant) were obtained from Tidewater Research Station, Suffolk, Virginia. Raw peanut cotyledons with intact skins were roasted in a 160°C oven. Cotyledons were collected at intervals of 7, 13, 16 and 19 minutes. Raw peanut cotyledons with intact skins were oil cooked in 160°C oil. Cotyledons were collected at intervals of 4, 8, 10 and 12 minutes.

Raw, oven-roasted, and oil-cooked peanut cotyledons were prepared for LM, SEM and TEM examination. Tissue blocks (1 mm³) of outer surface epidermis, mid-region parenchyma and inner surface epidermis were cut from peanut cotyledons and fixed in Karnovsky’s fixative (Karnovsky, 1965) as modified by Young and Schadel (1989). The 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. 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 six changes of 0.1 M sodium phosphate buffer (4°C, pH 7.0), the material was post-fixed for 1 hour in 1% osmium tetroxide in 0.1 M buffer (4°C, pH 7.0) and dehydrated at room temperature at 15 minutes intervals in a graded series of aqueous ethanol (10, 25, 50, 75 and 95%) and then finally at two 30 minute intervals in absolute ethanol.

Preparation for scanning electron microscopy (SEM)

Dehydrated peanut tissue was critical point dried in a Tousimis unit (Ladd) using liquid CO₂. Dried sections were mounted on aluminum specimen stubs with double-sided tape and silver conducting paint. Sections of peanut tissue on stubs were coated with palladium in a Tousimis unit (Ladd) using liquid H₂. Preparations were mounted on aluminum specimen stubs with a Technics Digital Thickness Monitor. Specimens were examined in a Phillips SEM.

Preparation for light microscopy (LM)

Dehydrated peanut tissue was embedded using the methodology of Spurr (1969) for long potlife resin. Sections, which were 7 μm in thickness, were cut using a Reichert ultramicrotome and glass knives. After mounting on glass slides, the sections were stained with 1% acid fuchsin and 1% toluidine blue using the methods of Feder and O'Brien (1968). Stained sections were observed using a Wild light microscope equipped for bright-field and polarized light microscopy and fitted with a 35 mm camera.

Preparation for transmission electron microscopy (TEM)

Dehydrated peanut tissue for TEM was also embedded in Spurr's resin. Ultrathin sections cut with a Reichert ultramicrotome were stained with 4.0% uranyl acetate for 1 hour, followed by 0.4% lead citrate for 4 minutes. Sections were examined with a JEOL 100S electron microscope.

Results

Scanning electron microscopy

To facilitate comparison of thermal modifications in similar anatomical areas for each heating method, the scanning electron micrographs (Figures 1-25) are presented in the following groups: (1) outer epidermal surface; (2) outer epidermal cross-section; (3) mid-region cross-section; (4) inner epidermal surface view; and (5) inner epidermal surface view. Each anatomical area is subgrouped so that SEM of raw peanut cotyledon is followed by SEM of oven roasted peanut cotyledon (13 and 19 minutes respectively) which in turn is followed by SEM of oil cooked peanut cotyledon (8 and 12 minutes, respectively).

SEM: Raw peanut cotyledon

The epidermal cells of the rounded outer surface (Fig. 1) are nearly rectangular in outline in the raw peanut. When the epidermal cells of the rounded outer surface are viewed in cross-section (Fig. 6), the smaller size of the epidermal cells can be compared with the larger parenchymal cells which subtend the epidermal cells. The parenchymal cells of the mid-region (Fig. 11) of the raw cotyledon contain a cytoplasmic network that surrounds the subcellular organelles which include starch grains, protein bodies and the spaces once occupied by lipid bodies (the lipids were removed during alcohol dehydration). The protein bodies and starch grains appear as large (3.0 - 10.0 μm) distinct spherical bodies but are indistinguishable from one another by SEM.

When the epidermal cells of the flat, inner surface are viewed in cross-section (Fig. 16), the subcellular organization in these cells can be seen. The epidermal cells of the flat, inner surface (Fig. 21) are characterized by their irregular shape and the presence of stomata.

SEM: Oven roasted and oil cooked peanut cotyledons

During both heating methods, the epidermal cells of the outer and inner surface became swollen as a result of escape of internal steam and oil released from lipid bodies during heating. Tissue areas gradually lost subcellular organization as cytoplasmic networks became increasingly disrupted with increased heating time.

Oven roasting at 160°C consistently induced similar thermal modifications in peanut cotyledon microstructure more slowly than oil cooking at 160°C. For example, epidermal cells on the rounded outer surface of an oven roasted peanut became swollen after 13 minutes at 160°C (Fig. 2) while epidermal cells on the rounded outer surface of an oil cooked peanut became swollen after only 8 minutes at 160°C (Fig. 4). Parenchymal cells which subtend the epidermal cells of the rounded outer surface of an oven roasted peanut exhibited nearly complete disruption of the cytoplasmic network after 19 minutes at 160°C (Fig. 8) while similarly located parenchymal cells of an oil cooked peanut exhibited nearly complete disruption of the cytoplasmic network after only 12 minutes at 160°C (Fig. 10).

All thermal modifications are summarized for both heating methods in Table 1, and the scanning electron micrographs are cross-referenced therein (Figs. 1-25).

Light microscopy: Raw, oven roasted and oil cooked peanut cotyledons

Under bright-field microscopy, an individual starch grain which had a clearly visible hilum in raw peanut (Fig. 26) retained a clearly visible hilum in all tissue areas after both heating methods. This included 19 minutes of oven roasting as well as 12 minutes of oil cooking (Fig. 27). Under polarized light microscopy, starch grains which were birefringent in raw peanuts, retained their birefringence in all tissue areas after both heating methods. Thus, the starch grains sustained no apparent thermal damage after either heating method.

During both heating methods however, most tissue areas progressively lost their subcellular organization as the cytoplasmic network became disrupted with...
Thermal modifications of peanut cotyledon

<table>
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<th>Raw Peanut</th>
<th>Oven Roasted Peanut (7 min, 160°C)</th>
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<th>Oven Roasted Peanut (19 min, 160°C)</th>
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Thermal modifications of peanut cotyledon

Figures 1-25. Scanning electron micrographs of peanut cotyledons.

Note: All micrographs are at same magnification as represented by the 10 \( \mu \text{m} \) bar in Figure 1.

Figure 1. Epidermal cells of the rounded outer surface of a raw peanut cotyledon.

Figures 2-3. Epidermal cells of the rounded outer surface after oven roasting at 160°C for 13 minutes (Fig. 2); and 19 minutes (Fig. 3).

Figures 4-5. Epidermal cells of the rounded outer surfaces after oil cooking at 160°C for 8 minutes (Fig. 4); and 12 minutes (Fig. 5).

Figure 6. A cross-section of the rounded outer surface of a raw peanut cotyledon. Epidermal cells (small arrow); parenchymal cells (large arrow).

Figures 7-8. Cross-sections of the rounded outer surfaces after oven roasting at 160°C for 13 minutes (Fig. 7); and 19 minutes (Fig. 8).

Figures 9-10. Cross-sections of the rounded outer surfaces after oil cooking at 160°C for 8 minutes (Fig. 9); and 12 minutes (Fig. 10).

Figure 11. A cross-section of parenchymal cells in the mid-region of a raw peanut cotyledon. Observe the cytoplasmic network (arrow) between the other subcellular organelles.

Figures 12-13. Cross-sections of parenchymal cells in the mid-region after oven roasting at 160°C for 13 minutes (Fig. 12); and 19 minutes (Fig. 13).

Figures 14-15. Cross-sections of parenchymal cells in the mid-region after oil cooking at 160°C for 8 minutes (Fig. 14); and 12 minutes (Fig. 15).

Figure 16. A cross-section of the flat, inner surface of a raw peanut cotyledon.

Figures 17-18. Cross-sections of the flat, inner surfaces after oven roasting for 13 minutes (Fig. 17); and 19 minutes (Fig. 18).

Figures 19-20. Cross-sections of the flat inner surfaces after oil cooking for 8 minutes (Fig. 19); and 12 minutes (Fig. 20).

Figure 21. Epidermal cells of the flat, inner surface of a raw peanut cotyledon. Note the presence of a stomate (arrow).

Figures 22-23. Epidermal cells of the flat, inner surfaces after oven roasting at 160°C for 13 minutes (Fig. 22); and 19 minutes (Fig. 23).

Figures 24-25. Epidermal cells of the flat, inner surfaces after oil cooking at 160°C for 8 minutes (Fig. 24); and 12 minutes (Fig. 25).
Figure 26. LM of a cross-section of parenchymal cells which subtend the epidermal cells of the rounded outer surface of a raw peanut cotyledon. Note the visible hilum (arrow) within the center of the starch grain. Bar = 10 μm.

Figure 27. LM of a cross-section of parenchymal cells which subtend the epidermal cells of the rounded outer surface of a peanut cotyledon after oil cooking at 160°C for 12 minutes. Note the visible hilum (arrow) within the center of the starch grain, which indicates structural resistance to thermal damage. Bar = 10 μm.

Figure 28. TEM of a cross-section of a cell-to-cell junction of parenchymal cells which subtend the epidermal cells of the rounded outer surface of a raw peanut cotyledon. Note the distinct middle lamellum (arrow). Bar = 0.5 μm.

Increased heating time. This disruption was initially small (< 10% disruption/cell) for the shorter heating intervals but became extensive (> 50% disruption/cell) for the longer heating intervals. During both heating methods, protein bodies gradually became more distended with increased heating time. During both heating methods some cell-to-cell separations occurred in the tissue areas approximately 1 mm beneath the outer and inner epidermis but not in the mid-region parenchymal tissue.

All thermal modifications are summarized for both heating methods in Table 1, and the light micrographs are cross-referenced therein (Figs. 26-27).

TEM: Raw peanut cotyledon

In the raw peanut cotyledon, the ultrastructure of the cell-to-cell junctions is characterized by a distinct middle lamellum (Fig. 28) and the protein bodies are nearly circular in outline with a distinct matrix (Fig. 29). The cytoplasmic network (Fig. 29) demarcates the spaces once occupied by lipid bodies. Alcohol dehydration removed the lipids contained in the lipid bodies during specimen preparation.

TEM: Oven roasted and oil cooked peanut cotyledons

During both methods the following thermal modifications were observed with increased heating time: (1) the cytoplasmic network became gradually disrupted; (2) the protein bodies became more distended; and (3) some
Thermal modifications of peanut cotyledon

**Figure 29.** TEM of a cross-section of a protein body (P) within a parenchymal cell in the mid-region of a raw peanut cotyledon. Note that the protein body (P) is almost circular in outline and is surrounded by the cytoplasmic network (arrows). Bar = 0.75 μm.

**Figure 30.** TEM of a cross-section of the separation of a cell-to-cell junction of two parenchymal cells which subtend the epidermal cells of the outer rounded surface of a peanut cotyledon after oven roasting at 160°C for 16 minutes. Note the remnants of a middle lamellum (arrow). Bar = 0.25 μm.

**Figures 31-32.** TEM of cross-sections of a severely distended protein bodies (P) within parenchymal cells which subtend the epidermal cells of the rounded outer surface of peanut cotyledon after oven roasting at 160°C for 19 minutes (Fig. 31) and after oil cooking at 160°C for 12 minutes (Fig. 32). Note the remnant of a disrupted protein body membrane (arrow) in Figure 31. Bars = 0.25 μm.
cell-to-cell junctions primarily within the first 1 mm beneath the cotyledon outer and inner surfaces disintegrated along the middle lamellae.

Oven roasting at 160 °C consistently induced similar thermal modifications in peanut cotyledons more slowly than oil cooking at 160 °C. For example, cell wall separation along the middle lamellae (Fig. 30) occurred within the parenchymal cells which subtend the epidermal cells of the rounded outer surface of an oven roasted peanut cotyledon after 16 minutes at 160 °C. These same thermal modifications occurred in similarly located parenchymal cells after only 10 minutes of oil cooking at 160 °C.

Although protein bodies never fused to one another after either heating method, severe protein body distension was observed within the parenchymal cells which subtend the epidermal cells of the rounded outer surface of an oven roasted peanut after 19 minutes at 160 °C (Fig 31). Severe protein body distension was observed in similarly located parenchymal cells after only 12 minutes of oil cooking at 160 °C (Fig. 32).

All thermal modifications are summarized for both heating methods in Table 1, and the transmission electron micrographs are cross-referenced therein (Figs. 28-32).

Discussion

The coordinated use of SEM, LM and TEM provided corroborative evidence of the thermal modifications of cytoplasmic network disruption, protein body distension and cell wall separation that gradually increased with increased heating time after oven roasting and oil cooking at 160 °C. A comparison of the effect of oven roasting and oil cooking indicated that similar thermal modifications occurred after both heating methods. Yet, oven roasting at 160 °C consistently induced similar thermal modifications more slowly than oil cooking at 160 °C.

For example, the thermal modifications which occurred in the outer epidermal cross-section of an oven roasted peanut after 19 minutes at 160 °C were characterized by greater than 50% disruption of the cytoplasmic network, severe protein body distension and the presence of cell wall separation along the middle lamellae. Equivalent thermal modifications in the same anatomical area of an oil cooked peanut occurred after only 12 minutes at 160 °C (see Table 1 for other comparisons).

Discussion with Reviewers

I. Heertje: How were the final product properties affected by the thermal treatments and related microstructural observations?

Authors: This is an important question which was, however, not addressed during the present investigation. We are currently evaluating the effects of thermal treatments on final product properties (and their correlation with the observed microstructure) and hope to report that in a separate publication.

References