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EFFECT OF ENVIRONMENT ON THE PHYSICAL STRUCTURE OF THE PEANUT (*Arachis hypogaea* L.)

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Abstracts

Peanuts produced under the drought conditions of 1980 were marred by off-flavors when processed. Several physical characteristics of these peanuts were noted to be related to these flavor problems. This paper deals with the investigation of these physical peculiarities using scanning electron microscopy. Major findings include previously unreported physical abnormalities such as: (1) tissue damage which appears as spotting on the outer surface of the cotyledon which is a result of cracking and fissuring; (2) tissue damage which appears as a narrow band along the interface of the outer rounded surface and the flattened edge of the inner surface; and (3) a flattened inner surface of the cotyledon.

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Introduction

Drought conditions persisted throughout the summer of 1980 in all three of the United States peanut growing regions (USDA, 1981). Temperatures were above normal and rainfall was well below average for the summer months. By August, temperatures were abnormally high setting records in some areas. In December, our peanut laboratory was involved in the evaluation of these peanuts because of a severe off-flavor problem. In the observation of these peanuts, the following abnormalities were noted: (1) tissue damage which appeared as spotting on the outer surface of the cotyledon; (2) tissue damage which appeared as a narrow band along the edges of the inner surface of the cotyledon; and (3) a flattened inner surface of the cotyledon without its characteristic indentation. Similar observations were made with peanuts grown in the ensuing years under similar conditions of growth. Thus, it appears that changes in the structural features may be related to changes in flavor of peanuts.

Peanut seed anatomy and cytology have been investigated by Woodroof and Leahy (1940), Yarbrough (1949), Bagley et al. (1963), Jacks et al. (1967), and Vaughan (1970). More recently, observations were made on the appearance of cell walls and the major subcellular components of both normal and pressed peanuts (Schadel et al., 1983). Also, light and scanning electron microscopy clearly showed pitting of parenchyma cell wall in normal peanut seed.

In accordance with the statement of Chabot (1979) that "the goal of the food scientist is to understand structural features of a material that are important in its functional role in food," we used scanning electron microscopy (SEM) for this study to examine structural differences between normal and environmentally stressed peanuts in an attempt to learn more about the physical defects in stressed peanuts and their possible role in causing flavor defects.

Materials and Methods

The cotyledons of resting peanut (*Arachis hypogaea* L. cv. Florunner) seed were examined with a dissecting microscope for physical structural characteristics. They were divided into two groups: (1) cotyledons with normal physical characteristics associated with environmentally unstressed peanuts; and (2) cotyledons with abnormal physical characteristics associated

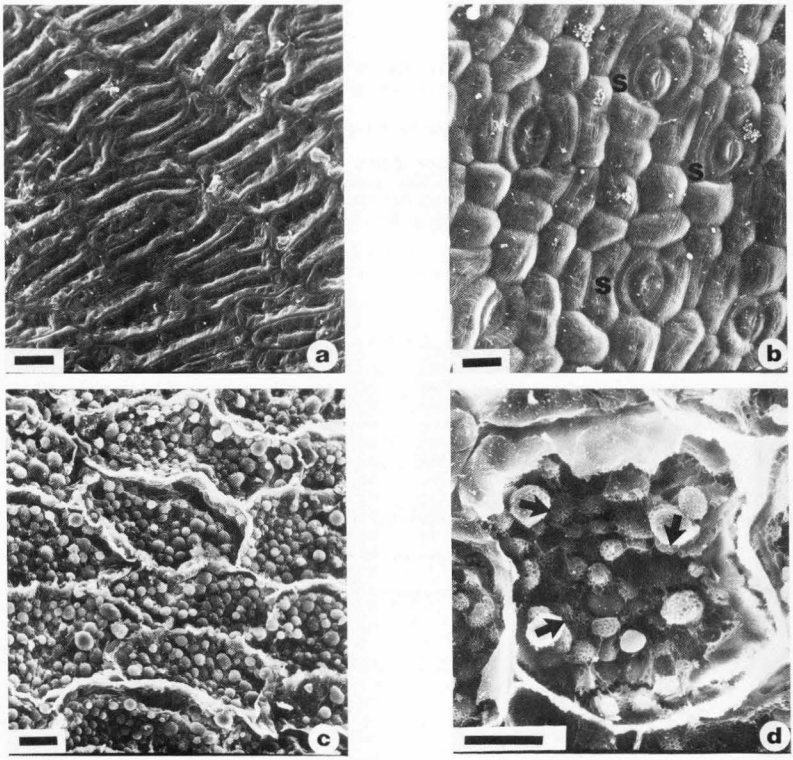


Fig. 1. Normal peanut structure: (a) Epidermal cells of the outer rounded surface of the cotyledon; (b) Epidermal cells with stomata (S) on the inner surface of the cotyledon; (c) Transverse section of the isodiametric parenchyma cells; and (d) Transverse section of a single parenchyma cell. Arrows point to the membrane-limited spherosomes (lipid bodies) about 1–2 μm in diameter, among the larger protein bodies and starch grains. Bars = 40.0 μm.

with environmental stress. Whole cotyledons, transverse sections of the mid-region of cotyledons, and tissue blocks (2 mm^3) from the mid-region and the outer and inner surfaces of the cotyledons were fixed in 4% glutaraldehyde in 0.05 M sodium cacodylate, pH 7.0, for 48 hours. The samples were then rinsed and post-fixed in 1% OsO_4 in 0.05 M sodium cacodylate, pH 7.0 for 2 hours. After washing in 0.05 M sodium cacodylate buffer, the tissue blocks were trimmed slightly to expose the cells for observation for which the cellular contents had not been disturbed by the initial pre-fixation cutting. The samples were dehydrated in a graded series of aqueous ethanol (10, 25, 50, 75, 95, and 100% ethanol) followed by a graded series of ethanol-amyl acetate (10, 25, 50, 75, 95, and 100% amyl acetate). Carbon dioxide was used as the transitional fluid in a Ladd Critical Point Dryer. The tissue was then gold-coated in a Polaron E 5000 Sputtering System. Samples were observed and photographed at 20 kV with an ETEC Autoscan microscope.

Results and Discussion

Normal Peanut Structure

The embryo of the resting peanut seed consists of two cotyledons and a small radicle and plumule known as the germ. Processors are primarily concerned with the tissue of the peanut seed cotyledons which constitutes about 96% of the seed weight.

Each peanut seed cotyledon consists of epidermal, vascular, and parenchymal tissue. Figures 1 thru 4 depict the morphological and anatomical features of peanut cotyledon structure pertinent to this study. The epidermis is made up of a single layer

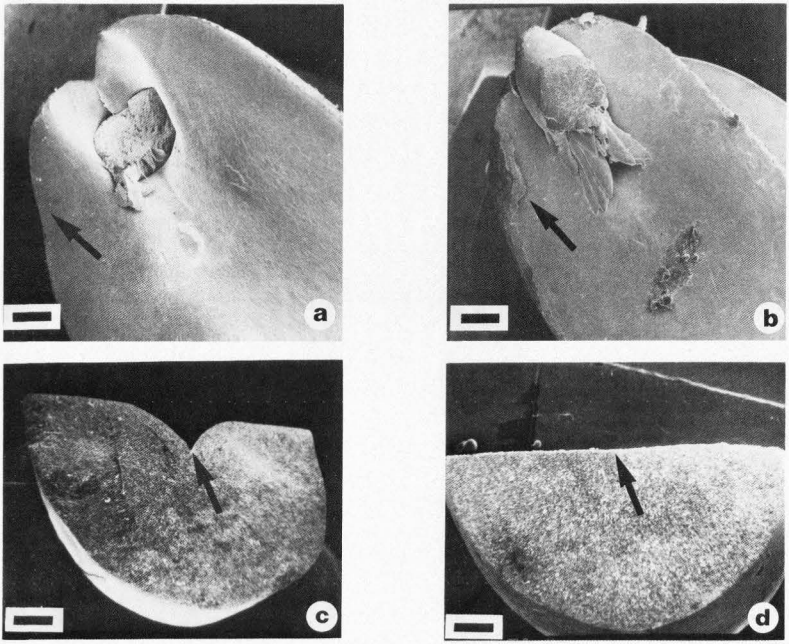


Fig. 2. Morphology of the inner surface of the cotyledon: (a) Normal cotyledon with smooth and regular edges (arrow); (b) Environmentally stressed cotyledon with rough and irregular edges (arrow) due to tissue damage; (c) Transverse section of the normal cotyledon which reveals the indentation (arrow) which transverses the longitudinal axis; and (d) Transverse section of the environmentally stressed cotyledon which reveals the flattened inner surface (arrow). Bars = 1.0 mm.

of cells which covers the surface of the cotyledon. The epidermal cells of the rounded outer surface are more or less rectangular in outline (Fig. 1a). The epidermal cells of the inner surface are irregular in outline and contain numerous stomata (Fig. 1b).

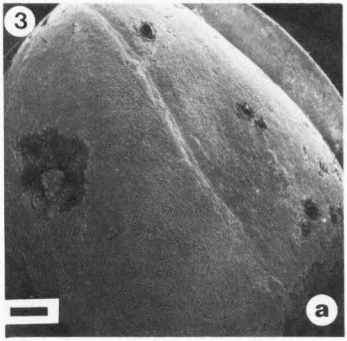
The vascular tissue of the peanut seed extends through each cotyledon of the embryo. Woodroof and Leahy (1940) described the vascular system as one series of six to eight bundles which follow the curvature of the outer surface and another series of four to six centrally located bundles. This vascular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

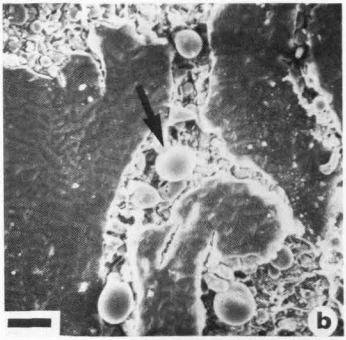
The majority of the tissue of the cotyledon is made up of rather large, almost isodiametric parenchyma cells (Fig. 1c–1d). The pitted walls of the resting seed parenchyma cells have conspicuous depressions (Fig. 4e). The wall depressions have been described by numerous workers (Woodroof and Leahy, 1940; Vaughan, 1970; Yatsu, 1981; Schadel et al., 1983).

The major subcellular organelles of the parenchyma cells are spherosomes (lipid bodies), protein bodies, and starch grains. The transmission electron microscope has been used by Jacks et al. (1967) and Neucere and Hensarling (1973) to characterize the spherosomes as particles about 1.0–2.0 microns in diameter bounded by a limiting membrane. After OsO_4 fixation, the limiting membranes of the spherosomes (lipid bodies) are observable with the scanning electron microscope (Fig. 1d) and create a "honeycomb effect" which appears around the protein bodies and starch grains.

Environmentally-Stressed Peanut Structure

One of the physical peculiarities observed in some environmentally stressed peanut cotyledons is tissue damage which appears as a narrow band along the interface of the outer rounded surface and the flattened edge of the inner surface. The unsectioned appearance of this interface in a normal peanut cotyledon is smooth and regular (Fig. 2a). The corresponding tissue has a typical ordered appearance when viewed in a transverse section (Fig. 4a). The unsectioned appearance of this interface in an environmentally stressed peanut cotyledon is rough and irregular (Fig. 2b). The corresponding tissue is characterized by cellular disruption and tissue disorganization when viewed in a transverse section (Fig. 4b).





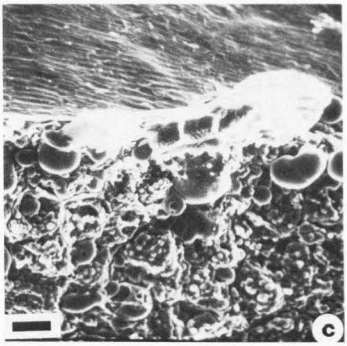
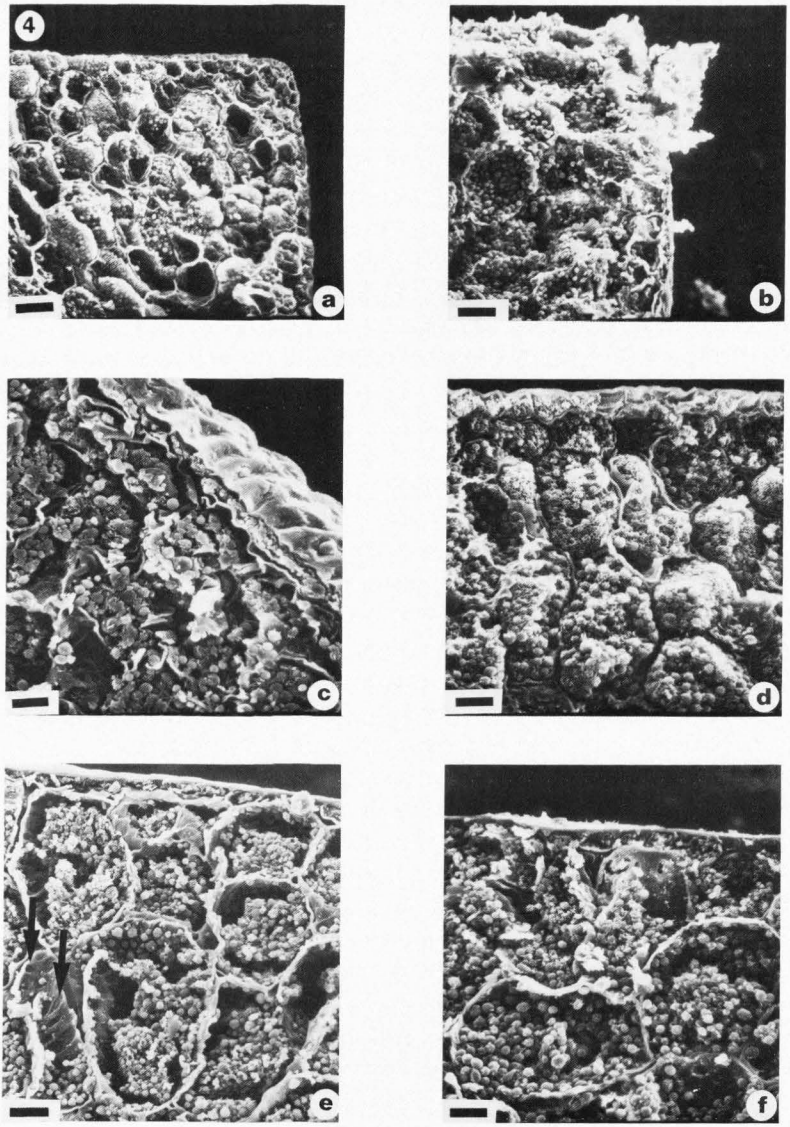


Fig. 3. Tissue disruption on the environmentally stressed cotyledon: (a) Outer rounded surface with tissue damage. Bar = 1.0 mm; (b) Fissure on the outer rounded surface with cellular contents extruded. Note the spherical thickened mass of extruded cellular contents (arrow). Bar = 50.0 μm; (c) Transverse section of a fissure on the outer surface. Bar = 50.0 μm; and (d) Transverse section of a fissure revealing amorphous coagulated cytoplasm. Bar = 20.0 μm.

Fig. 4. Internal tissue organization of normal and environmentally stressed peanut cotyledon: (a) Transverse section of the interface of the inner and outer surface of a normal cotyledon. Bar = 50.0 μm; (b) Transverse section of the interface of the inner and outer surface of an environmentally stressed cotyledon with tissue damage. Bar = 50.0 μm; (c) Transverse section of the tissue in the indentation of the inner surface of a normal peanut cotyledon. Bar = 20.0 μm; (d) Transverse section of the tissue of the flattened inner surface of an environmentally stressed cotyledon. Bar = 20.0 μm; (e) Transverse section of the outer rounded surface of a normal peanut cotyledon. Note the conspicuous depressions in the exposed pitted wall of a parenchyma cell (arrows). Bar = 20.0 μm; and (f) Transverse section of an undamaged area on the outer surface of an environmentally stressed peanut. Note the similarity of the cellular appearance to the normal peanut cotyledon. Bar = 20.0 μm.

A second physical peculiarity observed in some environmentally stressed peanut cotyledons is the absence of the characteristic indentation which traverses the longitudinal axis of the inner surface. This structural difference can best be observed in transverse section of the entire cotyledon (Fig. 2c–2d).

A third physical peculiarity observed in environmentally stressed peanut structure is the cracking and fissuring of the cotyledon surface which appears as spotting on the outer surface when viewed with the unaided eye. These physical disruptions are easily observed with the SEM and are characterized by the extrusion of coagulated cellular contents onto the surface of unsectioned cotyledon (Fig. 3a–3b). The cellular contents which have been extruded are referred to as "coagulated" in the sense of a fluid which has changed into a thickened mass. In transverse section, the coagulated cellular contents can be seen to extend to the depth of the fissures (Fig. 3c–3d). This fissuring of tissue and disruption of cellular contents is believed to be the primary source of the off-flavor problems associated with environmentally stressed peanuts. The fissuring is probably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caused by the lack of water. This would allow air to enter the tissue and promote oxidation of the cell contents, especially unsaturated lipid substances. Further investigations of the extruded cellular contents are necessary to characterize their chemical nature more specifically.

Transverse sections taken from the middle of the inner and outer surface of the normal peanut cotyledon and from identical regions in uncracked areas of environmentally stressed cotyledon revealed no unusual physical characteristics. The cellular contents and tissue organization of the middle of the inner surface in the normal cotyledon with its strong longitudinal indentation (Fig. 4c) were comparable to the cellular contents and tissue organization of the flattened inner surface of environmentally stressed cotyledons (Fig. 4d). Thus, it appears that the lack of the characteristic indentation in the environmentally stressed cotyledon is a manifestation of incomplete morphological development which does not result in cellular and tissue abnormalities. Cellular contents and tissue organization were also comparable in transverse sections taken from the outer rounded surface of normal cotyledons (Fig. 4e) and from uncracked areas of the outer rounded surface of environmentally stressed cotyledons (Fig. 4f).

Acknowledgement

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Discussion with Reviewers

W.J. Wolf: Have you tried washing stressed peanuts with various solvents to see what the extruded materials will dissolve in? Since peanut kernels contain about 50% oil, the extruded material (arrow, Fig. 3b) may likely be mostly lipid. It would be interesting to see whether the extruded materials would be removed by rinsing the cotyledons in hexane. Rinsing in water would leave the oil and starch but dissolve the proteins.

Authors: Our *future* investigation of the extruded materials to characterize their chemical nature more specifically will include not only a hexane rinse and a water rinse before fixation; but, we also plan to try a hexane rinse followed by a water rinse and a water rinse followed by a hexane rinse. This experimental design will allow us to investigate the alternative possibility that the extruded material may actually be a combination of oil and protein extruded as a result of cellular disruption.