Cotyledon Structure of Resting Peanut (*Arachis Hypogaea* L. cv. Florunner) Seed Before and After Hydraulic Pressing

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ABSTRACT

The cotyledon structure of resting peanut (*Arachis hypogaea* L. cv. Florunner) seed before and after hydraulic pressing was investigated with light and scanning electron microscopy. Observations were made of the appearance of cell walls and the major subcellular components: spherosomes (oil reserve bodies), aleurone grains and starch grains. Major findings include previously unreported cell wall damage and surface fissures that may be important to processors who express the oil from peanuts.

INTRODUCTION

VARIOUS PROCESSING CONDITIONS which utilize hydraulic pressing have been developed to obtain partially defatted peanuts (Vix et al., 1967; Pominski and Spadaro, 1980). This processing involves three mechanical operations: (1) pressing, (2) reconstitution, and (3) drying and roasting. Raw (with skins) or blanched peanuts are hydraulically pressed to remove the oil, which removes up to 80% of the oil in the peanuts (Vix et al., 1967), thereby reducing the calorie content while maintaining the protein value.

In order to achieve the maximum efficiency of the peanut oil pressing, it is important to understand the cotyledon structure of the resting peanut (*Arachis hypogaea* L.) seed. 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). The changes induced by the hydraulic pressing of peanut seed have been studied by Woodroof and Leahy (1940), Neucere and Hensarling (1963), and Yatsu (1981). The present study provides additional information of the cotyledon structure of the resting peanut seed before and after hydraulic pressing.

MATERIALS & METHODS

JUMBO, cv. Florunner, peanut cotyledons were hydraulically pressed at 1800–2000 psi in a 600-ton Albright Nell cage press. Oil content was reduced from 49.9 (w/w) to 32.6% (w/w) by this process. Tissue blocks (2 mm^3) of unpressed (native) and pressed cotyledons were fixed, embedded, sectioned, and stained for light microscopy using the methods of Yatsu (1981). Starch and aleurone grains (protein bodies) were stained differentially with 1% acid fuchsin specific for protein (Feder and O'Brien, 1968). Identically fixed tissue for scanning electron microscopy was 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 Diode Sputtering System. Samples were observed and photographed at 20 KeV with an ETEC Autoscan microscope.

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Unpressed peanut

The embryo of the raw, unpressed peanut consists of two cotyledons and a small radicle and plumule known as germ or "heart." Earlier workers (Woodroof and Leahy, 1940) reported that the blanching process removed the hearts as well as the skins (6–7.5% of the weight). However, in many cases today, the spin blanching process does not split the nuts and, thus, the hearts are retained (Pominski and Spadaro, 1980). The primary concern of processors of partially defatted peanuts is the tissue of the peanut seed cotyledons which constitutes about 96% of the seed weight.

The peanut seed cotyledon contains three kinds of tissue: (1) epidermis, (2) vascular, and (3) parenchyma. The epidermis consists of a layer of cells which covers the surface of the cotyledon. This epidermal layer is united by a thick cutin which is most abundant on the rounded, outer surface. The epidermal cells of the rounded, outer surface are more or less rectangular in outline. Woodroof and Leahy (1940) reported that stomata are scattered irregularly over the epidermal surface of the cotyledons of Spanish peanuts, and Yarbrough (1949) reported "somewhat simplified stomates occurring on the abaxial surface only" of Virginia Bunch cotyledons. The numerous stomata (Fig. 1) of Jumbo, cv. Florunner, peanuts are present only on the flat, inner (adaxial) surface of the resting seed cotyledon.

The vascular system of the peanut seed extends through each cotyledon of the embryo. Woodroof and Leahy (1940) stated that one series of six to eight bundles follows the curvature of the outer surface and another series of four to six centrally located. Our observations confirm that the vascular system makes up only a small part of the embryo.

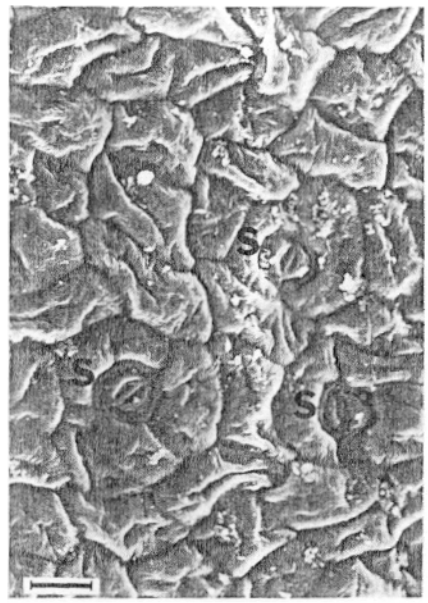

Fig. 1—Scanning electron photomicrograph of the epidermal cells with stomata (S) on the falt, inner surface of the unpressed cotyledon. Marker: 20μ.

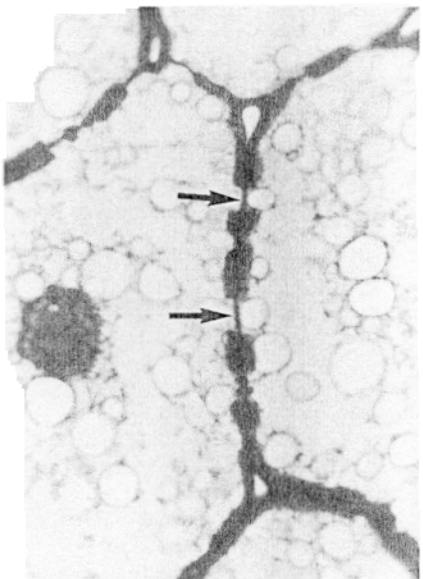

Fig. 2—Light photomicrograph of the parenchyma cell wall pitting (arrows) in the unpressed cotyledon stained with toluidine blue. Marker: 20μ.

Fig. 3—Scanning electron photomicrograph of the parenchyma cell wall pitting (arrow) in the unpressed cotyledon. Marker: 20μ.

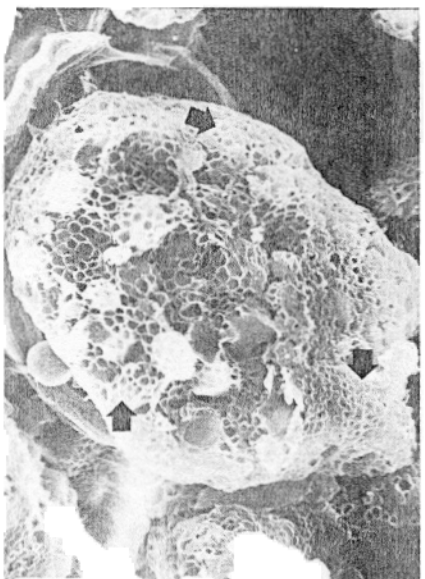

Fig. 4—Scanning electron photomicrograph of the entire cellular contents of a parenchyma cell of the unpressed cotyledon. Note the numerous 1–2 μ spherosome ghosts (arrows) surrounding the larger starch and aleurone grains. Marker: 20μ.

Woodroof and Leahy (1940) stated that the greater part of the embryo is made up of rather large, almost isodiametric parenchyma cells with pitted walls and small but distinct intercellular spaces. The pitted walls of the resting seed parenchyma cells have conspicuous depressions which give the walls a beaded appearance in sectional view with the light microscope (Fig. 2). These depressions have an elliptical to ovoid shape as seen with the scanning electron microscope (Fig. 3). The depressions in the walls of resting peanut seed parenchyma cells have been described by previous workers (Woodroof and Leahy, 1940; Vaughan, 1970; Yatsu, 1981).

The major subcellular organelles of the parenchyma cells are spherosomes (oil reserve bodies), aleurone grains, 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. Jacks et al. (1967) determined the composition of spherosomes to be 98.1% total lipid and 1.9% nonlipid residue. After OsO_4 fixation, the spherosomes appear as electron-dense bodies surrounded by electron-dense membranes when observed with the transmission electron microscope. We found that spherosomal ghosts were easily observed with the scanning electron microscope (Fig. 4), but that starch grains were more readily distinguished from aleurone grains with the light microscope (Fig. 5). The almost spherical starch and aleurone grains range in size from 2–10 microns in diameter. After treatment with 1% acid fuchsin stain, the aleurone grains stain a deep pink color, and the starch grains remain colorless.

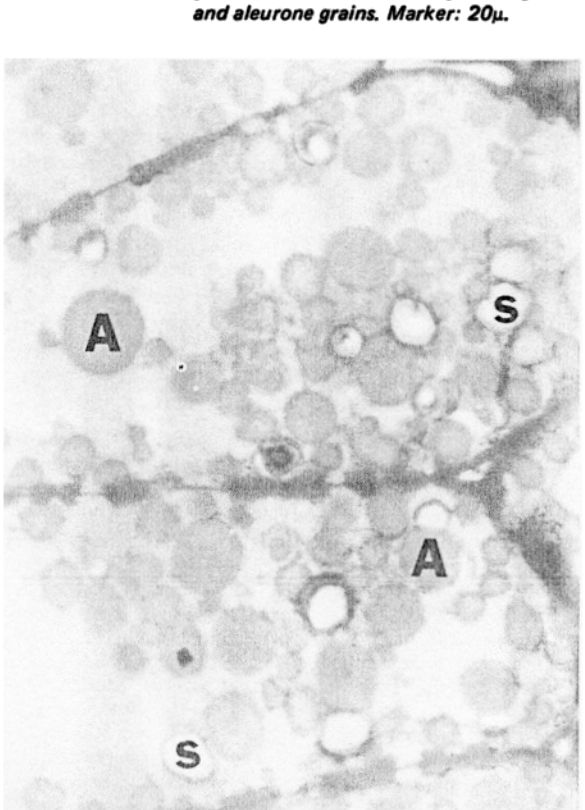

Fig. 5—Light photomicrograph of parenchyma cells of the unpressed cotyledon stained with toluidine blue and acid fuchsin. Compare the unstained starch grains (S) with the stained aleurone grains (A). Marker: 20μ.

Pressed peanut

Hydraulic pressing ruptures most of the spherosomes and creates a compact mass of aleurone grains, starch grains, and residual oil and cytoplasmic components within each cell. Distinct spherosomes can no longer be seen (Fig. 6). Neucere and Hensarling (1973) found that the pressing of peanut seed cotyledon tissue induced slight cellular aberrations and distention of membranes surrounding aleurone grains, but that starch grains and cell walls appeared unaltered. In the present study of pressed peanut seed tissue, we observed the unaltered appearance of the starch grains and the distention of membranes surrounding

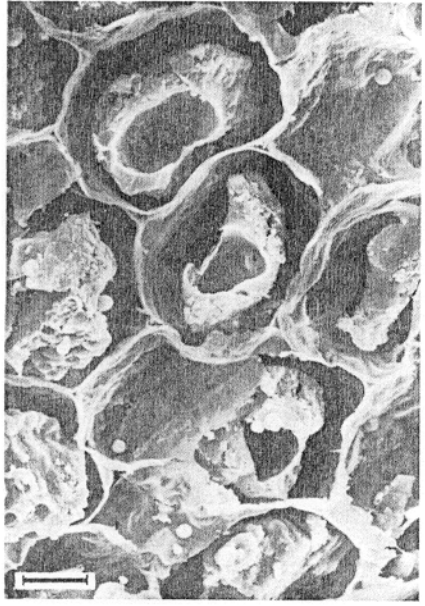

Fig. 6—Scanning electron photomicrograph of parenchyma cells of the pressed cotyledon. Note the absence of the majority of the distinct spherosomes. Marker: 20μ.

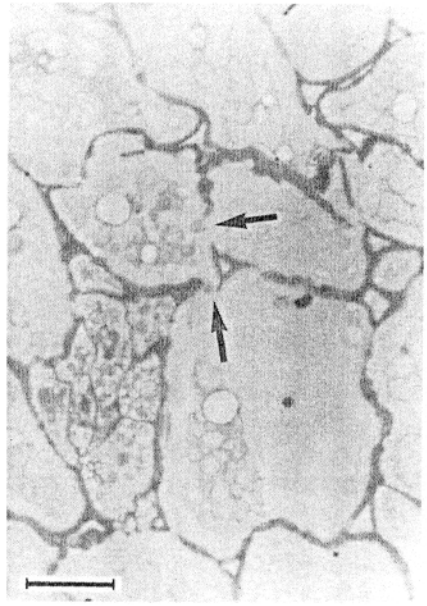

Fig. 7—Light photomicrograph of the pressed cotyledon stained with toluidine blue. Note the small, broken regions (arrows) of the cell walls and the compactness of the cellular contents. Marker: 20μ.

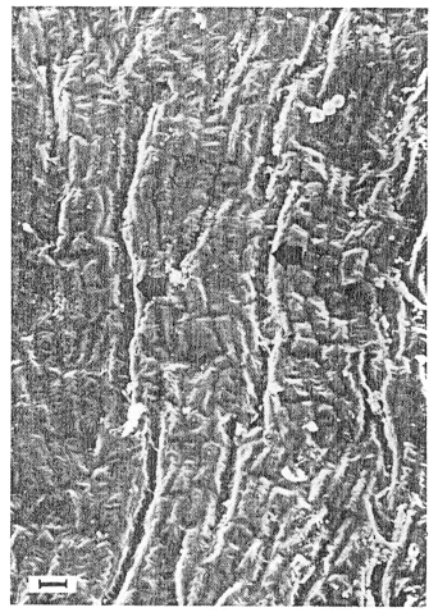

Fig. 8—Scanning electron photomicrograph of the rounded, outer surface of the pressed cotyledon. Note the absence of stomata and large fissures (arrows) along the epidermal cells cause by pressing. Marker: 20μ.

the aleurone grains, but unlike Neucere and Hensarling (1973), we observed that some cell walls were unaffected by pressing, while the majority of the cells had broken wall regions after pressing.

We believe that the broken cell wall regions which appear after pressing correspond to the depressions in the cotyledon cell wall of unpressed peanut. Esau (1965) stated that wall depressions previously referred to as pits may often be the sites of plasmadesmata, the tiny strand-like structures of cytoplasm that interconnect the living protoplasts of adjacent cells. Yatsu (1981) hypothesized that these depressions in the cell wall might impart structural strength (not unlike a keystone arch) and devised a crushing test to test this hypothesis. The results of his crushing test were inconclusive.

Our restuls indicate that these depressions, which are actually thin areas in the cell wall, are actually weak structural points. The small, broken cell wall regions appear to correspond to the thinner areas of the pitted wall (Fig. 7). Perhaps different peanut cultivars with different degrees of wall pitting, offer different resistances to release of oil during the pressing process. In addition to the broken wall areas of individual cells, we observed that the surface of the pressed cotyledon has numerous previously unreported fissures (Fig. 8), which may be involved in the release of oil during hydraulic pressing.

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