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Ultrastructure of Mature and Ripening Avocado (*Persea americana* Mill.) Fruit Mesocarp; Scanning, Transmission and Freeze Fracture Electron Microscopy

A Dissertation submitted in partial satisfaction
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Dissertation Committee:
Professor William W. Thomson, Chairman
Professor Roy E. Young
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ABSTRACT OF THE DISSERTATION

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by

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The ultrastructure of mature avocado *(Persea americana M*ill, var. Hass), fruit mesocarp cells, and changes in the ultrastructure during ripening were examined. The purpose of the study was to determine whether avocado fruit exhibit ultrastructural changes during the ripening process which are indicative of degradation. An answer to this question would help to determine whether or not ripening in avocados, and possibly other fruits, should be considered a senescence phenomenon, or whether ripening precedes senescence. Thin section transmission electron microscopy was complemented by investigations with freeze fracture and scanning electron microscopy (SEM).

A new technique for the preparation of biological material for examination in the SEM was modified and improved. This technique involved the use of thiocarbohydrazide (TCH) as a ligand, binding to osmium tetroxide (OsO_4). Alternate treatments of tissue with OsO_4 and TCH renders all cellular constituents (walls, organelles, and inclusions) conductive to electrons. This procedure therefore eliminates the need for metal coating of the specimen, and allows the direct observation of subcellular organelles in the SEM. The use of this technique for SEM, in conjunction with thin section and freeze fracture electron microscopy provided a multifaceted approach to the study of changes in the ultrastructure of ripening avocado fruit.

The primary, and essentially the only, degradative change observed was in the cell walls. This was manifested as a loosening of the wall striations, probably due to loss of pectins, and by a separation and apparent shortening of the cellulose microfibrils in the post climacteric fruit. An association of wall degradation with the plasmodesmata was not apparent.

The mature parenchyma cell of an avocado fruit contained the normal complement of cellular organelles; nuclei, plastids, mitochondria, microbodies, etc. The major

constituents of these cells were the lipid or oil bodies. These occurred in the cytoplasm and were apparently not surrounded by a membrane.

The structure of the plastids varied with the location of the cell with reference to the external environment. Thus, chloroplasts were found in the outermost cells, and etioplasts occurred in the inner cells of the mesocarp.

Mitochondria, endoplasmic reticulum (ER) and the plasma membrane all exhibited apparent ultrastructural changes during the ripening process. The mitochondria of unripe avocado mesocarp cells are fairly elongate, branching organelles. During ripening, an apparent increased elongation was observed in the SEM. No other obvious structural change occurred. The ER of unripe fruit, as seen by the three-dimensional view possible in the SEM appeared as tubular or as sheet-like structures. As ripening progressed, the ER apparently became vesiculated and was frequently in close apposition to the plasma membrane.

The changes in the ultrastructure of the plasma membrane were detected primarily by freeze fracture electron microscopy, and were manifested as an increase in the density of intramembranous particles (IMPs) of the EF face at the climacteric peak. This greater density of IMPs at the peak was followed by a decrease in density in the post climacteric fruits to a level similar to that of preclimacteric, unripe fruits.

Based on the lack of cellular or organelle degradation detectable at the ultrastructural level, the primary conclusion of this dissertation is that ripening precedes senescence, and should not be considered as a senscence phenomenon. The significance of the increased particle density in the EF face of the plasma membrane is uncertain at this time. However its coincidence with the climacteric peak in respiration is also coincident with the peak of activity of cellulase, and polygalacturonsase, and with the peak of ethylene production. Whether or not any of these physiological events can be directly related to the increased particle density depends on further studies.

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