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Anatomical and Morphological Characteristics of Laser Etching Depressions for Fruit Labeling

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SUMMARY. Fruit etching is an alternative means to label produce. Laser beam-generated pinhole depressions form dot-matrix alphanumeric characters that etch in the required price-look-up information. Pinhole depressions can disrupt the cuticular and epidermal barriers, potentially weakening the natural protection against pathogens. In the present study we describe the anatomical and morphological characteristics of the pinhole depressions in the cuticle/epidermis, and the changes taking place during storage of two fruits: avocado (*Persea americana*) and tomato (*Lycopersicon esculentum*). These fruits represent the extremes from a thick, non-edible peel to a thin edible peel. On both tomato and avocado, etching depressions were fairly similar in diameter and depth, averaging 200 μm and 25 μm , respectively, for energy impact durations of 30 μs for tomato and 45 μs for avocado. Immediately after etching, the two- to five-cell-deep depressions contained cuticle/wax deposits. Additional cuticle/wax material was deposited in and around the depressions during storage as demonstrated by confocal, fluorescent, and light microscopy. In addition, the cells underlining the etch depression increased phenolic and lignin deposits in their walls, creating a potential barrier against pathogenic organisms.

Price-look-up (PLU) labeling of fresh fruit and vegetables has become commonplace in the United States over the last 10 years. The implementation was first driven by the need to reduce cashier errors and integrate produce commodities into the information technology programs demanded by large grocery chains. More recently, traceability and food security concerns have been added elements to the need for source-applied coding. The PLU coding index is based on a

four-digit identification developed by the Produce Electronic Identification Board (1995). This board was established to improve electronic collection and communication of data for fresh fruit and vegetable sales. The traceback component relates to identifying the source and transfer links to retail market. It is cited as "good agricultural practice" (GAP) aimed at minimizing

liability and preventing occurrence of food safety problems (Center for Food Safety and Applied Nutrition, 1998). This concern has been broadened to encompass food security concerns from intentional contamination. Enhanced labeling, beyond the PLU number, can also lead to produce brand identification and additional information, such as country of origin for fresh produce.

Thus far, the most widely used labeling system consists of adhesive tags transferred to individual fruit and vegetables on the packing line (Varon and Paddock, 1978). Although apparently an effective system, adhesive tags are not permanent, and generate a series of secondary problems ranging from tag detachment, tag buildup along the packing line, fouling of processing equipment, to the need for special storage rooms. In addition, they are not permanent and may be removed at any postharvest handling stage.

An alternative means of fruit labeling consists of etching the required information on the produce surface using a low energy carbon dioxide laser beam (10,600 nm) (Drouillard and Rowland, 1997). The general etching process, used in electronics manufacturing and medical treatments, is described by Hecht (1994). A high absorption characteristic (>90%) for water of the 10.6- μm wavelength is noted. The etch marks become permanent, require no additional adhesives, and labeling information can be modified quickly without extensive delays. However, etched markings are formed in dot matrix style letters and numbers, each dot created by pinhole depression. Such depressions, applied after washing and waxing, disrupt the natural cuticular barrier and the protective commercial wax cover seemingly creating open cavities, although preliminary observations using tomato revealed that aqueous dyes were repelled from newly formed etch depressions, suggesting a possible self-sealing mechanism. Studies on the anatomical, morphological, and physical aspects of the etching cavities were prompted by the lack of information on the effect of etching on commodities as a form of labeling.

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
2.54	inch(es)	cm	0.3937
1	micron	μm	1
$(^{\circ}\text{F} - 32) \div 1.8$	$^{\circ}\text{F}$	$^{\circ}\text{C}$	$(1.8 \times ^{\circ}\text{C}) + 32$

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Fig. 1. Laser labeling machine used to etch fruit at the Citrus Research Center's packinghouse in Lake Alfred, Fla. The machine used a maximum energy level of 0.678 W per character at 35 μ s with a duty cycle range of 25%.

The study described below was aimed at understanding the structure and possible damage at the microscopic level caused by the creation of an ablated area on the surface of the produce.

Materials and methods

PLANT MATERIAL. Tomato and avocado fruit were purchased at a local grocery store. The waxed fruit were taken to the University of Florida's Citrus Research and Education Center in Lake Alfred, where a Durand-Wayland etching machine was located.

FRUIT ETCHING. A carbon dioxide laser unit (model XYmark 10; Durand-Wayland, LaGrange, Ga.) was assembled at the packinghouse as illustrated in Fig. 1. Individual fruit were stabilized on a 1-inch polyvinyl chloride (PVC) disk at approximately 4 inches from the laser's output. The laser maximum energy level was 0.678 W per character at 35 μ s with a duty cycle range of 25%. Two laser exposure times were selected for each fruit. The etching exposure times had been previously established by the manufacturer in field trials according to visual assessment of peel thickness. For tomato, etching times of 30 and 35 μ s were used, whereas avocado was etched using 40- and 45- μ s exposure times. Two fruit for each exposure time per variety were taken to the

microscopy lab for immediate tissue preparation and observation. Similar fruit samples were stored for 4 d at 20 °C and 95% relative humidity. These conditions are conducive to induce both physiological deterioration and pathological infections.

TISSUE PREPARATION. Tissue samples were prepared according to the type of microscopic observation intended (light, fluorescent, or confocal microscopy). The same preparation techniques were used for tissue observed at time 0 and after 4 d in storage.

CONFOCAL AND FLUORESCENT MICROSCOPY. A thin segment of fruit epidermis containing several etching depressions was sliced with a razor blade and mounted on a microscope slide. A drop of 0.01% solution of Auramine-O [a specific cuticle fluorescent stain (Heslop-Harrison, 1977)] in 0.05 M Tris buffer (pH 7.2) was applied over the etching depressions and allowed to penetrate the tissue for 1 h. After 1 h, the tissue was thoroughly washed with water, covered with a drop of glycerol, and immediately observed.

LIGHT MICROSCOPY. Small sections of fruit epidermis containing etching depressions were placed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). After 4 h, tissue was rinsed with phosphate buffer and post-fixed for 4 h in 2% osmium tetroxide dissolved in a similar buffer. Tissue was subsequently dehydrated in an acetone series and finally embedded in Spurr's resin (Spurr, 1969). For light microscopy, 1- μ m sections were mounted on microscope slides and stained with methylene blue/azure and counterstained with basic fuchsin (Schneider, 1981). Methylene blue/azure-basic fuchsin is a general polychromatic stain of tissue elements. Sections for electron microscopy (90 nm) were stained with uranyl acetate (Stempack and Ward, 1964) and lead citrate (Reynolds, 1963) after being mounted on copper grids.

Microscopy

CONFOCAL MICROSCOPY. Confocal microscope observations were made using a Leica TCS-SL (Leica, Heidelberg, Germany) with an emission wavelength of 488 nm and programmed to capture successive impressions 90 nm apart across the depth of the etch depression. Individual digital impressions were later combined

to create three-dimensional images. Fluorescence attenuation was kept constant for each fruit in order to avoid potential artifacts caused by variations in fluorescence intensities.

FLUORESCENT MICROSCOPY. A Nikon Eclipse TE 300 fluorescent microscope (Nikon Co., Tokyo) was used to observe live fluorescence from treated fruit surfaces. The microscope was equipped with a fluorescein H-2 filter and a Sony Cybershot 505 digital camera (Sony Corp., Tokyo).

LIGHT MICROSCOPY. Stained sections fixed on glass microscope slides were observed under a Leitz Laborlux light microscope. Photographic images were taken using a Sony Cybershot 505 digital camera mounted on the microscope.

Results

A typical avocado laser-etched label is presented in Fig. 2, demonstrating the overall visual characteristics of the etching marks. There were no essential physical differences between 40- and 45- μ s etch markings; therefore, we present only the visual data of one treatment. The light brownish color of the etching dots is likely due to the natural color of dry cellulose resulting from loss of water from the etched area. A similar appearance was observed for tomato (Fig. 3). However, for some fruit and vegetables, color enhancement is required for proper visualization of the label. This is accomplished by an immediate mechanical application of dye to the etched area.

The distance from the laser head to the fruit's surface was set at 10 cm. This distance was based on the manufacturer's recommendation considering focal length of the laser beam. To provide uniform dot size, the lens system of the carbon dioxide laser had been configured with a long depth of field. This provides a slightly wider focal spot but a more uniform application over an extended range, which was 1 inch for the standard laser settings. For the power intensity used to mark fruit and vegetables, the maximum capacity of the laser coding unit was approximately 20 objects per second. With fruit and vegetables the size of tomato or avocado, the application time is <0.05 s per object.

PHYSICAL PARAMETERS OF ETCHING DEPRESSIONS. A laser-etched area calculation was performed for a tomato of average size assuming an oblate

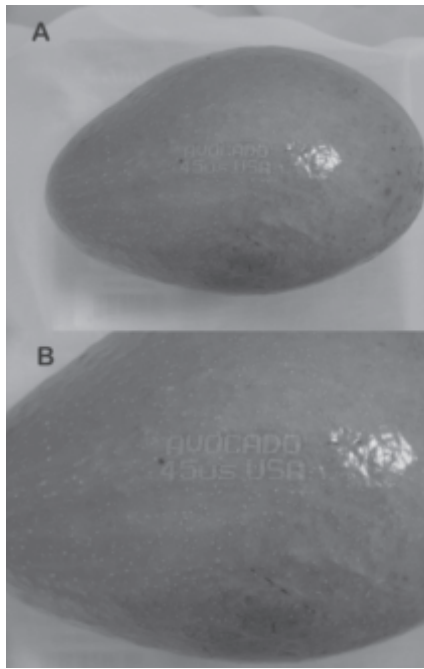


Fig. 2. Avocado fruit after labeling using an etching laser gun as described in materials and methods. Avocado was labeled with a maximum energy level of 0.678 W per character for 45 μ s. Images are presented in two magnifications for better view of the etched markings.

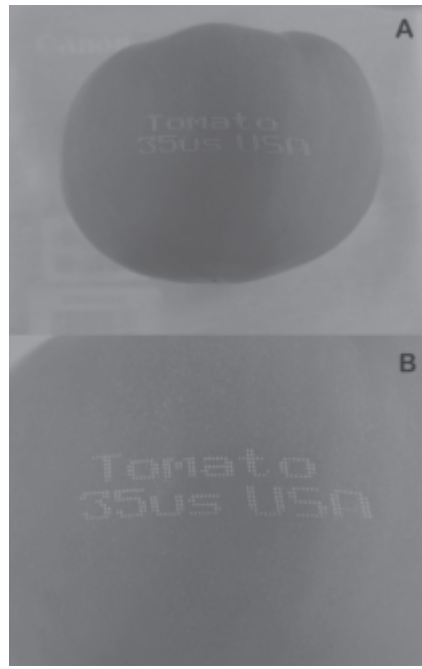


Fig. 3. Tomato fruit after labeling using an etching laser gun as described in materials and methods. Tomato was labeled with a maximum energy level of 0.678 W per character for 35 μ s. Images are presented in two magnifications for better view of the etched markings.

spheroid shape. An average major axis diameter (“a”) was measured for the test tomatoes as 3 inches while the minor axis diameter (“b”) was 2.5 inches. The tomato was selected as a test case because its surface area and

volume are less complex to calculate compared to avocado.

Each etched character is generated by an activated dot profile within a seven-high and eight-wide matrix (Table 1). The dots were estimated to be $\sim 200 \mu$ m in diameter from average measurements made in the microscopy study. Typically, the PLU produce identification code is four digits while the country of origin denotation would be most typically three characters. To bracket the expected laser-etched character, application of four, seven, and 10 characters are included in Table 2. The number “0” and alphabet character

“M” were considered as they require the highest number of dots, 40 and 38 dots, respectively (Table 1). A generic calculation program was developed in MathCad 12 (Mathsoft Engineering and Education, Cambridge, Mass.). Both the percentage of laser-treated area and volume ablated were calculated. The amount of laser-etched area for the expected seven-character application constituted less than 0.05% of the area of an average-sized tomato. For comparison, a large produce item (a = 6 inches, b = 5.5 inches), of similar oblate shape was also considered. The ablated volume was ascertained estimating the cylindrical depth at 50 μ m. Representative surface area and volumetric percentages for small and large produce items are presented in Table 2. Specific produce items were not evaluated because of their complex shape and the resultant difficulty in calculation of surface area and volume.

LIGHT MICROSCOPY. Light micrographs of untreated avocado exocarp show a distinctive epidermal layer composed of one to three cells in thickness (Fig. 4A). These cells are considerably smaller than the underlying parenchyma cells, and appear somewhat symmetrical. Cell walls are visibly thicker than those of storage parenchyma and the external surface is covered by a layer of cutin (grayish layer) that penetrates the anticlinal epidermal walls.

Tomato epidermal cells are elongated along the periclynal axis of the fruit. Although cells gradually increase in size towards the interior of the fruit, a four to five layer of epidermal cells can be distinguished (Fig. 5A). As in avocado, cutin also covered the exterior surface as well as the anticlinal cell walls of the outer epidermal cell layer. In either fruit, etching depressions rarely penetrated beyond the third

Table 1. Compilation of laser dots for individual alphanumeric characters used to label fruit and vegetables with two-dot line width in a 7 \times 8 matrix.

Character	Dots (no.)
0	40
M	38
B	37
N, Q	36
A, R, 8	34
G	33
H, O, W	32
5	31
D, E, U, V, 6, 9	30
K	29
P	28
3, 4	27
C, S, X, Z, 2	26
F, Y	24
I, J	22
1	21
L, T, 7	20

Table 2. Ablated surface area and volume estimates for laser-marked small and large produce. Estimates are based on an average diameter of 200 μ m for each pinhole depression.

Digits (no.)	Code ^y	Small fruit/vegetable (major radius = 3.8 cm, minor radius = 3.2 cm) ^z		Large fruit/vegetable (major radius = 7.5 cm, minor radius = 7.0 cm)	
		Surface area (%)	Volume (% $\times 10^{-6}$)	Surface area (%)	Volume (% $\times 10^{-6}$)
4	0	0.031	1.298	0.007	0.152
7	0000MMM	0.053	2.224	0.013	0.261
10	00000MMMMM	0.075	3.165	0.018	0.371

^z1 cm = 0.3937 inch

^yAlphanumeric characters selected for high dot density, 0 (40 of 56) and M (38 of 56).

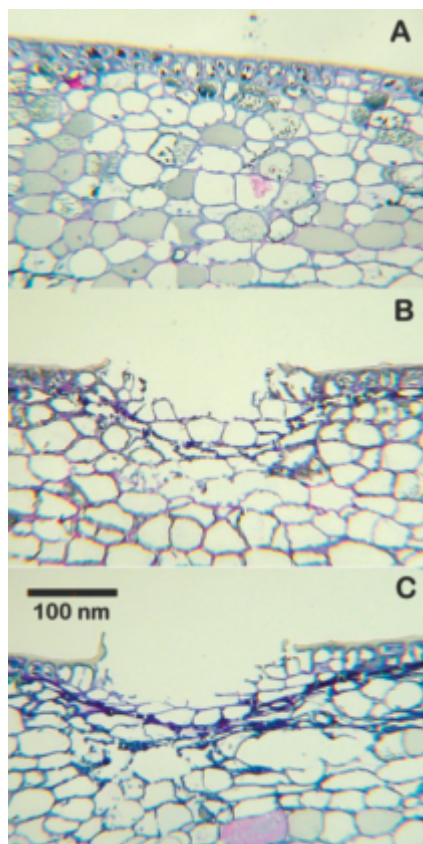


Fig. 4. Light micrographs of an anticlinal section of avocado epidermis before and after laser etching. Tissue was fixed and stained as described in materials and methods. (A) Undisturbed epidermis section; (B) pinhole depression soon after etching; (C) pinhole depression after 4 d in storage at 21 °C (69.8 °F) and 95% relative humidity.

layer of epidermal cells (Figs. 4B and 5B). Immediately after etching, cell walls around the depression looked indistinguishable from cell walls deeper in the tissue. After 4 d in storage, however, evident structural changes had occurred in wall composition of cells directly beneath the etch depressions in both fruit (Figs. 4C and 5C). The most visible change was extensive wall thickening, demonstrated by the darkening of the walls immediately below the first live cells. The increase in color intensity (purple) demonstrates deposition phenolics and other elements of lignification. Another visible change, albeit less evident in these micrographs but more evident under confocal fluorescent microscopy (next section), is a thickening of the cuticle layer around the etching depression. The changes in phenolics deposition are accentuated when the images in

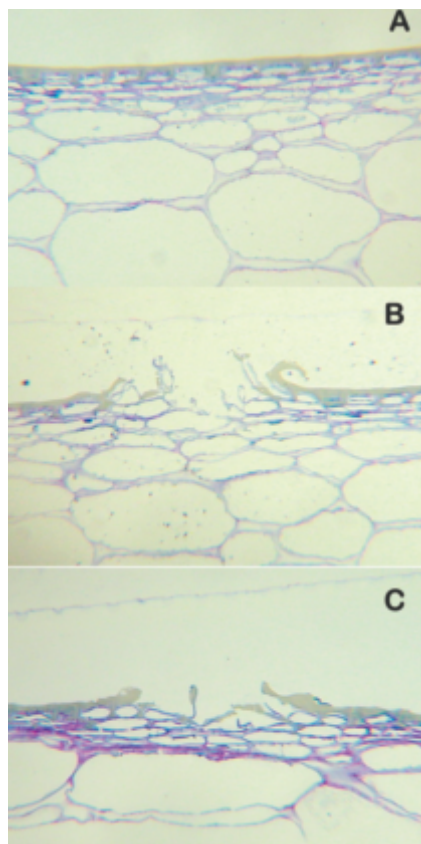


Fig. 5. Light micrographs an anticlinal section of tomato epidermis before and after laser etching. Tissue was fixed and stained as described in materials and methods. (A) Undisturbed epidermis section; (B) pinhole depression soon after etching; (C) pinhole depression after 4 d in storage at 21 °C (69.8 °F) and 95% relative humidity. Size scale as in Fig. 4.

Figs. 4 and 5 are observed under dark field (Figs. 6 and 7). In these images, new phenolic and lignin deposits manifest as green fluorescence under the outermost live cells (Figs. 6C and 7C). It is worth noting that under this dark field image, phenolic deposition and lignification in other peripheral cells become evident.

CONFOCAL AND FLUORESCENT MICROSCOPY. Confocal micrographs (Figs. 8 and 9) revealed the extent by which the etching depression is protected by heavy cutin deposits. Figures 8 and 9 show fluorescence from the etching depression at time 0 (A) and after 4 d (B) in storage. Aside from the marked increase in fluorescence intensity demonstrating heavy deposition of cutin during storage, fluorescence within the cavity at time 0 (Figs. 8A and 9A) also shows the presence of cutin in the exposed inner

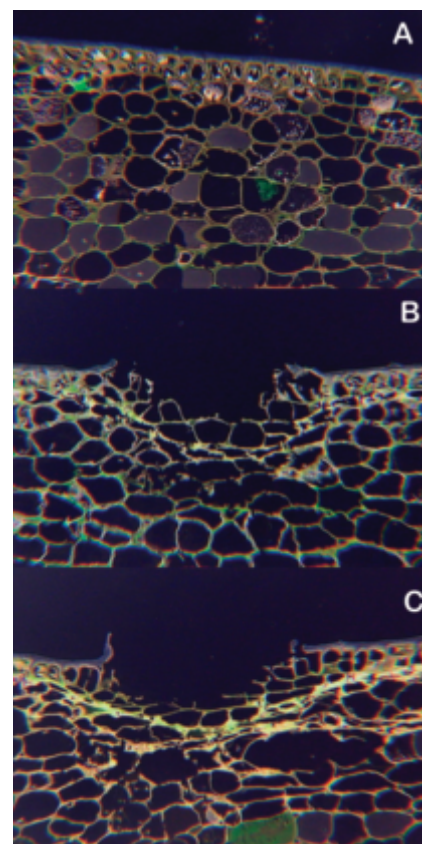


Fig. 6. Dark-field exposure of Fig. 4. Note the bright green fluorescence below the first layer of live cells in panel C, denoting heavy phenolics and lignin deposits after 4 d in storage at 21 °C (69.8 °F) and 95% relative humidity. Size scale as in Fig. 4.

layers. The presence of cutin (or waxes) within the etching depression at this time is significant in that it most likely extends protection against desiccation and pathogen penetration. There are two possible explanations for the presence of cutin at these depths within the depression. First, as seen in Figs. 4 and 5, cutin deposits can be found deep into the second epidermal layer. Second, the energy pulse from the laser source likely forced the epidermal wax inwardly impregnating the exposed cell walls.

The appreciable increase in wax deposition during storage visible in Figs. 8B and 9B was corroborated in Figs. 10 (close-up). This fluorescent micrograph shows the deposition of cutin inside the exposed walls of the depression as well as in the walls surrounding cells.

Discussion

In the present study, laser-etched depressions were characterized in two

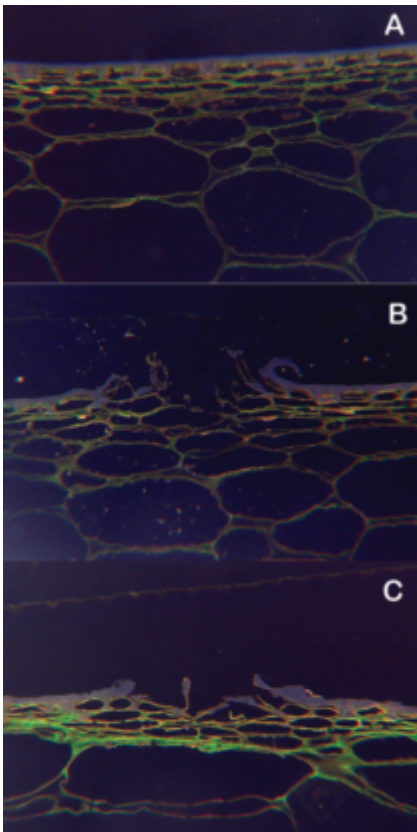


Fig. 7. Dark-field exposure of Fig. 5. Note the bright green fluorescence below the first layer of live cells in panel C, denoting heavy phenolics and lignin deposits after 4 d in storage at 21 °C (69.8 °F) and 95% relative humidity. Size scale as in Fig. 4.

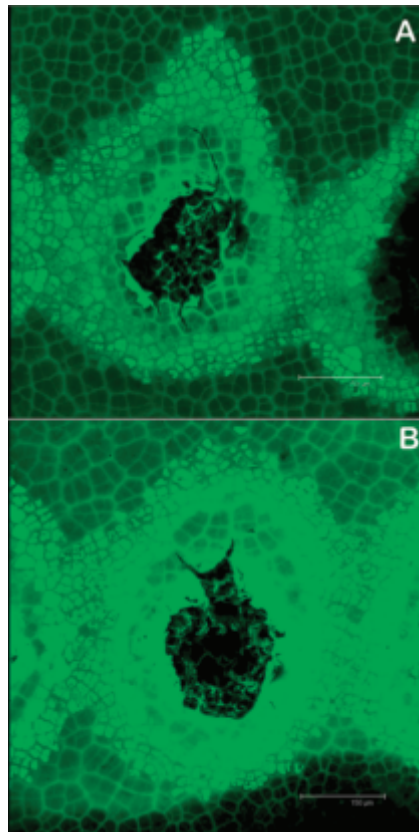


Fig. 8. Confocal fluorescent micrograph of a pinhole depression on avocado epidermis stained for cutin at (A) time 0 and (B) after 4 d in storage at 21 °C (69.8 °F) and 95% relative humidity. The increase in cutin, represented by green fluorescence, is evident in and around the depression.

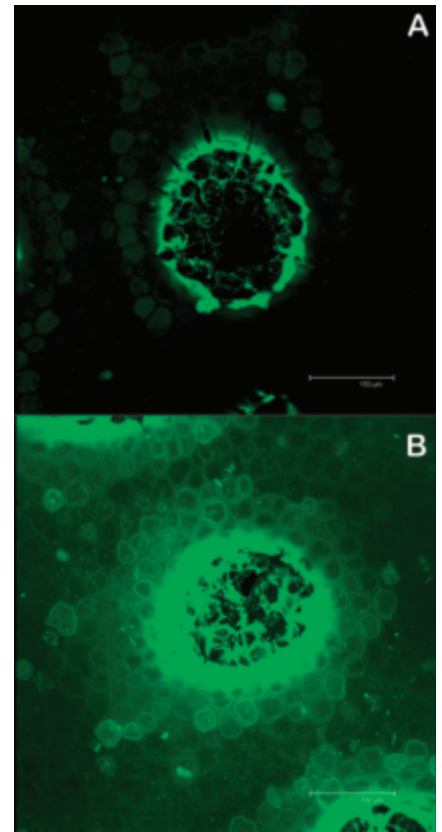


Fig. 9. Confocal fluorescent micrograph of a pinhole depression on tomato epidermis stained for cutin at (A) time 0 and (B) after 4 d in storage at 21 °C (69.8 °F) and 95% relative humidity. The increase in cutin, represented by green fluorescence, is evident in and around the depression.

produce items grouped by the edibility of the exocarp. For both tomato and avocado, etching depressions were fairly similar in diameter and depth, averaging 200 μm and 25 μm , respectively, for impact durations of 30 μs for tomato and 45 μs for avocado. Although varying with fruit size, the laser-etched area is a small percentage of the total fruit surface area estimated at approximately <0.05% for a tomato of 3 inches in diameter.

Of major concern is the unquestionable disruption of the fruit natural protective layer by the etching beam and the possibility that these open perforations may allow easy penetration by decay causing organisms. Using confocal microscopy we observed that, although initially stripped from their natural cuticle layer, etch depressions contained significant wax deposits along the walls of dead cells (Figs. 8–10). It is likely that the high-energy short time exposure of the laser beam

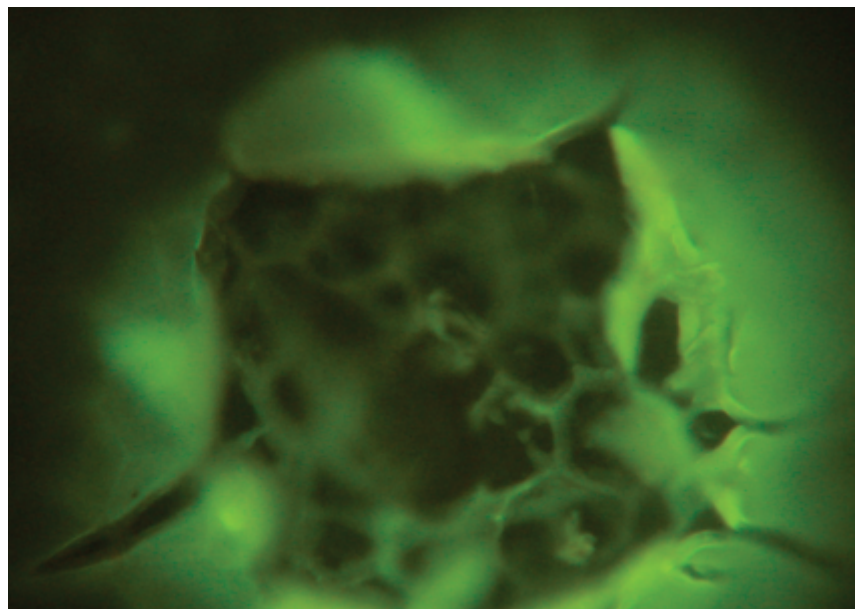


Fig. 10. High magnification confocal fluorescent micrograph of tomato pinhole depression stained for cutin taken after 4 d in storage at 21 °C (69.8 °F) and 95% relative humidity. Observe the substantial cutin/wax deposits in and around the depression.

momentarily vaporizes (or melts) both the natural and commercial waxes impregnating the exposed cell walls, thus creating an instant repellent shield. This may explain the repelling of aqueous dyes from the depressions after etching. With storage time, the amount of wax deposits increase substantially, further fortifying the protective cover.

In addition to wax, cells underlying the etched depression increase their wall's phenolic and lignin deposits (Figs. 4–7). Although the additional lignification presented here was taken after 4 d of storage, phenolic and lignin deposits most likely commenced shortly after etching. Taken together, the data from microscopic observations indicate that the pinhole depressions characteristic of the etching system for produce labeling initially possess some degree of wax protection. With storage time, underlying protective layers develop while additional natural waxes are deposited along the surface cell walls. Whereas the changes described here for tomato and avocado appear similar, it

is expected that differences in the rate of metabolic changes exist between species and storage conditions.

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