Proc. Fla. State Hort. Soc. 119:353-357. 2006.

CHARACTERISTICS OF LASER ETCHING DEPRESSIONS ON FLORIDA FRUITS AND VEGETABLES

ED ETXEBERRIA*, WILLIAM M. MILLER AND DIANN ACHOR
University of Florida, IFAS
Citrus Research and Education Center
700 Experiment Station Road
Lake Alfred, FL 33850

Additional index words. Lycopersicum esculentum, Persea americana, Citrus aurantifolia, Solanum tuberosum, PLU, postharvest, produce labeling

Abstract. **Fruit etching is rapidly becoming an alterative means to label produce. The alphanumerical characters are formed by laser beam generated dot-matrix pin-holes which disrupt the cuticular and epidermal barriers. The present study describes anatomical characteristics of the pin-hole depressions in the cuticle/epidermis of several fruits and vegetables grown in Florida. Descriptions were made immediately after labeling and after 4 days in storage at 20°C and 95% relative humidity. Regardless of the energy impact durations, pin-holes were fairly homogeneous averaging 200 μm in diameter and 25 μm in depth. 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.**

Used as an alterative means to label produce, laser beam generated dot-matrix characters can etch in the required price look-up (PLU) information directly into the peel product (Drouilliard, 2006). The PLU coding index is based on a four-digit identification developed by the Produce Electronic Identification Board (PEI Board, 1995). Etched markings are formed in dot matrix style letters and numbers, each dot created by pin-hole depressions. However, some concerns arise as the pin-hole depressions, applied after washing and waxing, disrupt the cuticular and epidermal barriers potentially weakening the natural protection against pathogens.

Commercial implementation of this technique requires a thorough understanding of the ramifications of the creation of pin holes on fruit surfaces, although preliminary observations using tomato revealed that aqueous dyes were repelled from newly formed etch depressions, whereas work with pears (Robert Sugar, personal communication) did not show a detectable increase in postharvest decay, suggesting a possible self-sealing mechanism. The present study was aimed at characterizing the laser generated pin-hole depressions in the cuticles of fruits and vegetables in addition to describing the changes that occur during storage. We included several fruits and vegetables with edible and non-edible peel such as tomato, avocado, potato, and citrus, which are produced in Florida.

*Corresponding author; e-mail: eetxeber@ufl.edu

Materials and Methods

Plant material. Tomato (*Lycopersicum esculentum* Mill.), avocado (*Persea americana* Mill.) acid lime (*Citrus aurantifolia* Swingle), and potato (*Solanum tuberosum* Linnaeus) were purchased at a local grocery store. Plant material was taken to the University of Florida's Citrus Research and Education Center in Lake Alfred, Fla., where a Durand-Wayland etching machine had been assembled.

Fruit etching. A carbon dioxide laser unit (Model XYmark 10, Durand-Wayland, LaGrange, Ga.) was assembled at the packinghouse. Individual fruit were positioned on a 1-inch PVC (polyvinyl chloride) disk at approximately 10 cm from the laser's output. The average laser energy level was 0.678 watts per character at 35 μs with a duty cycle range of 25%. Etching exposure times had been previously established by the manufacturer in field trials. For tomato and potato, etching time was of 30 μs, whereas avocado and lime were etched using 40 μs exposure times. After treatment, two fruit of each kind were taken to the microscopy lab for immediate tissue preparation and observation. A similar fruit sample was stored for 4 d at 20°C and 95% relative humidity (RH).

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.

Microscopy. For light microscopy, small sections of fruit epidermis containing etching depressions were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2), and prepared as described by Etxeberria et al. (2006). 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.

Confocal microscope observations were made using a Leica TCS-SL (Leica, Heidelberg, Germany) as described by Etxeberria et al. (2006). A Nikon Eclipse TE 300 fluorescent microscope (Nikon Co., Tokyo, Japan) was used to observed live fluorescence from treated fruit surfaces. The microscope was equipped with a fluorescin H-2 filter and a Sony Cybershot 505 digital camera (Sony Corp., Tokyo, Japan).

Results

Figure 1 shows an overview of several fruits and vegetables labeled during our initial trials, some not discussed in detail here. The beige color of the pin-holes in most fruits (tomato,

Fig. 1. Combined photograph of several fruits and vegetables labeled using the laser etching technique. For tomato, potato, pepper, cucumber and onion etching time was of 30 µs, whereas avocado and lime were etched using 40 µs exposure times.

avocado, cucumber, and pepper) likely represents the color of dry cellulose resulting from the laser etching. In some cases, however (onion, orange), the color of the fruit or vegetable can mask the pin holes creating the necessity for artificial staining with a commercial vegetable dye.

Physical characteristics. A laser-etched area calculation was performed for a fruit of average size assuming an oblate spheroid shape. An average major axis diameter (a) was measured for the test fruit as 3 inches, while the minor axis diameter (b) was 2.5 inches.

Each etched character is generated by an activated dot profile within a seven high and eight wide matrix (Table 1). Average diameter and depth of the pin-hole depressions are of 200 μm and 25 μm, respectively, regardless of the energy impact durations (30-45 μs) or peel thickness as demonstrated by a fluorescent micrograph (Fig. 2). 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 1. The number 0 and alphabet character M were considered as they require the highest number of dots, 40 and 38 dots, respectively (Etxeberria et al., 2006). Using a generic calculation program 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 approximately 0.05% of the area of an average sized tomato. For comparison, the amount of laser-etched area, a large produce item (a = 7.5 cm, b = 7.0 cm) of similar oblate shape would be of less than 0.02% (Table 1). The ablated volume was ascertained estimating the cylindri-

Table 1. Ablated surface area and volume estimates for laser marked small and large produce. Estimates are based on an average diameter of 200 μm and depth of 25 μm for each pin-hole depression.

		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)[z]	
Digits	Code	Surface area, %	Volume, %	Surface area, %	Volume, %
4	0000	0.031	0.65×10^{-6}	0.007	0.08×10^{-6}
7	0000MMM[y]	0.053	1.11×10^{-6}	0.013	0.13×10^{-6}
10	00000MMMMM	0.075	1.58×10^{-6}	0.018	0.18×10^{-6}

[z]1 cm = 0.3937 inch.
[y]Alphanumeric characters selected for high dot density, 0 (40 of 56) and M (38 of 56).

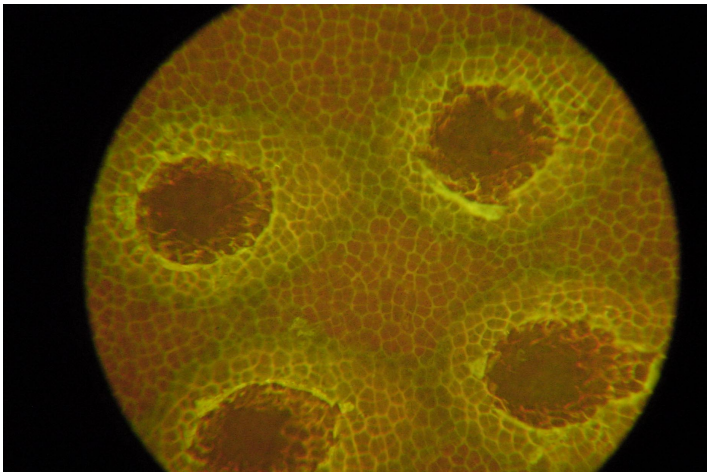

Fig. 2. Fluorescent micrograph of pinholes stained for waxes with auramine-O. The photograph is from an avocado soon after etching.

cal depth at 25 μm. Representative surface area and volumetric percentages for small and large produce items are presented in Table 1. Specific produce items were not evaluated because of their complex shape and the resultant difficulty in calculation of surface area and volume.

Confocal microscopy. Immediately after etching, the two- to five- cell-deep depressions contained cuticle/wax deposits (Fig. 3A, C, E, G). Three dimensional images of pin hole depressions in avocado (http://www.crec.ifas.ufl.edu/facilities/emlab/animation/avacado.htm) and tomato (http://www.crec.ifas.ufl.edu/facilities/emlab/animation/tomato.htm) revealed intense fluorescence within the broken cell walls. Fluorescent confocal microscopy revealed continuous deposition of additional layers of cuticle/wax around the depressions and underlying cell layers during storage for all tissues investigated (Fig. 3B, D, F, H), as demonstrated by the increase in fluorescence intensity.

Light microscopy. Light micrographs show distinctive epidermal layers composed of one to three cells in thickness (Fig. 4A, C, E, G). These cells are considerably smaller than

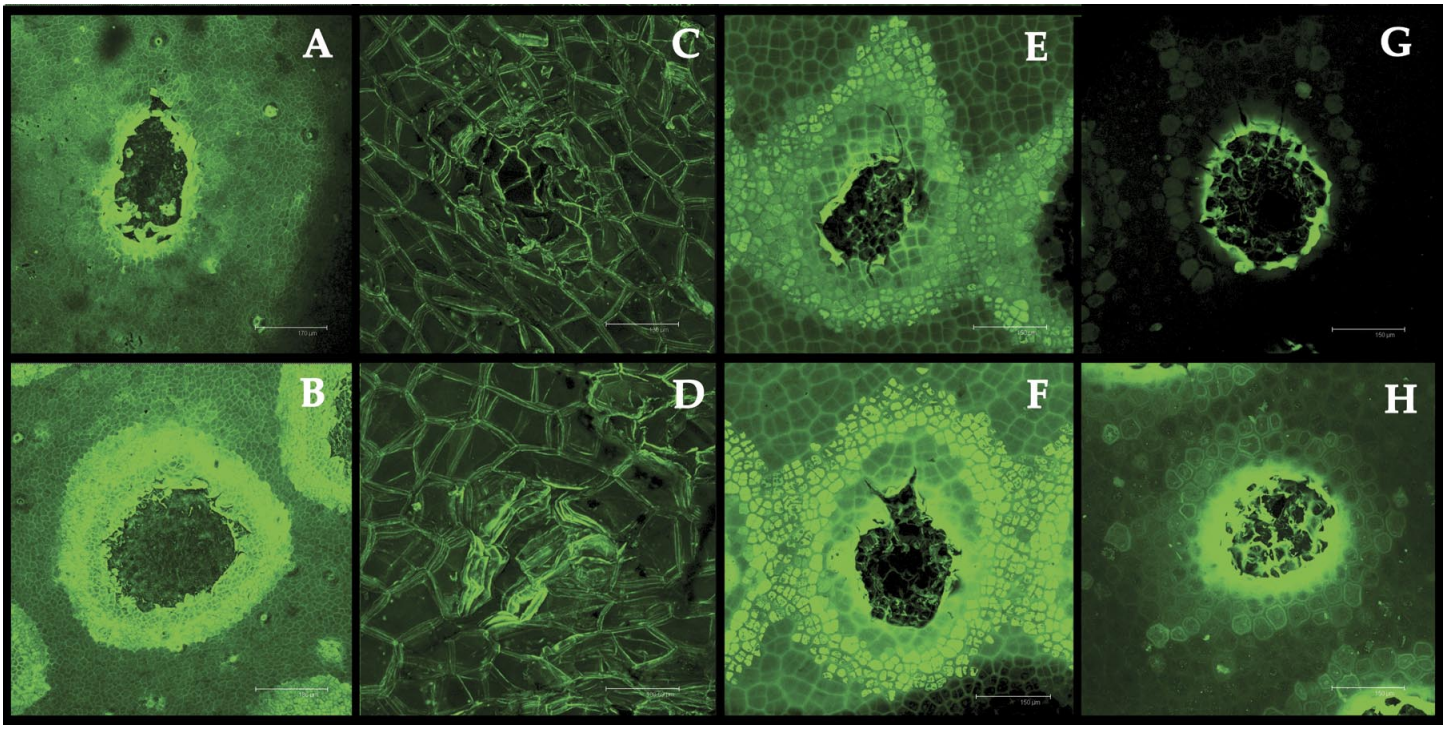

Fig. 3. Composite micrograph of pin-hole depressions stained for wax of avocado, tomato, lime, and potato taken soon after (A, C, E, G) and 4 d after etching (B, D, F, H). Tissue was stained with auramine-O and viewed using a confocal microscope.

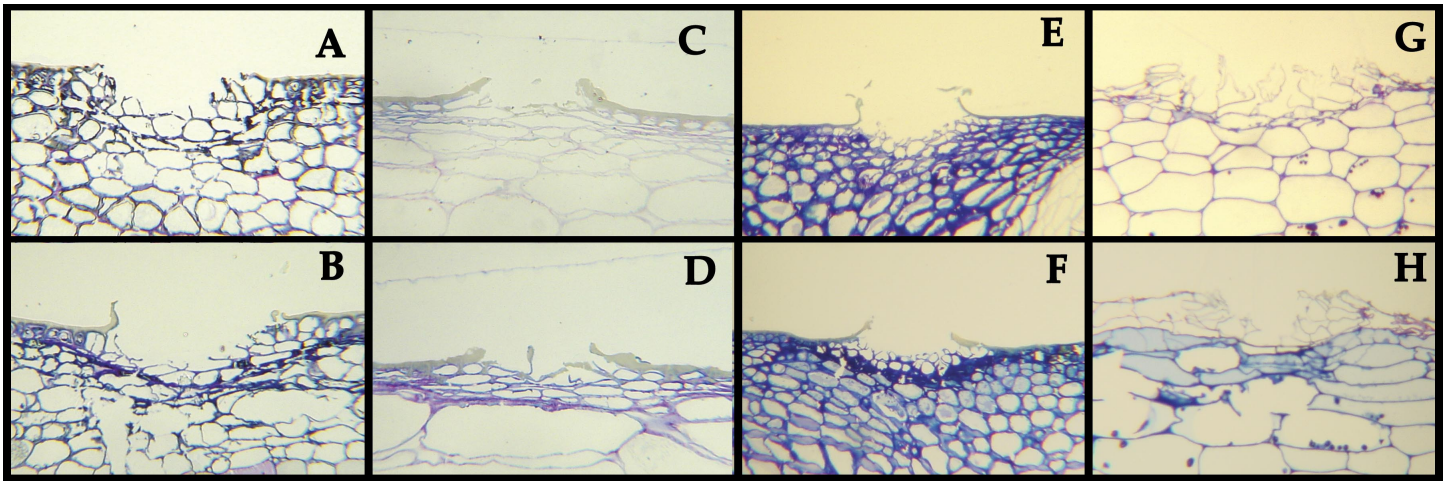

Fig. 4. Light micrographs of anticlinal sections of epidermis before and after laser etching. Tissue was fixed and stained as described in Materials and Methods. Pin-hole depressions were photographed immediately after etching for avocado (A), tomato (C), citrus (E), and potato (G), and after 4 d in storage at 20°C and 95% RH (relative humidity) for avocado (B), tomato (D), citrus (F), and potato (H).

the underlying parenchyma cells, which are more symmetrical and of various sizes depending on species. Epidermal 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. Etching depressions rarely penetrated beyond the fifth layer of cells.

Immediately after etching, cell walls of intact cells 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 all tissues investigated (Fig. 4B, D, F, H). The most visible change was the increase in color intensity (purple) which indicates the deposition of phenolics and other elements of lignification. The changes in phenolics deposition are accentuated when the images in Fig. 4 are observed under dark field (Fig. 5). In these images, new phenolic and lignin deposits manifest as green fluorescence under the outermost live cells. It is worth noting that under this dark field image, phenolic deposition and lignification in other peripheral cells becomes evident.

Discussion

In the present study, laser-etched depressions were characterized in several produce items grown in Florida. Etching depressions were fairly similar in diameter and depth, averaging 200 μm and 25 μm, respectively, for impact durations of 30 μs and 45 μs. Although varying with fruit size, the laser-etched area is a small percent of the total fruit surface area estimated at approximately <0.05% for a fruit/vegetable of 3.8 cm radius.

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 (Fig. 3). It is likely that the high-energy short-time exposure of the laser beam momentarily vaporize (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.

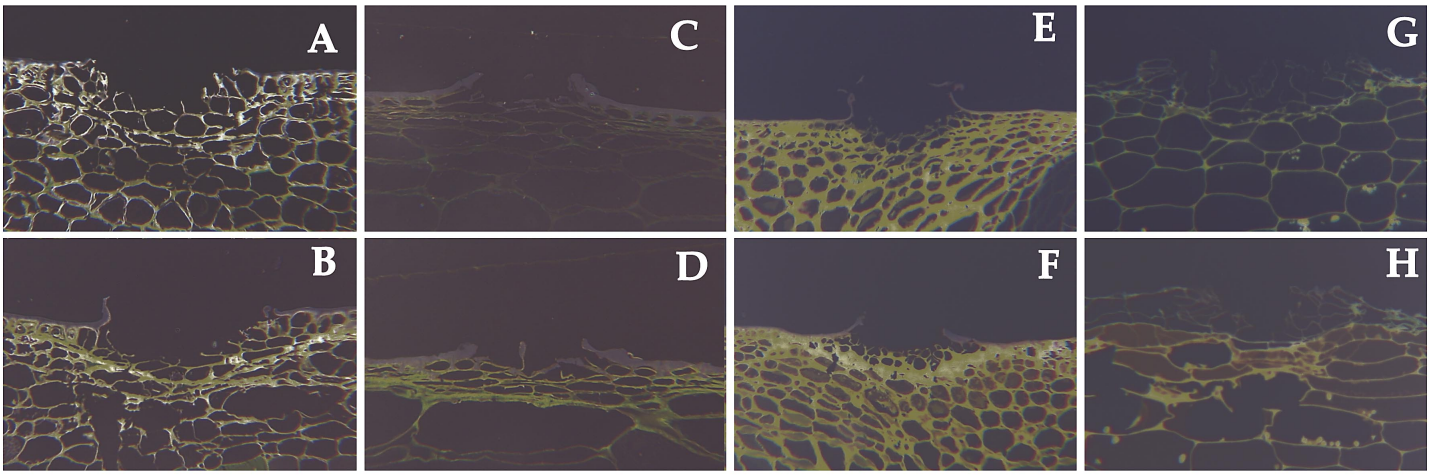

Fig. 5. Dark-field exposure of Figure 4. Tissue was fixed and stained as described in Materials and Methods. Pin-hole depressions were photographed immediately after etching for avocado (A), tomato (C), citrus (E), and potato (G), and after 4 d in storage at 20°C and 95% RH (relative humidity) for avocado (B), tomato (D), citrus (F), and potato (H).

In addition to wax, cells underlining the etched depression increase their wall's phenolic and lignin deposits (Figs. 4 and 5). 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 indicates that the pin-hole 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.

Literature Cited

Drouilliard, G. 2006. Laser technology to label produce. Proc. Fla. State Hort. Soc. 119: (in press).

Etxeberria, E., W. M. Miller, and D. Achor. 2006. Anatomical and morphological characteristics of laser etching depressions for fruit labeling. HortTechnology 16:527-532.

Heslop-Harrison, Y. 1977. The pollen-stigma interaction: Pollen tube penetration in crocus. Ann. Botany 41:913-922.

PEI Board. 1995. Guide to coding fresh produce. Produce Electronic Identification Board. Newark, DE.