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The Anatomy of a Laser Label

Ed Etxeberria[1*], Cody Narciso[1], Preeti Sood[1], Pedro Gonzalez[1], and Jan Narciso[2]

[1]*University of Florida, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33823*

[2]*USDA–ARS, Tropical and Subtropical Research Laboratory, Winter Haven, FL*

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Laser labeling of fruits and vegetables is an efficient alternative to adhesive tags. In general, the label consists of alphanumerical characters formed by laser-generated pinhole depressions that penetrate the produce's surface creating visible markings. The depth and morphology of the pinhole depression likely influence parameters such as water loss and clarity of the markings. Given the enormous differences in fruit anatomy, we investigated the anatomical characteristics of laser-generated pinholes on citrus peel. In general, the pinhole depressions are irregular in appearance, of approximately 0.3 mm in diameter and only 1–2 cells in depth. Although the external appearance remains fairly constant during cold storage, cell underlining cells layers below the pinhole depression appear to undergo metabolic changes as evidenced by their increasing darkening. In conclusion, the pinhole depressions on citrus fruits are very superficial in nature and not penetrating below the flavedo, therefore not enhancing the potential for penetration of pathogens or decay organisms.

Laser labeling of fruits and vegetables is an efficient alternative to adhesive tags (Sood et al., 2008; Yuk et al., 2007). The advantages of this system are numerous, and have been detailed in previous communications (Etxeberria et al., 2006; Sood et al, 2008). In general, the label consists of alphanumerical characters formed by laser-generated pinhole depressions that penetrate the produce's surface creating visible markings. However, the perceived consequences of these superficial ruptures on the cuticle and epidermis have been the primary concern of industry personnel and governmental regulatory agencies.

In a developmental study of etched markings on tomato and avocado, it was demonstrated that the laser-induced pinhole depressions did not penetrate beyond the 4th to 5th epidermal cell layer (Etxeberria et al., 2006). The depth and width of etched pinholes through the cuticle and epidermis of both tomato and avocado were fairly consistent despite the great differences in their respective anatomy. In both instances, lignin was deposited around the outermost living cells underlying the pinhole depressions after 4 d in storage, demonstrating a self-healing mechanism.

In citrus fruits, the exocarp (or flavedo) consists of a distinct layer of approximately 20 cells in thickness (~500 μm) containing numerous epidermal oil glands of average size of 50 μm. Disruption of these oil glands by the laser beam can not only create a much larger cavity, allowing for higher rates of water loss, but will distort the lettering pattern as well. The present study was aimed at detailing the anatomy of waxed and unwaxed etched labels on citrus at different magnification levels, and their changes during storage.

Materials and Methods

Plant material. 'Red Ruby' grapefruit was purchased from Haines City CGA (Citrus Growers Association) Packinghouse, Haines City, FL. The fruit had been washed and waxed with carnuba containing 15 ppm thiabendazole (TBZ) following established commercial practices.

Fruit labeling. Fruit was labeled as described by Sood et al. (2008) using a low-energy carbon dioxide laser etching machine (Model XY Mark-10, Sunkist Growers Inc., Fontana, CA) located at the University of Florida's Citrus Research and Education Center, Lake Alfred, FL. Individual fruit were placed against a polyvinyl chloride (PVC) rectangular frame stabilized 10 cm from the laser's output. The energy level used was the recommended 0.000752 W/dot per 35-μs exposure with a 25% duty cycle range.

Tissue preparation. Cubes of peel containing laser-generated pinholes were cut and fixed for 4 h in a 3% gluteraldehyde solution in phosphate buffer of pH 7.2 followed by 3 buffer washes of 10 min. The peel was then fixed 4 h in a 1% osmium tetroxide solution in buffer followed by 2 buffer washes of 10 min each. Peel was then dehydrated in an acetone series.

Microscopy. For light and transmission electron microscopy, peel was infiltrated with Spurr's resin overnight in 50%, 75%, and 100% resin solutions in acetone. Peel was then embedded in Spurr's resin and cut in 1-μm-thick sections with a Reichart Jung UltracutE model ultramicrotome on glass slides for light microscopy and 85-nm thin sections on No. 5 copper grids for electron microscopy. Sections for light microscopy were stained with methylene blue/azure A and basic fuschin for 15 and 20 s, respectively, and observed on Leitz LaborLuxS light microscope. Sections for electron microscopy were stained with 2% uranyl acetate for 15 min and washed with distilled water. The wash was followed by staining with 5% lead citrate for 5 min and another washed with 0.02 M NaOH and distilled water. Sections were observed on a FEI Morgagni 268 transmission electron microscope.

For scanning electron microscopy peel was fixed in a Ladd critical point dryer and mounted on studs. Tissue samples were plated for 90 s in a Ladd sputter coater with Au-Pd alloy and observed on an Hitachi S530 scanning electron microscope.

*Corresponding author; email: eje@crec.ifas.ufl.edu; phone: (863) 956-1151, ext. 1238

Fig. 1. A laser-generated dot-matrix label on a citrus fruit using 0.000752 W/dot and 35-μs exposure time with a 25% duty cycle range. To enhance resolution, the label was stained with blue vegetable color.

Results

A freshly-etched label on a mature Valencia orange is shown in Fig. 1. As customary with produce of lighter color, the etched markings were stained with dark vegetable color to enhance visibility. Closer examination of laser-generated pinholes shows the irregular edges of individual depressions and the appearance of uneven wax cover (Fig. 2). The corrugated appearance of the wax likely resulted from momentary melting by the laser beam and re-solidification of the wax.

A cross-section light micrograph of an untreated Valencia orange peel is presented in Fig. 3. The unaltered epidermis consists of approximately 3–5 layers of compact cells with dense cytoplasm and thick anticlinal walls. Random crystal deposits are seen on the outermost epidermal layer (arrow) which is covered by a thick cuticle (arrowhead).

The outermost layers of identifiable cells in a citrus peel comprise the flavedo (colored layer), whereas the lower tear if irregular cells make up the albedo (Fig. 4A). A laser-generated pinhole on a citrus peel does not penetrate beyond the outermost two cell layers (Fig. 4 A, B), with average surface diameter of approximately 0.5 mm. The shallow depth of penetration was consistent throughout

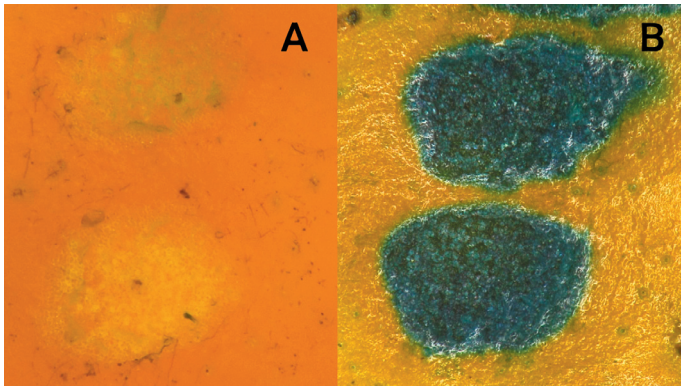

Fig. 2. A close up of an unstained (**A**) and stained (**B**) pinholes on a citrus fruit. Notice the uneven marking on **A** and the appearance of the reformed wax on **B**.

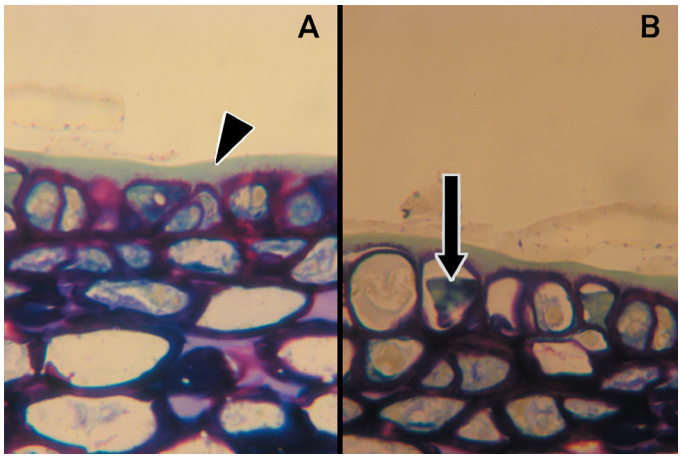

Fig. 3. Light micrographs of citrus peel epidermis showing dense cytoplasm, thick walls, sporadic crystals (**arrow**) and a conspicuous cuticle (**arrowhead**).

the many individual analyses including the apparent compression of underlying cells. Transmission electron micrographs (Fig. 5) and scanning electron micrographs (Fig. 6) confirm the superficial nature of the laser-generated pinhole in citrus. It is noteworthy that in tomato and avocado, up to 5 cell layers can be affected by the laser beam (Etxeberria et al., 2006).

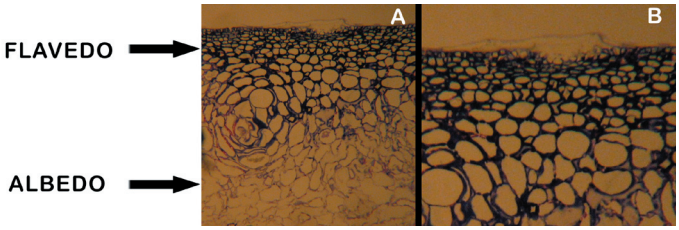

Fig. 4. Light micrograph of a cross-section of a citrus peel showing the albedo, flavedo areas (**A**) and a closeup of the pinhole depression (**B**).

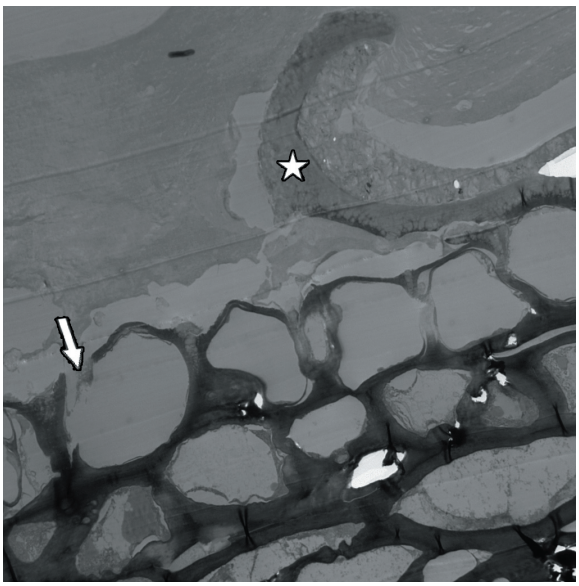

Fig. 5. Cross-section electron micrograph of a laser-generated pinhole depression on citrus fruit peel. Ruptured cell is indicated by the **arrowhead**, whereas the cuticle is marked by an **asterisk**.

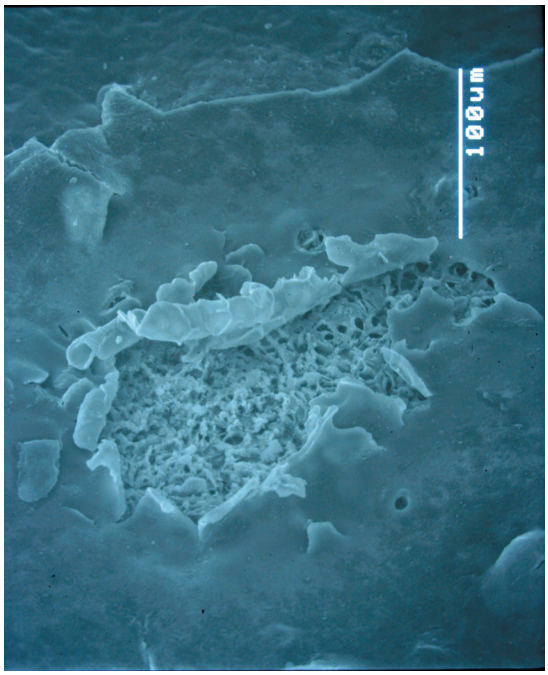

Fig. 6. Scanning electron micrograph of a of laser-generated pinhole depression immediately after etching on a citrus peel.

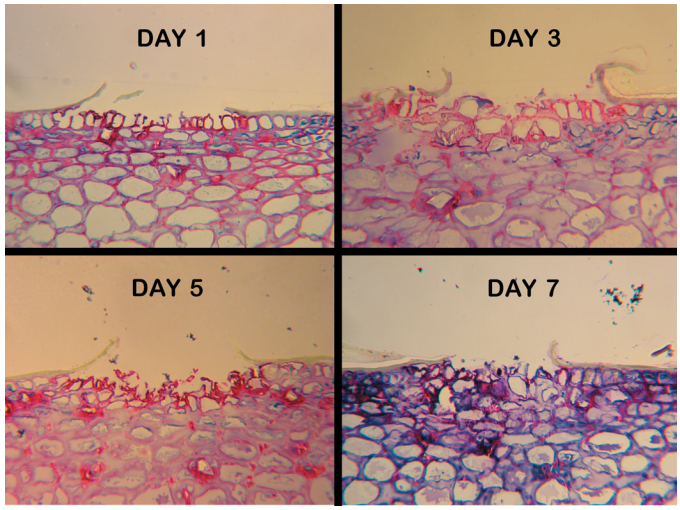

Fig. 7. Light micrographs of laser-generated pinhole depressions on citrus peel right after labeling and throughout 7 days in storage.

During storage at 10 °C and 95% RH, individual pinholes retain their morphological structure, even without an additional waxing treatment (Fig. 7). However, the sub-epidermal cells progressively stained darker presumably an indication of metabolic activity and development of protective deposits. A similar observation was made on stored tomato and avocado, where a distinctive protective layer was deposited below the pinhole (Etxeberria et al., 2006).

In conclusion, a laser-generated pinhole depression on citrus fruit does not penetrate beyond the second epidermal layer, decreasing the possibility of oil gland ruptures. During storage, the ruptured epidermal cells maintain their structure whether untreated or coated with an additional layer of wax. A noticeable feature of the underlining epidermal cells was the apparent reinforcement of cell wall components as demonstrated by the progressive darkening of the cell wall material when stained. The superficial nature of the pinhole and self-healing feature assist in protecting against pathogen invasion in long-term stored fruits and vegetables.

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