# EFFECTS OF LASER LABELING ON THE STORAGE QUALITY OF SELECTED FRESH FRUITS AND VEGETABLES

By

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To my late grandmother Mrs. Vedvati Sood, my parents Mr. Subhash Chandra Sood and Mrs. Subhash Sood and my lovable brothers Prashant and Dhanvesh

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| | |
|---|---|
| $CO_2$ | Carbon Dioxide |
| P.L.U. | Price Look Up |
| RH | Relative Humidity |

Abstract of Thesis Presented to the Graduate School
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EFFECTS OF LASER LABELING ON THE STORAGE QUALITY OF SELECTED FRESH FRUITS AND VEGETABLES

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Although in common practice since the 1990's, fruit labeling gained greater significance after 9/11 due to its potential relevance to food safety and bioterrorism. Labeling of fruits and vegetables is important for internal accounting, traceability and safety concerns. Most fruits and vegetables sold in the US are marked with non-permanent stickers or adhesive tags. These stickers usually contain names, identification numbers (three, four or five digit codes), country of origin, brands, and logos. They provide required information and help distinguishing among produce with similar appearance but a different quality value, resulting in a more efficient checkout process. Despite the many advantages, sticker labeling technology has several drawbacks. These adhesive labels are expensive to purchase and apply, and application equipment requires clean up after the season. In addition, produce are often left with glue residue on their surfaces after sticker removal, resulting in blemished fruits. An emerging technology called "Laser Labeling" could be considered a desirable alternative to the existing labeling system. Not only is each piece of produce permanently coded, but the specific information can be stored electronically for any period of time. The technique involves a low energy carbon dioxide laser beam which vaporizes the epidermal cells and reveals the underlying cells, preferably with a contrasting color. Produce epidermis is marked with alphanumerical dot matrix

characters formed by pinhole depressions (Drouilllard and Rowland, 1997). However, these depressions can promote water loss and increase the number of potential entry sites for decay-promoting organisms. The present study addresses the most vital of these postharvest concerns. The experiments were conducted to measure water loss, peel appearance, and potential decay in laser labeled citrus fruit namely "Honey tangerine" (*Citrus reticulata*), "Ruby Red" grapefruit (*Citrus paradisi*), tomato (*Lycopersicon esculentum*) and pepper (*Capsicum annum*) during storage. Laser labeled fruit stored at their respective optimum temperature and two relative humidities (i.e., 95% and 65% RH) for 3 to 5 weeks depending on the commodity, showed no increase in decay compared to non-etched control fruit, suggesting that laser labeling does not facilitate decay. This was confirmed by experiments where the spores of common postharvest pathogen (such as *Penicillium digitatum* in citrus and *Geotrichum candidum* in tomato and pepper) were coated on fruit surfaces before and after laser labeling. In either case, no decay was observed. Further, in agar plates which contain a lawn of *P. digitatum* spores, the laser labeling reduced germination of spores in contact areas. Water loss from etched areas and label appearance were determined during storage. Water loss from waxed and etched citrus peel declined to control levels after 24 hours in storage. Label appearance deteriorated slowly in citrus fruit as compared to tomato and pepper and was proportional to laser energy levels and ambient relative humidity. Waxing the laser labeled surface in citrus fruit reduced water loss by 35% to 94%, depending on the wax formulation used. This study concludes that laser labeling provides the produce industry a feasible alternative to adhesive sticker labeling without enhancing decay susceptibility.

# CHAPTER 1
# INTRODUCTION AND LITERATURE REVIEW

Fresh fruits and vegetables have been a part of the human diet in all cultures (Willis, et al., 1998). The nutritional importance of fresh fruits and vegetables was first recognized in England during the early part of the17$^{th}$ century for the ability of citrus to cure the 'scurvy' disease among the naval personnel. Their complete nutritional benefits were not known until recently however. Most fruits and vegetables are high in water content (80-90% of fresh weight) and low in protein and fat content. Carbohydrates content in fruits and vegetable is low except in certain starchy vegetables, for example cassava, potato, sweet potato, sweet corn and fruits such as dates. Lipids comprise less than 1% of most fruits and vegetables and are associated with cell membranes and the protective cuticular surface layer. Fruits and vegetables are also rich sources of certain vitamins, especially beta-carotene (precursor of vitamin A) and vitamin C (ascorbic acid). Approximately 95% of the human dietary vitamin C comes from fruits and vegetables. Fruits, such as citrus, strawberry and kiwifruit, are outstanding sources of this vitamin. All vegetables contain small amounts of the B-complex vitamins, but their nutritive value is attributed mainly to beta carotene, vitamin C and folic acid. The most abundant mineral found in fruits and vegetables is potassium followed by calcium. Minerals like magnesium, iron and phosphorus are also present at substantial levels. Their high water and fiber content helps in digestion and utilization of foods. Fruits and vegetables are good sources of naturally occurring antioxidants that may help protect against free radicals and oxidative damage, thereby lowering cancer and cardiovascular disease risk (Cao et al., 1996; Cohen et al., 2000). These antioxidants include vitamin C, glucosinolates, flavonoids , carotenoids and polyphenolics (Larson, 1988). For example limonin and nomilin in citrus fruits are believed to help inhibit the development of certain forms of cancer (Lam and Hasegawa, 1989; Miller et al., 1989). Many studies have

stated that a frequent intake of cruciferous vegetables, such as broccoli, cauliflower, and cabbage, could also be helpful in protecting against cancer (De Long et al., 1986, Zhang et al., 1995). Avocado is a very rich source of monounsaturated fats (50-75%) and recent studies reported the benefits of avocado with respect to heart diseases. The United States Department of Agriculture food pyramid or "5 A Day- For Better Health" dietary program suggests 2 servings of fruits and 3 servings of vegetables every day per individual (Center for Nutrition Policy and Promotion, 2000).

World fruits and vegetable production is estimated around 388 MT and 486 MT respectively, with China being the world's largest producer of both fruits and vegetables followed by India. According to FAO, China contributes nearly 50% of the world's vegetable production and 16% of the fruit production. India accounts for about 8% of the world's fruit production and 15% of the total vegetable production. US is a dominant player in the international trade of fruits and vegetables and accounts for about 25% of world trade. As far as production within the US, California leads by producing more than 50% of the all fruits and vegetables. Florida is the second largest producer with 8% and 14% of the US vegetable and fruit production respectively (NASS, 2000). In general, production of fruits and vegetable crops worldwide has increased over the past few years.

### 1.1 Fruit Anatomy and Classification

Botanically, 'fruit' is defined as a ripened or mature ovary including seeds and other associated plant plants such as the receptacle (e.g.,strawberry), bracts (e.g., apple) and peduncle (e.g., pineapple). Fruits can be classified various ways. Based on the growing conditions, fruits are divided into temperate, tropical and sub-tropical types. Temperate fruits are grown in places with distinctly cold winter. These are suited to higher elevations and can withstand frost such as apple, plum, peach and pear. Tropical fruits require moist warm climate and can bear dry

weather (e.g., banana, mango, guava, papaya). Fruits such as citrus, grapes, loquat etc., are sub-tropical in nature. These fruits are grown in between temperate and tropical climates, and can withstand low temperature and frost but only for short period.

Based on their respiration patterns and ethylene production during maturation and ripening, fruits and vegetables are categorized as either climacteric or non climacteric (Biale, and Young, 1981). The term 'climacteric' was first used in apples for the characteristic rise in the respiration rate that accompanies maturation and ripening (Kidd and West, 1924). The ripening process of climacteric fruits (e.g., apple, banana, mango) is accompanied by a peak in respiration and a simultaneous burst of ethylene production (Giovannoni, 2004). When exposed to exogenous ethylene, climacteric fruits undergo autocatalytic ethylene production. On the contrary, non climacteric fruits (e.g., citrus, strawberry) do not show increased ethylene production and respiration during ripening (Knee et al., 1977). Vegetables in general are divided into three main groups: 1) seeds and pods; 2) flowers, buds, stems and leaves; and 3) bulbs, roots and tubers. Some immature fruit (cucumber, zucchini, beans) and ripe fruit types (tomato, avocado, capsicum, egg plant) are also consumed as vegetables.

### 1.2 Postharvest Concerns

Fresh fruits and vegetables are living tissues and therefore highly perishable in nature. They continue their biological processes after harvest and subject to changes (Kader, 2002). Their quality rapidly deteriorates after harvest. During handling and transportation, these fresh commodities are subjected to impact, compression, vibration, bruising, low/ high temperature, humidity conditions and pathological attacks. These may lead to loss in the quantity and quality of these fresh commodities between harvest and consumption. The magnitude of these postharvest losses is higher in developing countries (20-50%) than in developed countries (5-25%) depending on the commodity, cultivar, infrastructure and handling practices. These losses

can be reduced by implementing proper handling and storage techniques. Proper postharvest management facilitates the continuous and timely supply of fresh fruits and vegetables.

The deterioration of fresh commodities can be the result of physiological breakdown due to natural ripening, water loss, temperature injury, or invasion by pathogens (Thompson et al., 1998). These harvested fresh commodities exhibit enhanced respiration and ethylene production. Respiration is a process by which organic compounds are broken down into simple sugars and further with a release of heat ATP and energy resulting in reduced nutritional quality as well. Within the range of $4^{o}$ to $35^{o}$C every 10 $^{o}$C rise in temperature causes 2-3 fold increase in the respiration rate thereby shortening the shelf life of the commodity (Pittenger, 2002). Fresh commodities are stored at low temperature to slow down respiration and senescence which in turn lengthen the postharvest shelf life (Kader, 2002). Since fresh horticultural commodities vary in composition, morphology (roots, stems, leaves, flowers, fruits) and physiology, they have different postharvest requirements and recommendations for maintaining maximum postharvest shelf life. Storage life can vary from < 2 weeks to > 16 weeks depending on the commodity. For example, green onions can be stored for less than 2 weeks as compared to dried onions which can be held at 0 $^{o}$C for 8-10 weeks. Citrus fruits in general can be stored for moderate periods of time. Grapefruit can be stored for 6-8 weeks at 12-15 $^{o}$C and 90-95% RH. Fully ripe tomatoes can be stored optimally for 3-5 days at 7-10 $^{o}$C and 85-95% RH, in contrast to mature green which can be kept for 14 days at 12.5 -15 $^{o}$ C. Storage of peppers at 7.5 $^{o}$C is best for maintaining maximum shelf life of 3-5 weeks.

Fresh horticultural crops are a “package of water”. Water loss equates to loss in saleable weight, textural quality (softening, flaccidity, loss of crispiness, juiciness) and nutritional quality. Weight loss of only 5 % can make the fresh produce appear wilted or shriveled and may render

them unmarketable. However, high (RH) (90-95%) in conjunction with low temperature and low surrounding air velocity helps in reducing water loss. In general, fruits store best at 85 to 95% RH and vegetables at 90 to 98% RH in order to retain freshness. Besides holding the produce at high humidity, transpiration or water loss can be reduced by applying waxes and other surface coatings or wrapping with plastic films. Fruits that are normally waxed include citrus, apples, pear, avocado, cucumber, tomato and pepper (Baldwin, 1994).

Packaging is one of the most important postharvest treatments required for marketing of fresh produce. It also plays a crucial role in preventing deterioration losses and improving shelf life of produce. More than 1,500 types of packages are used for produce in the US. Packaging material includes corrugated fiber board boxes, plastic trays, mesh bags, woven sacks, stretch films, shrink wrapping etc. Commodities such as potatoes, onions, citrus, sweet corn are packed in mesh bags. Besides making attractive displays for supermarket, these bags provide adequate ventilation for produce. Plastic bags (polyethylene films/wraps) are commonplace in packing fresh fruits and vegetables such as wrapping of an individual stalk of cauliflower, cabbage, and lettuce. High value produce items such as berries (strawberry, blueberry, raspberry), small fruits and datil peppers are most often packed in clamshells. Besides being inexpensive, clamshells provide great protection to the produce and make a very pleasing consumer package.

### 1.3 Traceback

The “Center for Disease Control and Prevention” (CDC) estimated that food borne diseases causes approximately 76 million cases illnesses, 350,000 hospitalizations and 5,000 deaths annually in US. Produce is considered a leading vehicle of foodborne illnesses and was responsible for more than half of illnesses associated with foodborne outbreaks between 1998 and 2004. From 1995-2006, 22 produce outbreaks have been reported in the US. Hepatitis cases by consumption of green onions from Mexico in 2003 and Salmonella outbreak of 2004 in Roma

tomatoes led to deaths of thousands. Furthermore, in 2006, 205 cases of E. coli O157:H7 illnesses transmitted by bagged spinach were reported in 26 states. The same year, Salmonella outbreaks linked to tomatoes accounted for the death of thousands. Salmonella is the most common bacterial agent causing food borne illness. Approximately, 1.4 million illnesses and 600 deaths are caused by Salmonella each year in US (Mead, et al., 1999). In 2005, US Center for Disease Control and Prevention's Emerging Infectious Program under the Food-borne Diseases Active Surveillance Network reported 6471 confirmed cases of Salmonella (Center for Disease Control and Prevention, 2006). However, difficulty in tracing the source of tainted food items stifled the investigation of these past outbreaks. Due to the difficulties in tracing back produce, more emphasis has been lately placed on traceability and other food safety concerns of the fresh produce industry.

Traceability is the ability to trace food items back to their source (growers, packers) and through all commercial channels. It is considered as "good agricultural practices" (GAP) and intended to minimize the liability and preventing occurrence of food security problems. (Center for Food Safety and Applied Nutrition,1998). Owing to the current food safety concerns, track and trace systems for the product in international trade, particularly in sea and air freight are becoming increasingly important. There is a need for high quality identification (labeling) and information systems.

### 1.4 Produce Labeling

In 2002, US congress passed the Public Health Security and Bioterrorism Preparedness and Response Act, also known as "Bioterrorism Act". The purpose of the Act was to protect the country's food supply against the intentional contamination. Section 305 of this Act specifies the requirement for labeling of products including produce. Since then price look up (P.L.U.) labeling of produce has become commonplace. Price –look –up (P.L.U.) labeling of the fresh

fruits and vegetables has gained marked attention in the United States over the last decade. PLU codes are normally used on items that are sold in loose state or bunch (for example, an individual orange or bunch of greens). The PLU index coding contains four-digit identification number developed by the Produce Electronic Board (PEIB) to identify the variety of fruit and vegetables (PEIB, 1995). This board was established to upgrade the electronic data collection and communication of fresh produce sales (Etxeberria et. al., 2006).

So far, the most commonly used labeling system consists of adhesive tags/stickers applied to individual fruit and vegetable on the packing line (Varon and Paddock, 1978). The first fruit label is dated back in 1929 and was created by a British company Elders & Fyffes Ltd. (Anonymous). Produce labeling started with the labeling of bananas. Labeling of oranges, grapefruit, tomatoes, pepper and others with individual stickers is accomplished by passing the individual fruit beneath a cassette of gummed labels applied with gentle pressure. The PLU stickers usually contain names, identification number (three, four or five digit code), country of origin, brands and logos. These are helpful for grocery clerks, making it easy to distinguish among produces with similar appearance, but a different quality value. As a consequence, sticker labeling also saves time at the checkout counter.

Despite the many advantages, sticker labeling technology has several drawbacks. Besides being expensive, these stickers require clean up after the season, as they gummed up along the packingline. Fruits are often left with glue residue on its surface after sticker removal resulting in blemished fruits. In addition, these stickers are not permanent and can be detached at any postharvest handling stage. It is also not possible to make P.L.U. number changes in a timely manner.

## 1.5 Laser Labeling

Laser labeling is a technology where low-energy CO2 lasers beam (10,600 nm) (Drouilllard and Rowland, 1997) is used to mark a produce. The general etching process is similar to the one used in electronics manufacturing and medical treatments and explained by Hecht (1994). The laser labeling is a sterile, non-contact, high speed, efficient method which provides a sharply defined permanent mark. Laser beam etches the outermost pigment layer of the produce skin to reveal a contrasting sub-layer. Etched markings are formed in dot matrix pattern letters and numbers, each dot created by pinhole depressions (Etxeberria et al., 2006). The laser labeling apparatus has an articulating arm with an optical head attached to it (Figure 1-1) and can be positioned at a variety of positions related to the piece of produce. The laser head emits a high intensity and controlled light beam which is directed along a predetermined path to etch the epidermis of the produce. The mark is produced by "vaporizing" the wax portion of the skin and desiccating the underlying cell layers under where the high intensity beam contacts the skin. The contact time of the beam is yet limited to reduce or prevent transfer of heat energy or thermal degradation or breakdown of the underlying tissues. The whole system is controlled by a programmable electronic interface, which is a high speed programmable logic controller (PLC). The graphic interface create files to store specific equipment settings required for different varieties of fruit, different PLU codes, and other related information. Such settings of the equipment allow the system to sense differences in surface texture and color and make necessary adjustments.

In order to operate the laser labeling machine, the user enters the desired code and system parameters using a keypad. The PLC uses this input and the one from the attached external sensors to make the necessary calculations and adjustments in order to emit laser beam. The system can etch PLUs, trace codes, and date and time stamps in 14 different languages; it can

also convert any kind of image into a matrix that can be printed. The processing speed of the laser labeling machine is up to 17 fruits per second. It can be used in various fruits and vegetables such as cucumber, pepper, potato, avocado, tomato, apple, citrus, pear, watermelons and even onions (whose epidermis is only 18 µm deep) (Figure 1-2A and Figure 1-2B). For fruits such as citrus that do not have good contrast, FDA-approved food coloring is used for contrast enhancement.

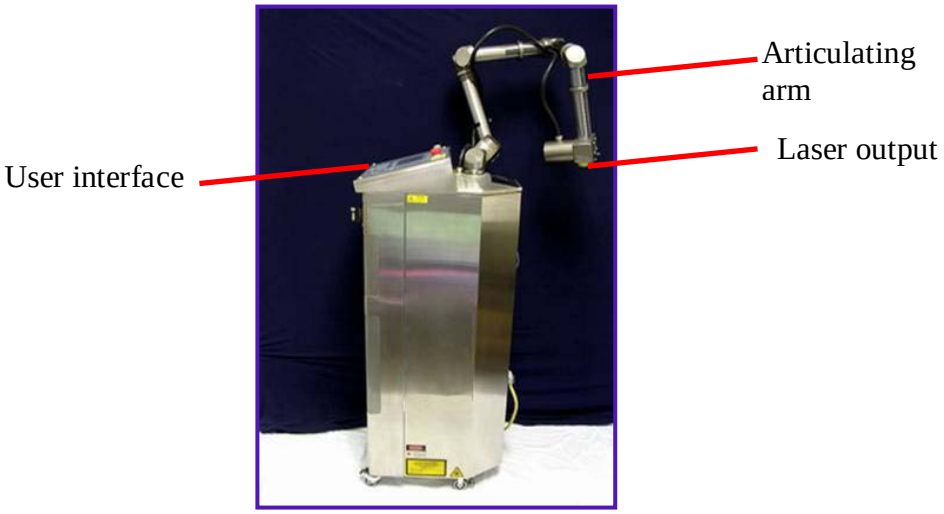
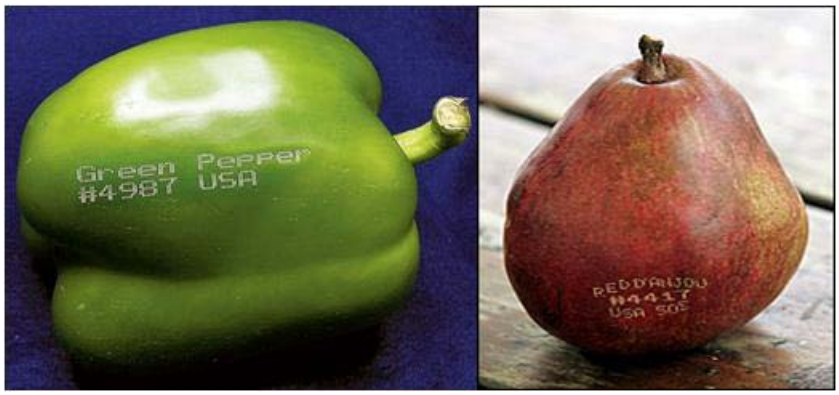



Figure 1-1. Laser labeling machine




A B

Figure 1-2. Laser Labels. A) Laser labeled pepper. B) Etched pear.

Other advantages of this laser technique are that the label are permanent, do not require additional adhesives, and labeling information can be stored electronically for any period of

time. In the long run, laser system offers production cost cutting as well. The machine requires little or no supervision, thereby reducing operating cost to a greater extent. Furthermore, these labels do not create any clutter, leave no glue residues on the produce surface, and above all, offers the potential to track and trace the origin of the produce. The system is more flexible as PLU information can be changed number of times before labeling the fruit. Furthermore, the laser labels are environment friendly.

The barriers to the acceptance of this technology are the presumptions that it is not safe and may leads to early produce decay, as pinhole depressions make the surface more susceptible to water loss, potential pathogen attack and other postharvest losses. Water loss from harvested fruit occurs continuously and may be responsible for loss of quality and marketability of fruit. The natural cuticular wax layer of citrus fruit protects against water loss. Laser labeling pinholes rarely penetrate beyond the third layer of epidermal and/or underlying cells. Underlying tissues deposit additional wax, lignin and phenolic suggesting a self healing process as noticed previously in avocado and tomato (Exteberria et.al, 2006). Brown (1973) also reported lignin deposition in the injured citrus peel. Significant deposition of lignin was noticed around the wounds inoculated with *Geotrichum candidum* in lemon peel (Baudoin and Eckert, 1985). Some changes were observed at the wound and in surrounding peel tissues after 1-4 days of wounding in satsuma mandarins (Kinay et al., 2005). The lignin-like material (L-LM) is induced by wounding of fruit in cucumber (Walter et al., 1990) and impedes the hyphal penetration of Penicillium in lemons (Stange and Eckert, 1994).

The effectiveness of natural cuticular barrier diminishes by the soaking, washing and brushing of the fruit during packinghouse operations. Therefore, commercial wax coating is applied to the fruits to compensate the loss of natural protection. In addition, waxes applied for

other reasons such as reduction of water loss, improved appearance and some level of protection against decay. The composition and properties of a typical self-polishing, fungicide-containing water wax was described by Newhall and Grierson (1955). In general the rate of water loss from waxed fruit is comparable to unwashed fruit. Commercial use of waxes is extensive for fruits, especially apples and citrus, and some vegetables such as tomatoes, melons and cucumbers. Limited use is observed in asparagus (*Asparagus officinalis*) peppers, carrots, radish, potatoes, squash and turnip (Baldwin, 1994).

Produce may be exposed to pathogens both prior to harvest in the field and also after harvest while handling, storage and transit. Most pathogens required wounds or other unprotected areas to enter the fruit tissue. Laser labeling disrupts the natural protective barrier seemingly creating open wounds. Studies on anatomical, morphological and physical aspects of the laser labeling cavities states that the cells underlining etch depression increase phenolic and lignin deposits in their walls (Etxeberria et al., 2006) , yet the degree of protection has not been investigated. The microbial community of bacteria and yeasts on the surfaces of fruit and vegetables can influence the development of postharvest rots of fruit and vegetables (Blakeman, 1985; Spurr, 1994). Although it was shown previously that the alphanumeric codes produced by the laser beams do not support the infiltration and survival of spoilage organism like *Salmonella* spp. on tomato surface (Yuk et. al., 2007) little additional information is available regarding the decay of laser labeled fruits. The present study addresses potential complications brought about by microflora population and various other postharvest losses on the laser labeled fruit in comparison to non laser labeled.

# CHAPTER 2
# EFFECTS OF LASER LABELING ON THE QUALITY OF TANGERINES DURING STORAGE

## 2.1 Introduction

Citrus is the major fruit crop of Florida. The state contributes nearly 58% of all U.S. tangerine production, 78% of which goes to the fresh market. With the possibility of bioterrorism and other economic concerns, labeling of fresh market produce has become increasingly relevant in the last few years. In addition to making 'check-out' easier, labeling helps with the tracking and traceability of the produce. Laser labeling is emerging as an alternative to traditional stickers/adhesive labels. With this method, a low energy $CO_2$ beam creates pinhole depressions into the product surface that forms the alphanumeric label information (Drouilliard and Rowland, 1997). However, these pinhole depressions disrupt the protective barrier of the produce and can potentially become entry sites for decay organisms and sites for enhanced water loss, despite the significant amounts of wax and lignin deposited by surrounding and underlying cells (Etxeberria et al., 2006). Little information is available on the impact of this new technology on the overall quality of the labeled produce, especially its effect on water loss and decay during storage. The present study investigates the effects of laser labeling on the quality of "Honey tangerine" (*Citrus reticulata* Blanco) during storage.

## 2.2 Materials and Methods

### 2.2.1 Plant Material

"Honey tangerine" (*Citrus reticulata* Blanco) fruit was purchased in October 2008 from Haines City CGA, Haines City, Fla. after commercial washing and waxing. Fruit was labeled using a low energy $CO_2$ laser labeling machine (Model XY mark 10, Sunkist Growers Inc., Fontana, Calif.) at the Citrus Research and Education center in Lake Alfred as described earlier (Etxeberria et al., 2006).

### 2.2.2 Exposure Time Selection

A label code "M1" was etched on fruit surfaces using 18 different exposure times corresponding to 30 µs to 140 µs label duration (Etxeberria et al., 2006). The energy level used was the recommended 0.000752 W/dot. To enhance resolution, labels were dyed with fruit-based black color. Five replicates per exposure time were used. Images of the dyed label were taken using a Canon Powershot S31S digital camera mounted on a Wild Heerbrugg 165083 stereoscope. Surface area altered by the label was measured using an Image Processing Software. Total area was calculated based on number of pixels within the area covered by the laser depressions with 1 pixel corresponding to 50 $\mu m^2$.

### 2.2.3 Water Loss

Water loss from the fruit surface was measured using a modified leaf porometer (Decagon Devices, Pullman, Wash.). To estimate water loss as a function of exposure time, an etched rectangle of 7 x 8 dots matrix pattern was used for each of four exposure times, namely low (35 µs), commercially recommended (45 µs), medium (85 µs ), and high (120 µs). The modified leaf porometer was placed on the top of the treated area immediately after laser labeling until a stable reading was obtained.

Water vapor diffusion from fruit and vegetables was calculated using Eq. 2-1 based on Fick's law where "water vapor flux density" or "evaporation rate" is the product of vapor conductance (gv) and the difference between vapor concentration at the evaporating surface (Cvs) and water vapor concentration in atmosphere (Cva). Values are expressed as mmol $m^{-2}$ $sec^{-1}$. The modified leaf porometer (Decagon Devices, Pullman, Wash.) estimates the value for water vapor conductance (gv) which can be computed in the equation below to obtain "evaporation rate."

$$F = gv\ (Cvs - Cva) \qquad (2\text{-}1)$$

Vapor concentration was calculated using Eq. 2-2, where $e_a$ is the vapor pressure (kPa) which is a function of temperature, and $p_a$ is the atmospheric pressure (kPa) (information courtesy of Dr. Doug Cobos, Decagon Devices, Inc., Pullman, Wash.).

$$Cv = e_a / p_a \quad (2\text{-}2)$$

### 2.2.4 Effects of Waxes on Water Loss from Labeled Areas

Nine different commercial waxes were tested for moisture loss reduction from the labeled area. Fruit were labeled using a single exposure time (45 µs) and wax applied using a painter's brush. Evaporation rate measurements of the labeled surface were determined before and after waxing along with control (no label on fruit). Each experiment was replicated 30 times. The second part of the experiment followed water loss from the waxed etched area during storage. Half of the total number of etched rectangles (2 rectangles per 30 fruit) were waxed with one of the waxes tested (highest reduction in water loss) and kept at 10 $^{o}$C and 95% RH for 7 d. Daily measurements of evaporation rate of the waxed label, unwaxed label, and control (no label on fruit) were carried out with the porometer.

Table 2-1. Nine commercial waxes tested for water loss reduction from laser labeled areas

| Names of waxes | Names of Manufacturers |
|---|---|
| Deco shellac | Deco, Monrovia, CA |
| Carnuba 505 | Deco, Monrovia, CA |
| Carnuba + TBZ | Deco, Monrovia, CA |
| Carnuba 231 | Deco, Monrovia, CA |
| Carnuba blend | Deco, Monrovia, CA |
| Citrus wax shellac | HDH Agriproducts, Tavares, FL |
| HDH Carnuba | HDH Agriproducts, Tavares, FL |
| Pace Carnuba | Pace International, Visalia, CA |
| Organic Carnuba | Pace International, Visalia, CA |

### 2.2.5 Peel Stability

The label 'Florida citrus' was etched on the fruit using 45 µs for exposure time, and fruit kept at 10 oC in two different relative humidity levels (i.e., 65% and 95% RH) for 4 weeks. Five

replicates (1 replicate = 1 box = 60 fruit) per exposure time and control (non-labeled fruit) were used. Weekly examination of the label appearance and surrounding area was done. Peel stability was determined on the basis of a visual rating scale according to the shrinkage of the skin around label as follows: 0 (no shrinkage), 1 (very low), 2 (low), 3 (medium), 4 (high), and 5 (very high) (Figure 2-1). For better visualization, we show the scale using grapefruit.

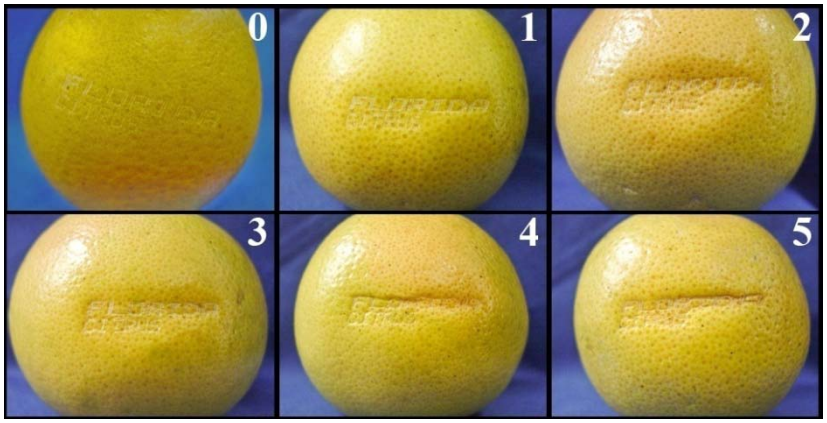



Figure 2-1. Shrinkage rating scale ranges from 0 (no shrinkage) to 5 (total label collapse)

### 2.2.6 Decay Study

Fruit were labeled using the commercially suggested exposure time (45 µs) and stored for 5 weeks at 10 $^{o}$C and 95% RH. Five replicates of labeled and control (non-labeled fruit) (1 replicate = 1 box = 60 fruit) were used. Fruit were examined weekly for decay.

### 2.2.7 Inoculation Study

Experimental areas on the fruit were subjected to four treatments. The four treatments were: 1) inoculation of fruit prior to labeling, 2) inoculation after labeling, 3) inoculation on waxed label, and 4) waxing of inoculated label. Fruit were inoculated with a spore suspension of *Penicillium digitatum*. Link ($10^5$) was used as inoculum. Inoculum was prepared by growing *P. digitatum* Link on potato dextrose agar (BD/Difco, Sparks, MD) plates for 7 d. One hundred microliters of sterile 0.1% Tween 20 was placed on the plate surface and the spores were liberated from the colony and placed in sterile phosphate buffer (0.1%; 7.2 pH). Spore

concentration was determined by counting cells with a hemocytometer (Hausser Scientific, Horsham, PA). Inoculum was adjusted to $10^5$ cells /mL. Inoculation was carried out by spreading a thin layer of spore suspension onto the fruit peel before and after labeling using a sterilized small brush. Inoculation was carried out by applying a thin layer of spore suspension over the experimental areas using a painters brush. Inoculated fruit was stored at 10 $^oC$ and 95% RH for 3 weeks and examined for decay weekly. Labeling was done using one exposure time (45 µs). Thirty replicates consisting of one fruit each were used for all four treatments.

In a separate experiment, *Penicillium* spore suspension was spread on potato dextrose agar plates (BD/Difco, Sparks, Md.). The plates were laser labeled and observed under the microscope immediately and 72 h after labeling. Each experiment was repeated five times.

### 2.3 Results and Discussion

The visual effect of increasing laser labeling exposure time on tangerine peel is shown in Figure 2-2. At the lowest possible exposure time of 30 µs, the label was faint and hardly visible, whereas at the highest exposure time of 120 µs, the etch markings merged into solid lines. Calculations of laser etched surface area were done using Image processing software. In general, the area covered by the etched markings increased with increase in exposure time (Figure 2-3). The rate of peel surface area disruption declined at higher exposure times as etch markings began to merge. Etched markings created with 45 µs exposure time were selected as the best among all energy levels on the basis of visual appearance and area covered. This exposure time (45 µs) creates less surface disruption while generating readable code. However, from Figure 2-2 and 2-3, higher exposure times create darker labels without significantly increasing peel disruption.

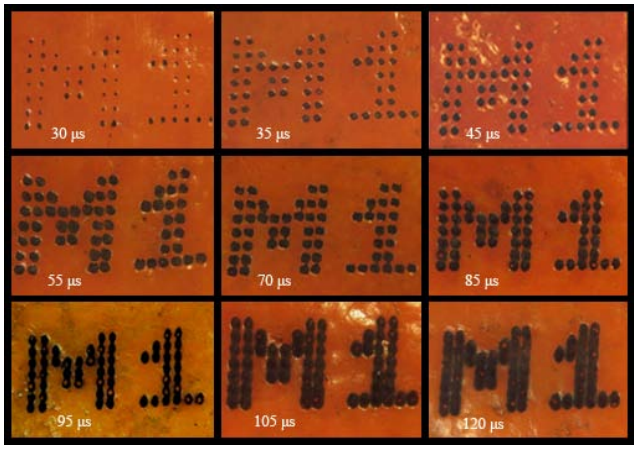



Figure 2-2. A group photograph of labels etched using increasing exposure times

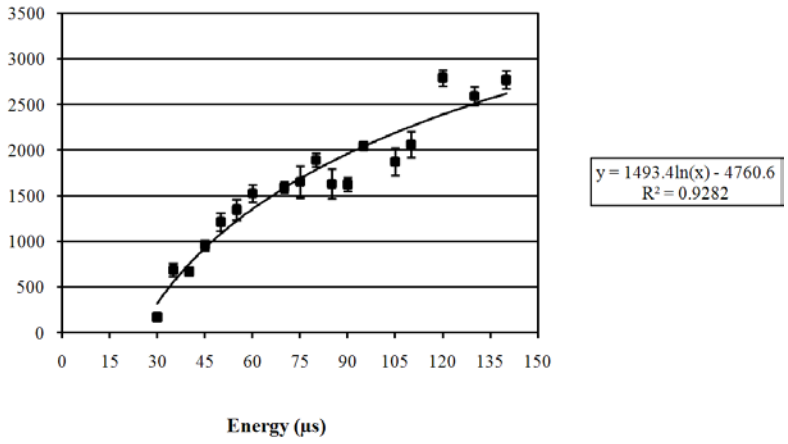



Figure 2-3. Surface area covered by 100 dots etched with exposure times ranging from 30 µs to 140 µs. Each point represents an average of five replicates. Vertical lines represent SE

### 2.3.1 Water Loss

Moisture content is one of the important factors determining the marketable quality of produce. Fruit and vegetables start losing moisture immediately after harvest (Ben Yehoshua and Rodov, 2002). However, laser-generated etched markings are physical pinholes penetrating through the cuticle and into the epidermis rendering the produce surface more susceptible to water loss than non etched surfaces. There was a sharp increase in the rate of water loss between the 35 and 45 µs exposure times (Figure 2-4). The difference in the rate of water loss was less pronounced at higher exposure times.

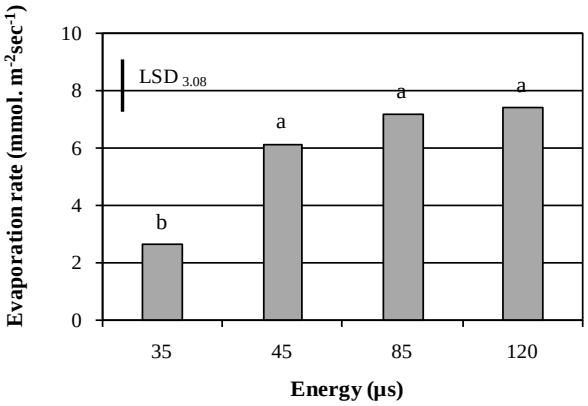



Figure 2-4. Water loss associated with labels etched using four different exposure times. Each bar represents an average of 20 samples

### 2.3.2 Effects of Waxes on Water Reduction from Labeled Areas

Natural waxes on the epidermis play a vital role in retaining moisture in fruit and vegetables (Kader, 2002). The openings created by laser labeling disrupt the natural waxy coating on the surface of produce. In this experiment, all nine commercial waxes that were applied helped in reducing water loss by sealing the etched area. However, significant differences in the capability of these waxes to prevent water loss were observed. Among all waxes, three reduced water loss by >80%, with Carnauba 505 resulting in the highest protection (86% reduction in water loss) (Figure 2-5).

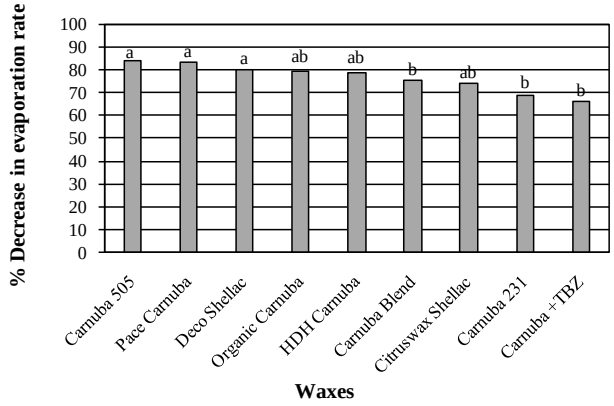



Figure 2-5. Percentage decrease in evaporation rate resulting from wax application on labeled areas. Each wax was tested on 30 separate labels

Rate of water loss from unwaxed labeled areas declined with time of storage. There was a sharp decline in the rate of water loss 24 h after labeling, which continued to decrease before reaching a constant value (Figure 2-6). Water loss from waxed labeled (using Carnuba 505) areas was nearly equal to control unlabeled areas, which confirms the importance and necessity of waxing the fruit after labeling.

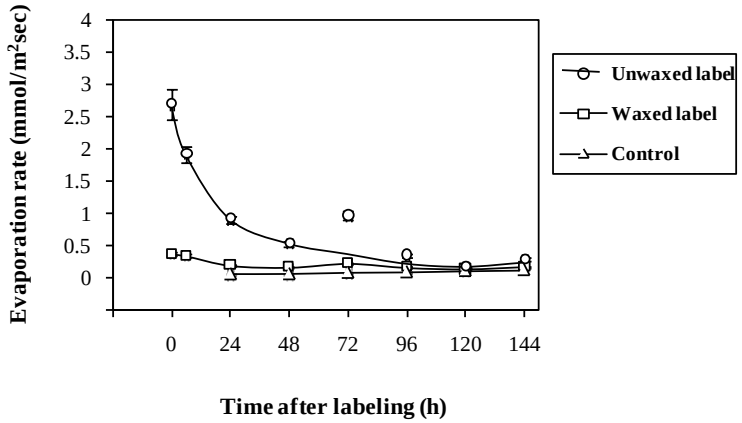



Figure 2-6. Evaporation from unwaxed laser label, waxed laser label (using Carnuba 505), and control (no label) during 7 d storage. Each point represents 30 samples. Vertical lines represent SE

### 2.3.3 Peel Stability

The rate of water loss from fruit depends upon the vapor pressure deficit between the commodity and surrounding air, which is influenced by temperature and relative humidity. Relative humidity has a direct effect on rate of moisture loss from produce (Forbes and Watson, 1992). Fruits are commonly stored at ≥ 90% RH (Nuñes, 2007). In the present study, 45 µs labels at both 65 and 95% RH, began to show low levels of shrinkage within a week (Figure 2-7 and 2-8). Degree and percent fruit showing visible signs of shrinkage on the label increased with storage time, exposure time (data not shown), and was inversely proportional to RH. However, even at the end of 5 weeks, labeled fruit peel at 95% RH had minimal shrinkage.

Table 2-2. Peel stability of laser labeled "Honey Tangerine" using 45µs and stored at 10 °C and 65% RH

| Percentage of total fruit | | | | | |
|---|---|---|---|---|---|
| Time after labeling (weeks) | 1 (very low) | 2(low) | 3(medium) | 4(high) | 5(very high) |
| 1 | 5.28 | 1.66 | 0.32 | 0 | 0 |
| 2 | 6.3 | 3.66 | 0.66 | 0 | 0 |
| 3 | 2.64 | 5.28 | 2.98 | 0.32 | 0 |
| 4 | 2.96 | 4.64 | 4.62 | 1.96 | 0 |

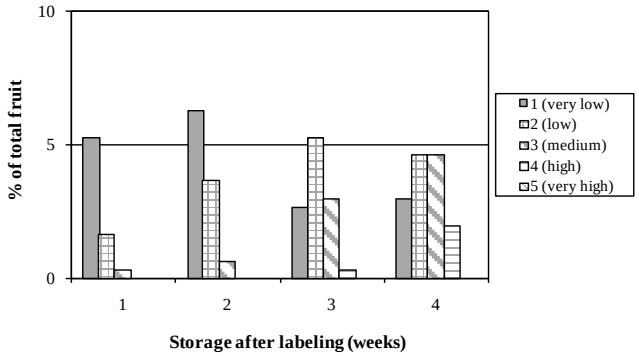



Figure 2-7. Peel stability of etched fruit (45 µs) during storage at 10 °C and 65% RH

Table 2-3. Peel stability of laser labeled "Honey Tangerine" using 45µs and stored at 10 °C and 95% RH

| Percentage of total fruit | | | | | |
|---|---|---|---|---|---|
| Time after labeling (weeks) | 1 (very low) | 2(low) | 3(medium) | 4(high) | 5(very high) |
| 1 | 1.28 | 0.32 | 0 | 0 | 0 |
| 2 | 2.6 | 0.66 | 0 | 0 | 0 |
| 3 | 0.98 | 1.96 | 0 | 0 | 0 |
| 4 | 1.64 | 1.96 | 0.64 | 0.32 | 0 |

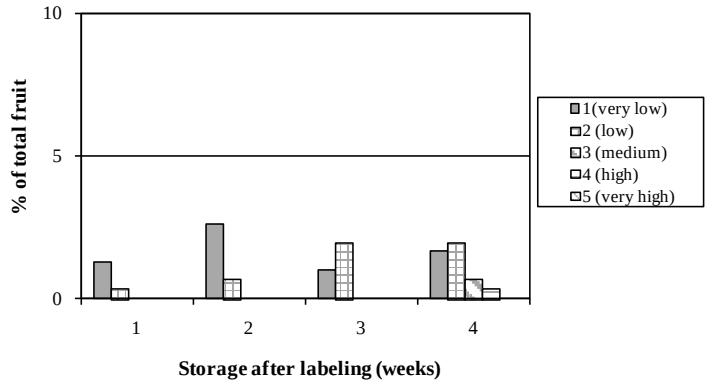



Figure 2-8. Peel stability of laser labeled fruit (45 µs) during storage at 10 °C and 95% RH

### 2.3.4 Decay Study

Citrus fruits are relatively non-perishable and can be stored for long periods. Tangerine, in general, can be stored at 5-8 $^{o}$C at 90-95% RH for 2-6 weeks (Arpaia and Kader, 2006). Decay of citrus fruit in storage is one of the major factors responsible for postharvest losses. Citrus fruits in storage are vulnerable to various decay organisms such as fungi (e.g., *Penicilium* spp.) and bacteria (Kader, 2002). Laser labeled fruit held at 10 ºC and 95% RH conditions showed no decay around etched area (Figure 2-9). All decay during the storage period was independent from the labeled areas, the most common being stem end rot. In the present study, decay rate of the fruits labeled with all the four exposure times were similar to the control (non-labeled fruit) which confirms that laser labeling does not enhance decay (Figure 2-9). These results are in accordance with those of Yuk et al. (2007) where laser labeling did not facilitate *Salmonella* infiltration and survival in tomato.

Table 2-4. Decay of non laser labeled and laser labeled "Honey Tangerine" using four different exposure time

| | Total Decay (%) | | | | |
|---|---|---|---|---|---|
| Time after labeling (weeks) | Control | 35 µs | 45 µs | 85 µs | 120 µs |
| 1 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 |
| 3 | 5 | 7.5 | 5.31 | 5 | 6.87 |
| 4 | 8.12 | 10.62 | 11.25 | 9.37 | 10.62 |
| 5 | 14.06 | 15.62 | 15.62 | 15.31 | 15.62 |

In a separate experiment, fruit were inoculated before and after laser labeling with a suspension of *P. digitatum* ($10^5$). In fruit inoculated prior to and after labeling, no symptoms of decay appeared after 3 weeks in storage. When viewed under the microscope, laser labeling appeared to prevent spore germination as indicated by the lack of fungal growth at the points of

laser impact (Figure 2-10A). The laser also destroyed mycelial strands after germination as demonstrated in Figure 2-10B.

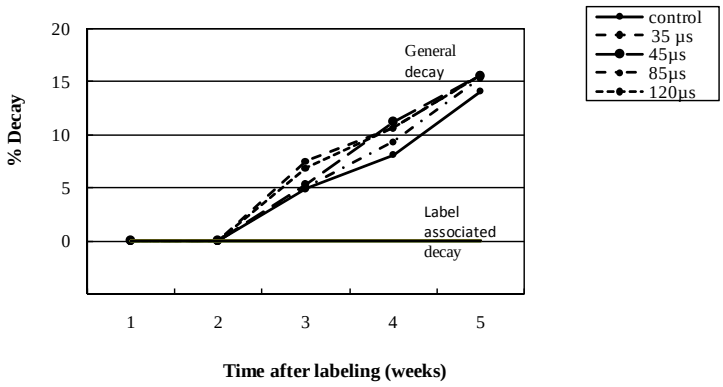



Figure 2-9. Decay of labeled fruit in storage. Fruit were labeled with four exposure times and stored at 10 ºC and 95% RH. Each point represents an average of five boxes, each containing 50 fruits. There was no decay associated with the laser label

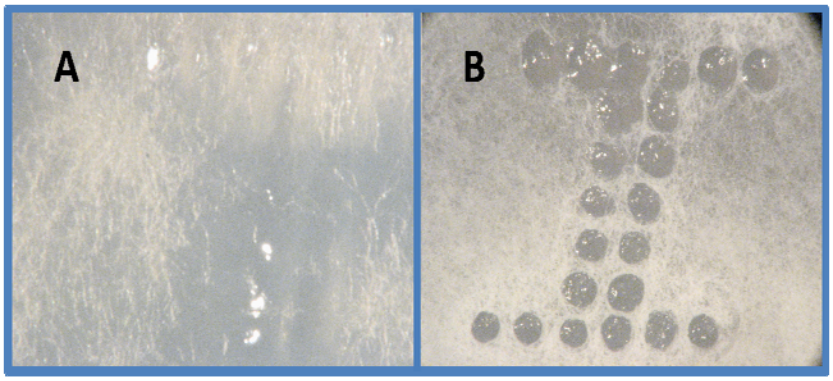



Figure 2-10. Fungal spores were spread on agar plates and laser labeled at 45 µs (A) and laser labeling of 72 h grown hyphae (B)

The results obtained in the present study demonstrate that the epidermal openings created by the laser labeling do not promote decay in tangerines, as no decay symptoms associated with the laser etched area were observed. In fact, laser labeling appeared to prevent mold decay. Analysis of the data allows us to estimate an optimum exposure time that produces readable labels with minimal water loss (Figure 2-11). For tangerines, a label using 45-65 µs exposure

times is optimal. It is important that labels are protected with a wax coat to control water loss, or significant label collapse may occur.

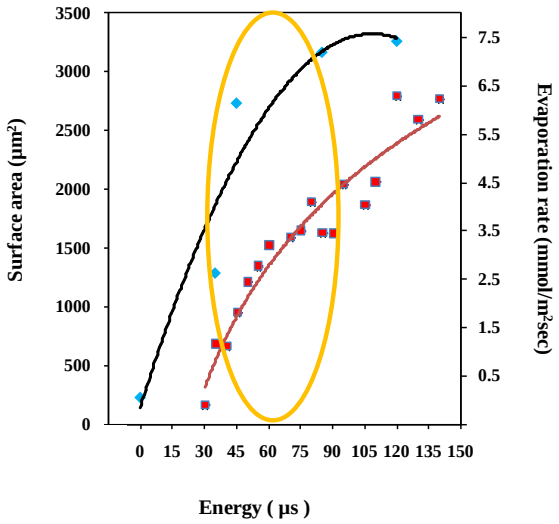



Figure 2-11. Combined data from Figure 2-3 and 2-4 showing water loss and label exposure time and the oval area includes all readable labels

# CHAPTER 3
# LASER ETCHING: A NOVEL TECHNOLOGY TO LABEL FLORIDA GRAPEFRUIT

## 3.1 Introduction

Laser labeling has become an alternative means of fruit labeling in many areas of the world (e.g., New Zealand, Australia, Pacific Rim countries), approved in others (e.g., South Africa, Mexico, Canada, Argentina, Chile, EU), and currently in final approval stages by the Food and Drug Administration. The technique 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). Etched markings are formed in dot matrix style letters and numbers, each dot created by a pin-hole depression. The advantages of laser labeling have been described previously (Etxeberria et al., 2006), yet some reservations linger about potential adverse effects during storage. The pinhole depressions applied after washing and waxing disrupt the natural cuticular barrier and the protective commercial wax cover, seemingly creating open cavities that would allow for increased water loss and facilitate entrance of decay organisms.

In previous anatomical studies using tomato (*Lycopersicum esculentum*) and avocado (*Persea Americana*) (Etxeberria et al., 2006), it was demonstrated that cells under the affected area of a laser pinhole developed a protective layer mostly of lignin and phenolics when stored for 4 d at 10 °C and 95% relative humidity (RH). This rapid healing response, accompanied by phenolic deposition, has been observed in Valencia oranges (*Citrus sinensis*) (Brown et al., 1979). When 'Ruby Red' grapefruit (*Citrus paradisi*) were damaged by friction with sandpaper, penetration by *Penicillium digitatum* was inhibited where cells at the surface produced lignin before fungal entry (Brown et al., 1979). In a related study, Yuk et al. (2007) challenged tomato fruit with *Salmonella* immediately after labeling and observed no migration into the tissue by the organism, suggesting that some protection is supplied by the labeling process itself.

Little information is available on the impact of this new technology on the overall quality of labeled produce, especially its effect on water loss and decay during prolonged storage. In Florida, grapefruit represents 43% of the citrus fresh market (Florida Citrus Mutual, 2008), a condition that requires extended storage especially when transported to international destinations. The present study determined the effects of laser labeling on water loss and decay susceptibility during prolonged storage.

## 3.2 Materials and Methods

### 3.2.1 Plant Material

"Ruby Red" grapefruit was procured 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.

### 3.2.2 Fruit Labeling

Fruit was labeled as described by Etxeberria et al. (2006) using a low energy carbon dioxide laser labeling 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. Differences in applied energy are expressed by variations in the exposure time to the laser, and varied according to individual experiments as described below.

### 3.2.3 Selection of Optimal Exposure Time

A label code containing one letter and one number ("M1") was etched on fruit surfaces using different exposure times ranging from 30 µs to 140 µs. To enhance resolution, labels were rubbed gently with a cotton-tipped swab dipped in a dark, fruit-based colorant. Images of the

dyed label were captured using a Canon Powershot S31S digital camera (Cannon, Lake Success, New York) mounted on a Wild Heerbrugg 165083 stereoscope (Leica Microsystems GmbH, Wetzlar, Germany). Surface area affected by the label was measured using image processing software developed by Dr. Arnold Schumann (Citrus Research and Education Center, University of Florida/IFAS, Lake Alfred, FL). Total area was calculated based on number of pixels within the area covered by the laser depressions with 1 pixel corresponding to 50 $\mu m^2$. Five replicates per energy level were used.

### 3.2.4 Determination of Water Loss

Water loss from the fruit surface was measured using a modified leaf porometer (Decagon Devices, Pullman, WA). To estimate water loss as a function of energy level, an etched rectangular matrix pattern of 7 X 8 dots was used for each of four pre-selected exposure times, low (30 µs), commercial standard (45 µs), medium (80 µs) and high (120 µs). The modified leaf porometer was placed on the top of the treated area immediately after etching until a stable reading was obtained.

Water vapor diffusion from grapefruit was calculated using equation 3-1 based on Fick's law where "water vapor flux density" or "evaporation rate" (F) is the product of vapor conductance (gv) and the difference between vapor concentration at the evaporating surface (Cvs) and water vapor concentration in atmosphere (Cva). Values are expressed as millimoles per square meter per second. The modified leaf porometer estimates the value for water vapor conductance (gv) which can be computed in the equation below to obtain 'Evaporation rate'.

$$F = gv\ (Cvs - Cva) \qquad (3\text{-}1)$$

Vapor concentration was calculated using equation 3-2, where $e_a$ is the vapor pressure (kPa) which is a function of temperature, and $p_a$ is the atmospheric pressure (kPa).

$$Cv = \frac{e_a}{P_2} \quad (3\text{-}2)$$

### 3.2.5 Effect of Different Waxes on Water Loss from Labeled Areas

Nine different commercial waxes were tested for their effect on moisture loss reduction from a labeled area. Waxes were obtained directly from the manufacturers. Fruit were labeled using a single exposure time (45 µs) then waxed using a sponge paint brush. Evaporation rate measurements of the labeled and unlabeled (control) surfaces were performed before and after waxing. Each measurement was replicated 30 times.

Table 3-1. Nine commercial waxes tested for water loss reduction from laser labeled areas

| Names of waxes | Names of Manufacturers |
|---|---|
| Deco shellac | Deco, Monrovia, CA |
| Carnuba 505 | Deco, Monrovia, CA |
| Carnuba + TBZ | Deco, Monrovia, CA |
| Carnuba 231 | Deco, Monrovia, CA |
| Carnuba blend | Deco, Monrovia, CA |
| Citrus wax shellac | HDH Agriproducts, Tavares, FL |
| HDH Carnuba | HDH Agriproducts, Tavares, FL |
| Pace Carnuba | Pace International, Visalia, CA |
| Organic Carnuba | Pace International, Visalia, CA |

A separate experiment was conducted to evaluate water loss from waxed and unwaxed etched areas during storage. Etched rectangles were waxed and fruit kept at 10 °C and 95% RH for 7 d. Daily measurements of 'evaporation rates' of the waxed label, unwaxed label and control (no label on fruit) were carried out. For each treatment, 30 separate measurements were made.

### 3.2.6 Peel Stability

The label "Florida citrus" was etched on the fruit using four exposure times (35 µs, 45 µs, 85 µs, 120 µs), and fruit was kept at 10 °C and 65% or 95% RH for 4 weeks. Five replicates (1 replicate = 1 box = 35 fruit) per exposure time and control (non labeled fruit) were used. Weekly

examination of the label appearance and surrounding area was performed. Peel stability was determined on the basis of a visual rating scale according to the shrinkage of the skin around label as follows: 0 (no shrinkage); 1 (very low); 2 (low); 3 (medium); 4 (high); 5 (very high) (Figure 3-1).

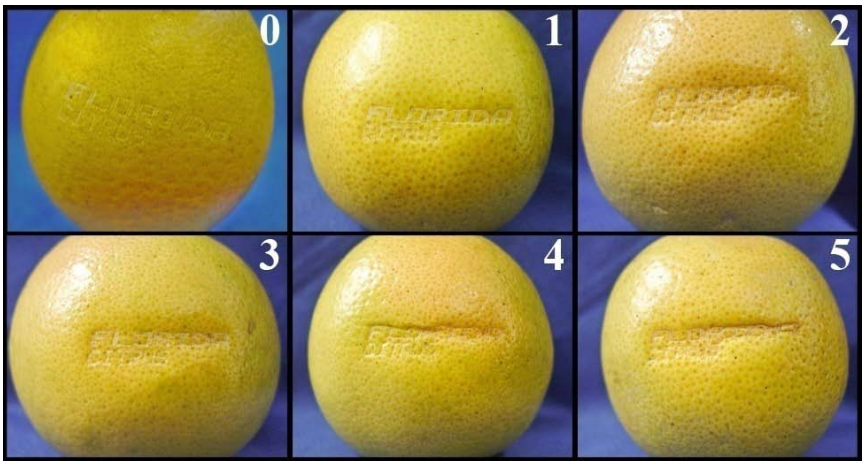



Figure 3-1. Shrinkage rating scale for laser-labeled 'Ruby Red' grapefruit peel. Scale ranges from 0 (no shrinkage) to 5 (total label collapse). Fruit were labeled at 45 µs with the label "Florida Citrus"

### 3.2.7 Decay Study

Fruit treated as indicated above and stored at 95% RH were examined weekly for decay symptoms for 5 weeks. Decay was categorized individually, but reported as total decay.

### 3.2.8 Mold Inoculation Study

Fruit labeled "Florida Citrus" were subjected to four treatments: 1) Inoculation of fruit prior to labeling; 2) Inoculation after labeling; 3) Inoculation on waxed label; 4) Waxing of inoculated label. Inoculum was prepared as described in chapter 2. Inoculated fruit were stored at 10 °C and 95% RH for 3 weeks and examined for decay weekly. Thirty replicates of one fruit each were used for all four treatments. Labeling was performed using one exposure time (45µs).

In a separate experiment, 250 µL of the P. digitatum spore suspension was spread on agar plates, allowed to dry and incubated at 23 °C. In a separate experiment, spores were allowed to

germinate for 72 h and plates were then labeled as indicated above. Laser labels were observed under microscope immediately and 48 h after labeling.

## 3.3 Results

### 3.3.1 Optimization of Exposure Time for Grapefruit Labeling

A previous study demonstrated that the dot matrix style forming the alphanumerical characters of laser labels constitute superficial ruptures (pinholes) of the epidermal layer exposing the contrasting underlying tissue (Etxeberria et al., 2006). On citrus fruit, however, because the colored epidermis (flavedo) is thicker than the depth of the pinholes, vegetable dye was applied to provide contrast. The visual effect of increasing laser labeling exposure time on grapefruit peel is shown in Figure 3-2. At the lowest possible exposure time of 30 µs, the label was faint and hardly visible, whereas at the highest exposure time the pinhole depressions merged into solid lines.

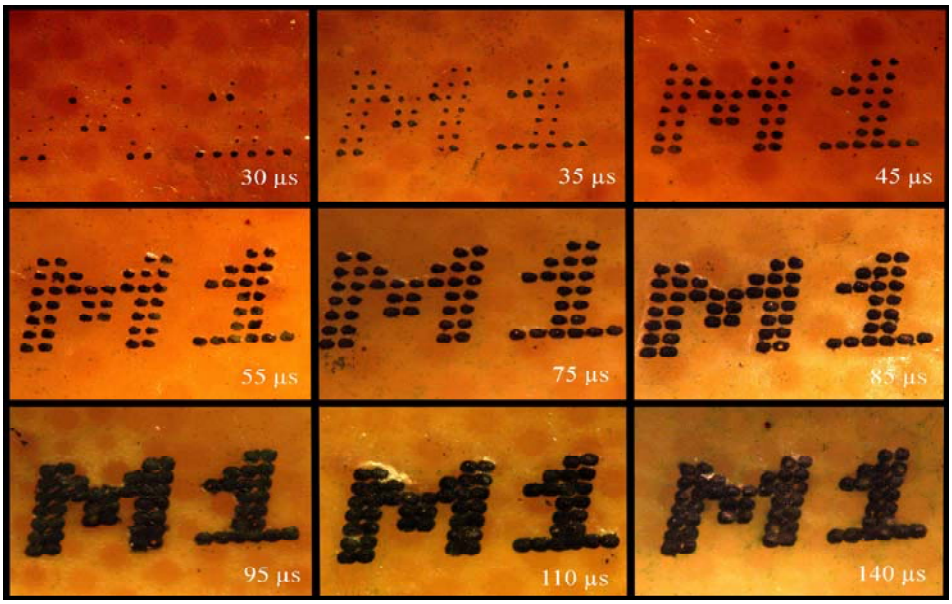



Figure 3-2. A group photograph of labels etched on 'Ruby Red' grapefruit using different exposure times/energy levels. The 45 µs picture represents the energy level generally recommended for commercial use. Contrast of etched label was enhanced using fruit based color

Using image processing software, affected surface area was calculated as a factor of increasing exposure time. In general, area covered by the etched marking increased with higher exposure times (Figure 3-3). As expected, the rate of peel surface area disruption declined at higher exposure times as pinholes began to merge.

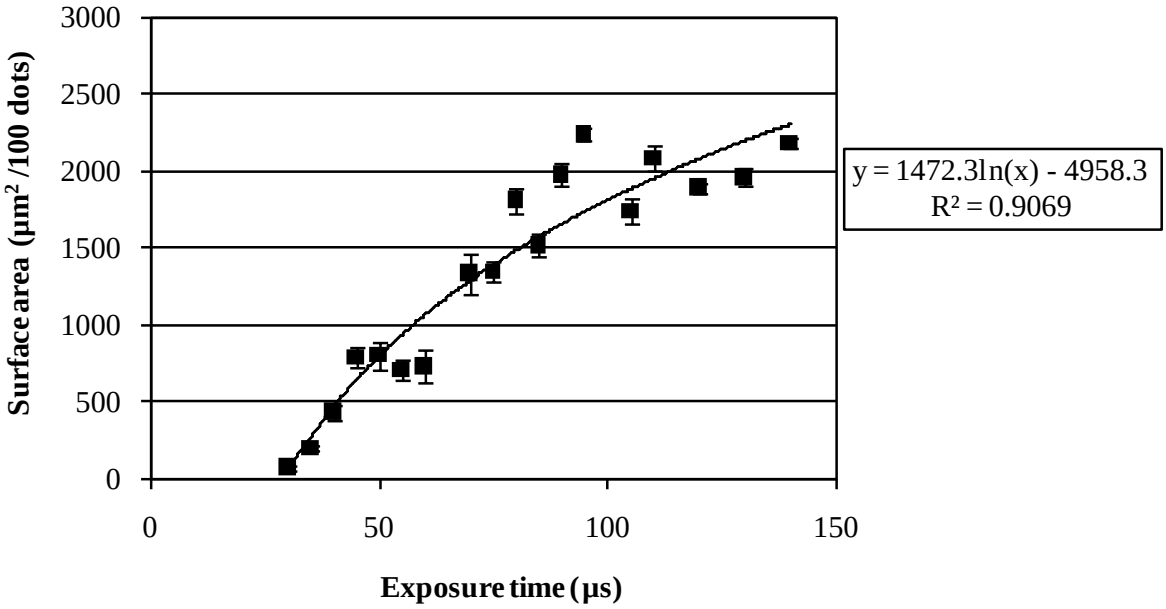



Figure 3-3. Relationship between exposure time (30 to 140 µs) and laser-labeled surface area (covered by 100 dots) of 'Ruby Red' grapefruit. Each point represents the average of five replicates labeled at ambient temperature. Vertical lines represent the standard error

### 3.3.2 Measurements of Water Loss

Water loss from etched surfaces was measured as a function of exposure time and pinhole size (Figure 3-4). For this experiment, four exposure times were selected representing low (35 µs), commercially recommended (45 µs), medium (85 µs) and high (120 µs). Water loss measurements were made immediately after labeling. As shown in Figure 3-4, there was a rapid increase in the rate of water loss between 45 and 85 µs. Afterwards, increase in the rate of water loss was negligible.

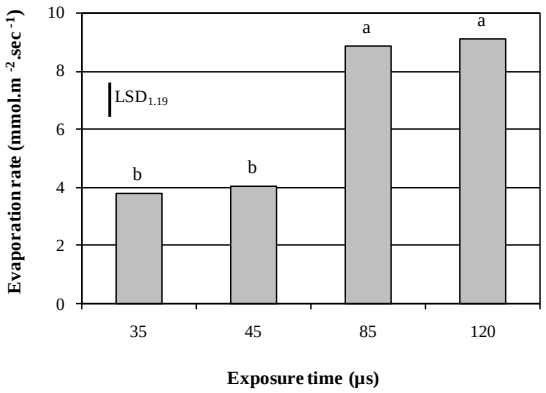



Figure 3-4. Water loss from laser-labeled 'Ruby Red' grapefruit peel using four different exposure times. Water loss from the etched surface was measured at ambient temperature immediately after laser labeling 30 fruit per exposure time. Letters atop bars indicate statistical groupings. Bars with different letters are significantly different (P=0.05)

### 3.3.3 Effect of Waxes on Water Loss Retardation in Labeled Area

The effect of waxing on reducing water loss from etched fruit surfaces was investigated using different waxes. All nine waxes tested reduced water loss by 25% to 94% with four coatings showing over 90% reduction (Figure 3-5). Citrus wax shellac resulted in the highest reduction of water loss (94%; Figure 3-5) whereas shellac resulted in moderate water loss reduction as previously described by Hagenmaier and Shaw (1991).

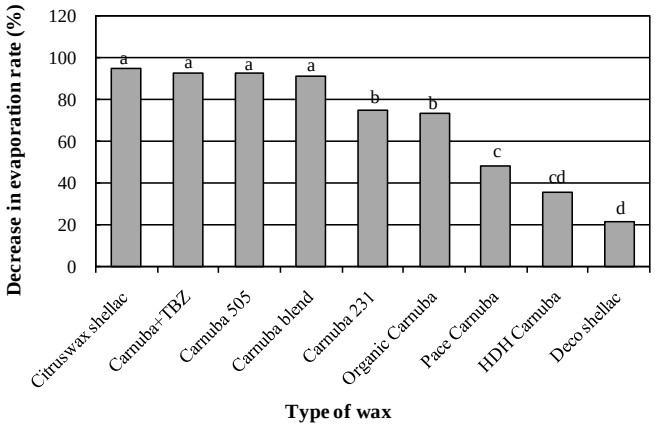



Figure 3-5. Percentage decrease in the rate of water loss from laser-labeled 'Ruby Red' grapefruit peel by different commercial waxes compared to unwaxed label. Bars with different letters are significantly different by t Tests (LSD= 14.7, P=0.05)

Water loss from unwaxed labeled areas declined with time in storage (Figure 3-6). There was a steady decline in the rate of water loss up to 72 h after labeling which continued more gradually thereafter. Water loss from unwaxed labeled areas never reached control levels (Figure 3-6) as they did with tangerines (*Citrus reticulata*) 4 d after labeling (Sood et al., 2008). During the first 24 h, the rate of water loss from waxed etched areas was approximately 40% higher than control. Afterwards, water loss from control and waxed labeled surface remained nearly identical.

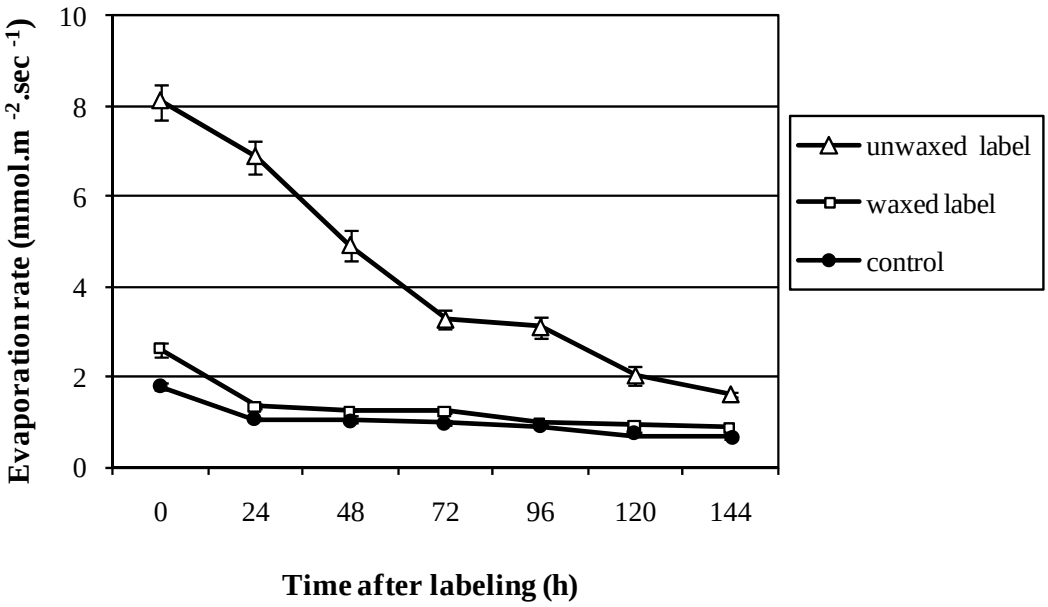



Figure 3-6. Water loss from unwaxed label, waxed (carnuba) label and control 'Ruby Red' grapefruit (no label) during 7 d storage at 10 °C (50.0 °F). Initial measurements were taken on 30 fruit immediately after labeling at ambient temperature. Vertical lines represent standard error of the averages.

### 3.3.4 Peel Stability

The potential effect of water loss from the etched area on the label stability during storage was investigated at two levels of RH. To determine the physical effect on label stability, a visual scale was created (see Materials and Methods; Figure 3-1). As expected, degree of label distortion increased with time and was inversely proportional to ambient RH (Figures 3-7 and 3-

8). After 4 weeks in storage, approximately 40% of etched labels in fruit stored at 95% RH showed some degree of shrinkage, although most were classified as very low (Figure 3-7). On the contrary, storage at 65% RH resulted in substantial label shrinkage with approximately 98% of the fruit affected (Figure 3-8). This is in sharp contrast to tangerines which showed minimal shrinkage when stored at similar RH (Sood et al., 2008).

Table 3-2. Peel stability of laser labeled "Ruby Red" grapefruit using 45µs and stored at 10 $^{o}$C and 95% RH

| | Percentage of total fruit | | | | |
|---|---|---|---|---|---|
| Time after labeling (weeks) | 1 (very low) | 2(low) | 3(medium) | 4(high) | 5(very high) |
| 1 | 15.38 | 1.14 | 0 | 0 | 0 |
| 2 | 28.55 | 2.28 | 0 | 0 | 0 |
| 3 | 28.54 | 5.13 | 0 | 0 | 0 |
| 4 | 27.39 | 10.26 | 1.14 | 0 | 0 |

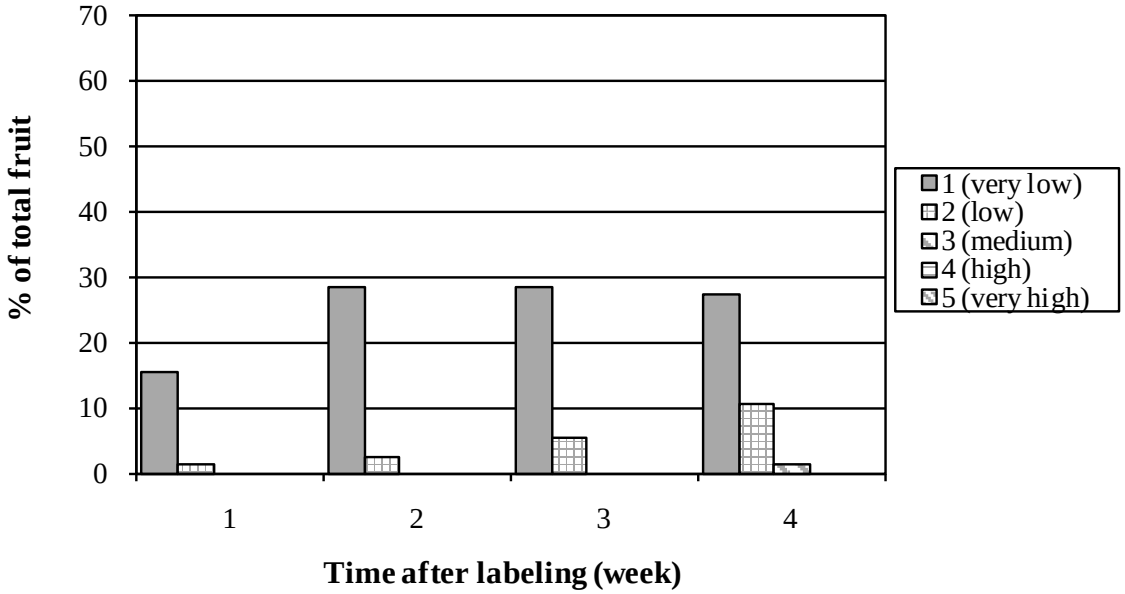



Figure 3-7. Peel shrinkage of etched 'Ruby Red' grapefruit during storage at 10 °C (50.0 °F) and 95% relative humidity. Shrinkage severity was determined on 175 unwaxed fruit per exposure time using a visual scale of 0 (very low or no shrinkage) to 5 (very high or total label collapse) depicted in Figure 3-1.

Table 3-3 Peel stability of laser labeled "Ruby Red" grapefruit using 45µs and stored at 10°C and 65% RH

| Percentage of total fruit | | | | | |
|---|---|---|---|---|---|
| Time after labeling (weeks) | 1 (very low) | 2(low) | 3(medium) | 4(high) | 5(very high) |
| 1 | 19.96 | 13.68 | 3.99 | 0 | 0 |
| 2 | 58.24 | 25.1 | 5.13 | 0 | 0 |
| 3 | 50.24 | 24.54 | 13.68 | 2.28 | 0 |
| 4 | 55.4 | 23.4 | 11.4 | 4.56 | 1.14 |

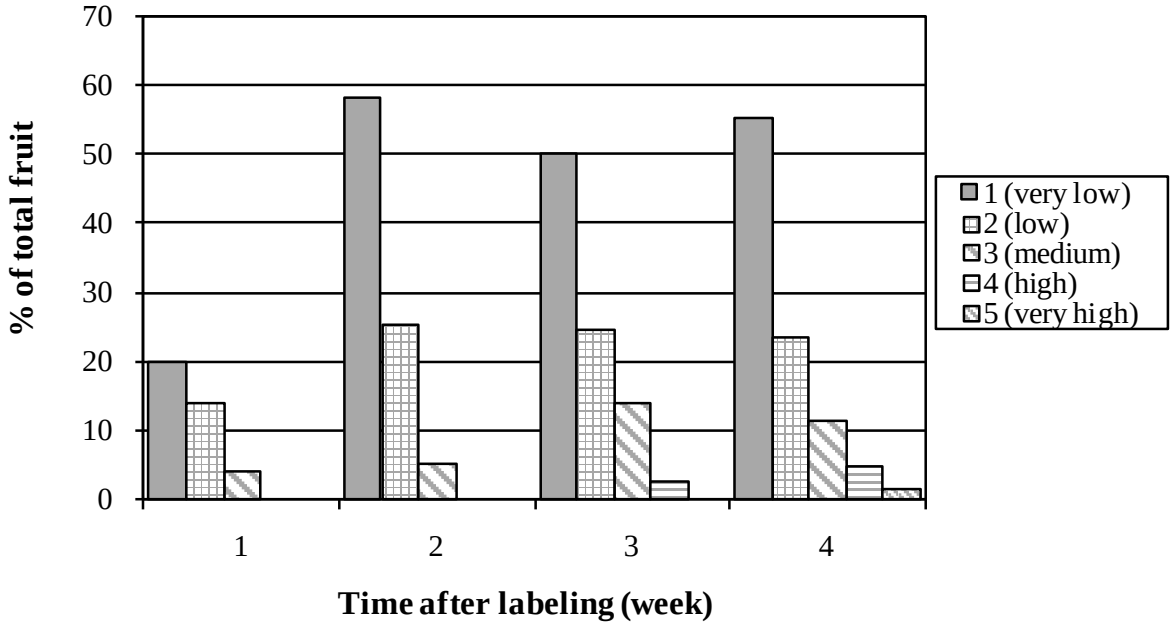



Figure 3-8. Peel shrinkage of etched 'Ruby Red' grapefruit during storage at 10 °C (50.0 °F) and 65% relative humidity. Shrinkage severity was determined on 175 unwaxed fruit per exposure time using a visual scale of 0 (very low or no shrinkage) to 5 (very high or total label collapse) depicted in Figure 3-1.

### 3.3.5 Decay Study

Fruit decay was followed for 5 weeks in packed grapefruit stored at 10 °C and 95% RH. Close attention was placed to the sites of decay with special emphasis on the labeled area. Although different kinds of decay were noted, we report total decay for simplification. In the present study, fruit decay in fruits labeled with all four exposure times was similar to control non-labeled fruit (Figure 3-9). All decay present during the 5-week storage period was

independent from the labeled areas, the most common being stem end rot. These results are analogous to those of Yuk et al. (2007) who demonstrated a lack of *Salmonella* migration and survival in tomato laser labels.

Table 3-4 Decay of non laser labeled and laser labeled "Ruby Red" grapefruit using four different exposure time

| | Total Decay (%) | | | | |
|---|---|---|---|---|---|
| Time after labeling (weeks) | Control | 35 µs | 45 µs | 85 µs | 120 µs |
| 1 | 0 | 0 | 0 | 1.14 | 0 |
| 2 | 0.57 | 0 | 0 | 1.14 | 0 |
| 3 | 1.71 | 0 | 0.57 | 1.14 | 0.57 |
| 4 | 1.71 | 0.57 | 0.57 | 1.71 | 1.41 |
| 5 | 1.71 | 0.57 | 0.57 | 1.71 | 1.41 |

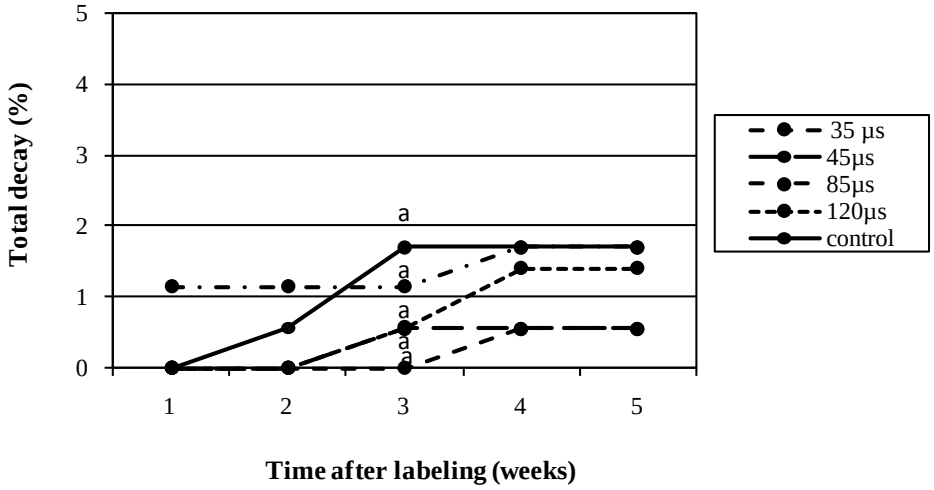



Figure 3-9. Total decay of labeled 'Ruby Red' grapefruit in storage. Fruit were labeled using 4 exposure times (35 µs, 45 µs , 85 µs and 120 µs) at ambient temperature and stored at 10 °C (50.0 °F) and 95% relative humidity. There was no decay associated with the laser label. Each point represents the average of five boxes, each box containing 50 fruit. Vertical lines represent standard error of the averages

In citrus fruits, green mold caused by *P. digitatum* is the most common postharvest decay. Surprisingly, fruit inoculated prior to or after labeling with a *P. digitatum* spore suspension showed no symptoms of mold growth even after 3 weeks in storage. On the contrary, the control fruit which was wounded and inoculated with the same spore suspension decayed within a week.

In a separate experiment, laser labeling appeared to destroy mold spores layered on dextrose agar plates, as no germination occurred on the labeled pinholes while the area around sustained a dense network of fungal hyphae (Figure 3-10A). When spores were allowed to germinate and were then subjected to laser labeling, vegetative hyphae was eliminated from the labeled areas (Figure 3-10B).

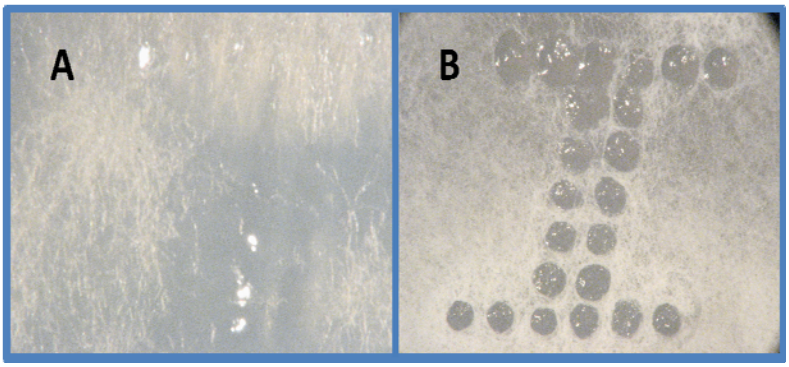



Figure 3-10. Fungal hyphae grown for 72 h on potato dextrose agar. A) after a lawn of spores was laser labeled at 45 µs, B) laser labeling of 72 h grown hyphae

## 3.4 Discussion

Laser labeling of fruits and vegetables is based on etching the required information on the produce surface with permanent dot matrix alphanumerical characters or symbols. In doing so, the physical disruption of the natural epidermal and cuticular protection may introduce artifacts with potential detrimental consequences. For example, epidermal waxes, which play a vital role in retaining moisture in the fruit and vegetables (Riederer and Schreiber, 2001), are disrupted by the laser generated pinholes potentially rendering the produce surface susceptible to water loss and secondary invasion by decay organisms.

The relationship between pinhole size and water loss was investigated as a means to establish optimal label boundaries in terms of maximal readability with minimal water loss. Our results showed that increasing labeling time beyond 45 µs substantially increased water loss (Figure 3-4) proportional to pore size (Figure 3-3), yet readability was optimal between 55 to 85

μs. Therefore, optimizing readability at the lowest range of water loss between the range 45 to 55 μs can be achieved without risking additional detrimental effects due to water loss.

During commercial operations at the packinghouse, waxing of citrus fruits precedes fruit labeling, packing, storage, and long-distance transport. Whereas sticker labels do not suffer physical deterioration in storage, the loss of water from the etched labels (Figure 3-6) may alter the physical appearance of the fruit's surface, thereby distorting the label (Figures 3-1, 3-7, 3-8) and making it less effective and appealing. As reported, RH has a direct effect on the rate of moisture loss from produce surfaces (Forbes and Watson, 1992). The data indicated that a considerable portion of the fruit showed some level of shrinkage making the label unreadable, especially fruit stored at 65% RH for 4 weeks. Even the lowest shrinkage (or shriveling) obtained at 95% RH can be sufficient to make the fruit less appealing. It is likely that at some point in the commercial chain of events, fruit may encounter lower than 65% RH. Although the natural healing process of the label pinholes reduced water loss to control levels after 4 d (Figure 3-6; Etxeberria et al., 2006), addition of a secondary wax coat immediately after labeling reduced water loss to control levels (Figure 3-6) which delayed or prevented unnecessary shrinkage. In as much as a second wax application is not commercially feasible at the moment, methods should be developed to address this problem in the future to avoid loss of visual appeal and functionality.

Citrus fruits are relatively non-perishable, and can normally be stored for long periods (Kader, 2002). Grapefruit can be stored at 10 to 15 °C and 90% to 95% RH for 5 to 8 weeks (Grierson, 1974). In general, citrus fruits are vulnerable to various decay organisms during storage, especially *Penicillium sp.* (Kinay et al., 2001). *Penicillium sp.* growth necessitates rupture of the produce surfaces (Eckert and Brown, 1986) a condition seemingly created by the

laser pinholes. Our data from fruit held at 10 ºC and 95% RH for 5 weeks showed no decay symptoms associated with the etched area. Brown et al. (1979) found that accumulation of lignin occurred rapidly in injuries at 30 ºC and RH over 90%, likely creating rapid protection. Although our experiments were conducted at 10 ºC, either formation of lignin or loss of humidity from the pinhole area appeared sufficient to deter decay. This is probable because *P. digitatum* is a wound parasite and does not actively invade intact plant tissue. There were some decayed fruit in the etched fruit samples; however, it was no different than that of non-etched fruit control samples. The lack of decay associated with etched areas was further substantiated by exposing the fruit to four possible conditions : 1) Inoculation of fruit prior to labeling; 2) Inoculation after labeling; 3) Inoculation on waxed label; 4) Waxing of inoculated label. Whether coated with a high concentration of *P. digitatum* spores before laser labeling or a layer of spores coated over the freshly etched surface, no fruit decay took place from the labeled area during 4 weeks in storage. The lack of decay by mold was unexpected given that, in one instance, fruit were essentially inoculated with *P. digitatum* spores immediately after laser labeling. These data, and especially those of Figure 3-10 showing lack of spore germination on etched areas and the elimination of vegetative hyphal growth by laser labeling, demonstrate that the laser labeled areas do not have increased susceptibility to decay organisms in grapefruit.

There was an appreciable difference in the postharvest behavior of laser labeled fruit between grapefruit and tangerines, especially on the efficacy of different waxes in preventing water loss and in label shrinkage (Sood et al., 2008). Differences in wax efficiency are difficult to reconcile considering that surface area affected by different labeling times was nearly identical in both types of citrus and coverage by waxes is expected to be alike. However, previous work by the authors showed that any treatment of fruit peel affects the cuticle, and therefore, could

have an effect on the way that additional coatings are laid down. Re-arrangement of the epicuticular waxes, even in different areas on the same fruit, will determine the coverage of waxes applied to the peel (data not shown). Also the differences in peel topography between the tangerine and the grapefruit may be an added factor in the uniformity of the applied coating. The larger number of grapefruit affected by label shrinkage, however, is likely due to the higher percentage of water loss through the labeled areas as compared to non-labeled areas.

Although restricted to one commodity, this study shows that laser labeling of grapefruit is a viable option to identify produce with a permanent tag. When compared to sticker labeled fruit, a laser labeled commodity is relatively tamper free and the fruit quality remains high as the invasion of the epidermis does not incite decay, provide an avenue for food pathogens and water loss is easily controlled.

# CHAPTER 4
# EFFECTS OF LASER LABELING ON THE POSTHARVEST STORAGE BEHAVIOUR OF TOMATO AND PEPPER FRUIT

## 4.1 Introduction

Produce consumption is considered as the prime source of food borne illnesses in the US (Center for Disease Control and Prevention, 2006). The difficulty to trace the past outbreaks such as Salmonella in tomatoes, E.coli O157:H7 in apples etc. has made the labeling of produce a necessity. Labeling helps track and trace the product to its original source (i.e. grower and packer). Thus far, the most widely used labeling system for produce is Price Look Up (P.L.U.) stickers/adhesive labels. These stickers offer several advantages such as making the checkout process easier, helps distinguishing between similar products and tracing back to the original sources. However the non permanency of these stickers urged industry and scientists to develop a more efficient and permanent labeling method called as laser labeling. Laser labeling, as mentioned in previous chapters, involves the use of low energy $CO_2$ to etch information directly onto the fruit surface by creating pinhole depressions. These etched openings can promote undesirable water loss and serve as an entry site for pathogen invasion. However, anatomical and morphological studies of laser etched depressions in tomato and avocado showed the accumulation of additional waxes and phenolic compounds around exposed cell walls and underneath tissues, thereby proposing the self healing mechanism of laser etched areas (Etxeberria et al., 2006).

Initial studies to investigate the postharvest effect of the laser labeling were conducted on citrus fruit such as tangerine and grapefruit. The results helped in identifying the optimum laser energies for commercial use in both the fruits. Additionally, it was shown that laser labeling does not promote decay and only allows minimal water loss in citrus fruits. The appearance of the label does change with the time unless wax coated. The present study deals with fruit with

edible peel such as tomato and pepper. Because of the variation in the morphology of the fruits with non-edible peel fruit like citrus, laser labeling was anticipated to behave differently.

Tomato (*Lycopersicon esculentum*) is one of the most widely consumed vegetable crops worldwide (Chapagain and Wiesman, 2004) .Botanically, a tomato fruit is a berry consists of seed enclosed in fleshy pericarp developed from ovary. Pericarp is further divided into exocarp or fruit skin, parenchymatous mesocarp and a single celled layer of endocarp. The fruit skin or exocarp composed of the outer epidermal layer and two to four layers of thick walled hypodermal cells with collenchymatous thickenings. Epidermal layer of a tomato fruit is covered with thin waxy cuticle. Green pepper (*Capsicum annum*) is a berry fruit with capsule shaped hollow structure. The berry is formed by a thick juicy pericarp and a placenta binding the seeds. The pericarp is further composed of an epicarp or external layer, mesocarp or fleshy intermediate zone and a membranous endocarp.

This study was focused on the effects of laser labeling on water loss and other postharvest concerns (e.g., decay, label shrinkage) in thin skinned peel-edible produce such as tomato and green peppers and to contrast the results with those obtained for citrus fruit.

## 4.2 Materials and Methods

### 4.2.1 Plant Material

Tomato fruit variety “BHN602” was purchased from Taylor and Fulton Packinghouse, Palmetto, Floridaon 10 June, 2008. Tomato fruit was procured at mature green stage. On the other hand, pepper fruit was purchased from and J&J Ag Products, Inc. Packinghouse, Clewiston, Florida on 5 th July, 2008. Both the fruits had been washed and coated with mineral oil based waxes following established commercial practices.

### 4.2.2 Fruit Labeling

Fruit was labeled as described in chapter 3. Applied energy or exposure time varied depending on the type of experiments conducted.

### 4.2.3 Selection of Optimal Exposure Time

A label code containing one letter and one number ("O1") was etched on fruit surfaces using different exposure times ranging from 30 µs to 120 µs. A dark, fruit- based colorant was rubbed gently over the labels with a cotton-tipped swab to enhance resolution. Total area covered by the laser depressions was calculated by following the procedure described in chapter 3. Five replicates per energy level were used.

### 4.2.4 Determination of Water Loss

Water loss was measured as described in chapter 3 except the exposure times used. Tomato and pepper fruit were labeled using four pre-selected exposure times, low (35 µs), standard (55 µs), medium (75 µs) and high (100 µs).

### 4.2.5 Peel Stability

The label "Florida tomato" was etched on tomato fruit using an optimum exposure time and fruit was kept at 12.5°C and 65% or 95% RH for 3 weeks. Five replicates (1 replicate = 1 box = 50 fruit) per exposure time and control (non labeled fruit) were used. Similarly, pepper fruit was etched with the label "Florida pepper" and stored at 7$^{o}$C and 65% or 95% RH for 3 weeks. Five replicates (1 replicate = 1 box = 30 fruit) per exposure time and control (non labeled fruit) were used. Fruit was examined for label appearance and surrounding area for 3 weeks. Peel stability of both the fruit types was determined on the basis of a visual rating scale according to the shrinkage of the skin around label as follows: 0 (no shrinkage); 1 (very low); 2 (low); 3 (medium); 4 (high); 5 (very high) (Figure 4-1) and (Figure 4-2).

A separate experiment was conducted to evaluate peel stability of waxed and unwaxed etched areas in tomato fruit during storage. Daily visual observations of the unwaxed and waxed laser labels on tomato fruit was done for 7 days.

### 4.2.6 Decay Study

Fruit treated as indicated above and stored at 95% RH were examined for decay symptoms nearly every alternate day for 3 weeks. Results were reported as total decay.


Figure 4-1. Shrinkage rating scale for laser-labeled tomato peel. Scale ranges from 0 (no shrinkage) to 5 (total label collapse). Fruit were labeled at 55 µs with the label "Florida tomato"

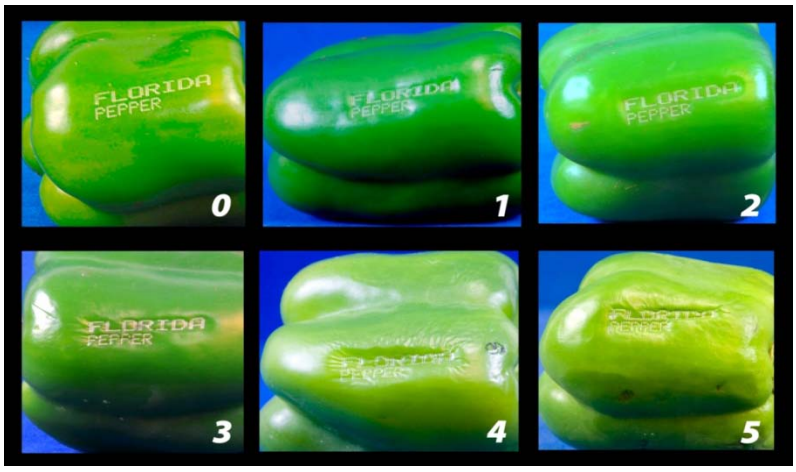



Figure 4-2. Shrinkage rating scale for laser-labeled pepper peel. Scale ranges from 0 (no shrinkage) to 5 (total label collapse). Fruit were labeled at 55 µs with the label "Florida pepper

### 4.2.7 Inoculation Study

Pathogen inoculation study on fruit labeled "Florida tomato" and "Florida pepper" was conducted as described in chapter 3. Tomato and pepper fruit were inoculated with *Geotrichum candidum Link*. The inoculum was prepared using the same method as with the *Penicillium digitatum*. Inoculated tomato and pepper fruit were stored for 3 weeks at 95% RH and at 12.5 $^{o}$C and 7 $^{o}$C, respectively. Weekly examination of the inoculated fruit was carried out. Labeling was performed using one exposure time (55µs).

## 4.3 Results

### 4.3.1 Optimum Exposure Time for Labeling

Previous anatomical and morphological studies demonstrated that the dot matrix style forming the alphanumerical characters of laser labels represent surface ruptures (pinholes) of the epidermal layer exposing the contrasting underlying tissue (Etxeberria et al., 2006). The visual effect of increasing laser labeling exposure time on tomato and pepper peel is shown in Figure 4-3 and Figure 4-4. As in previous tests on tangerine and grapefruit, the lowest possible exposure time of 30 µs generated faint, very superficial and slightly visible label, whereas the highest exposure time caused the merging of the pinhole depressions into solid lines.

These results were similar to as seen in citrus fruit (Sood et al, 2007). However, because of the difference in skin morphology, the labels on tomato and pepper were clearer and more visible than on citrus.

The affected surface area as a function of increasing exposure time was calculated using the same image processing software developed by Dr. Arnold Schumann. As previously demonstrated in citrus fruit, (Chapter 1 and 2), area covered by etched markings increased in response to higher exposure times (Figure 4-5 and Figure 4-6). As expected, the rate of peel

surface area disruption in both the fruit types did not increase significantly at higher exposure times due to merging of the pinholes.

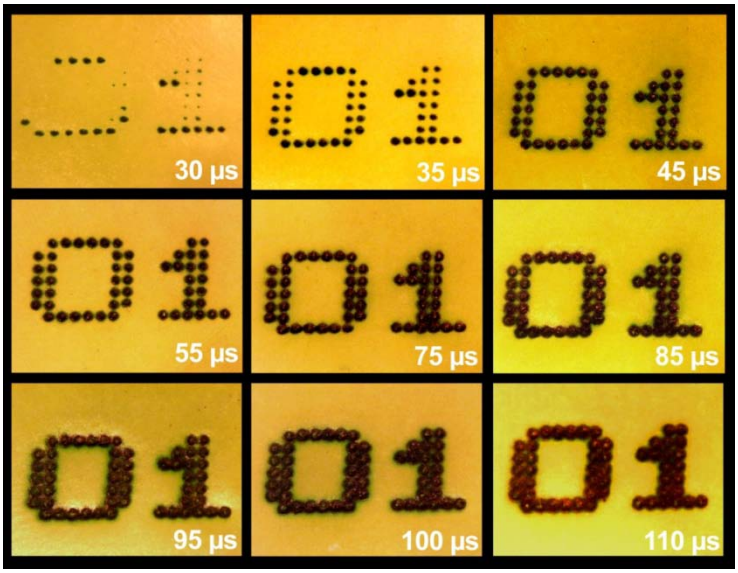



Figure 4-3. A group photograph of laser labels on tomato fruit using different exposure times/energy levels. Contrast of etched label was enhanced using fruit based color.

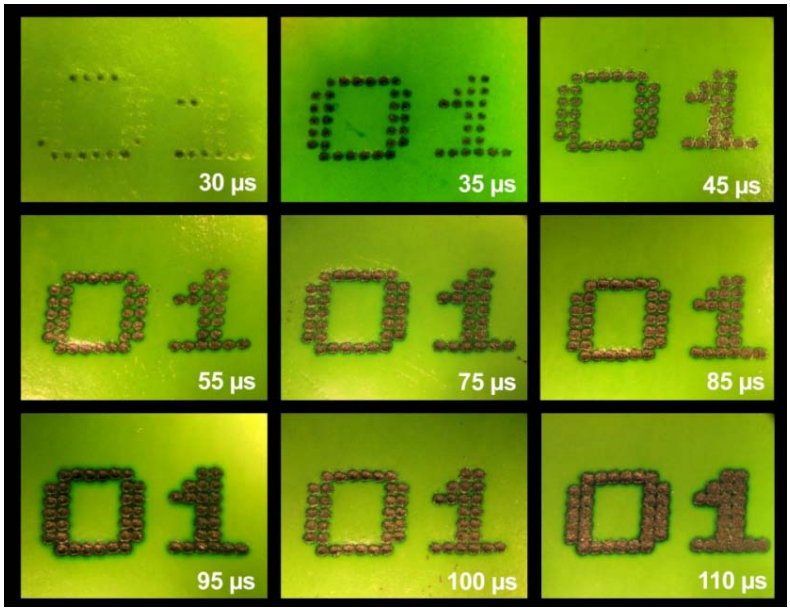



Figure 4-4. A group photograph of labels etched on pepper fruit using different exposure times/energy levels. Contrast of etched label was enhanced using fruit based color

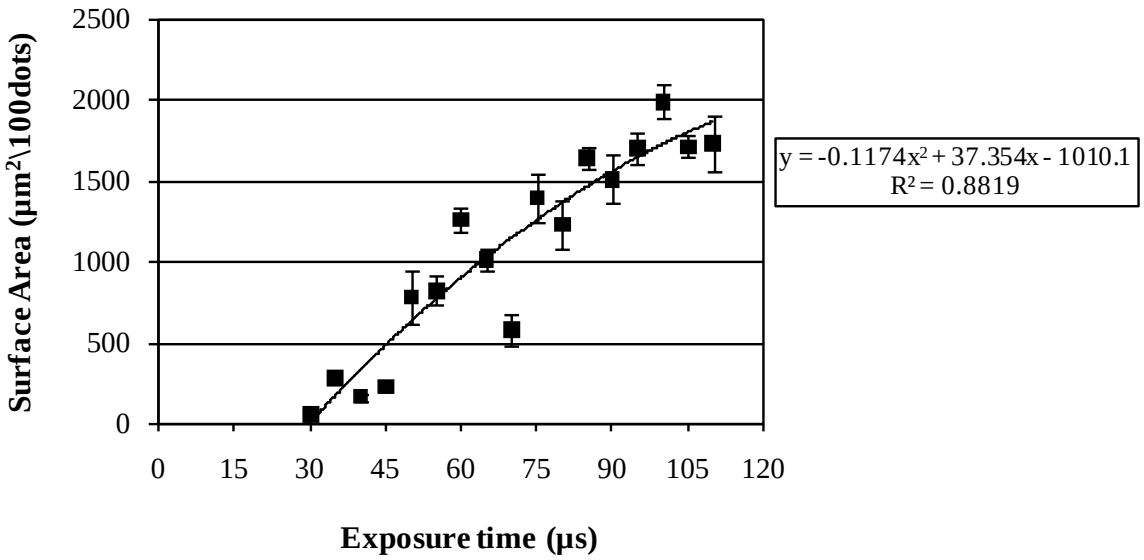



Figure 4-5. Relationship between exposure time (30 to 120 µs) and laser-labeled surface area (covered by 100 dots) of tomato fruit. Each point represents the average of five replicates labeled at ambient temperature. Vertical lines represent the standard error.

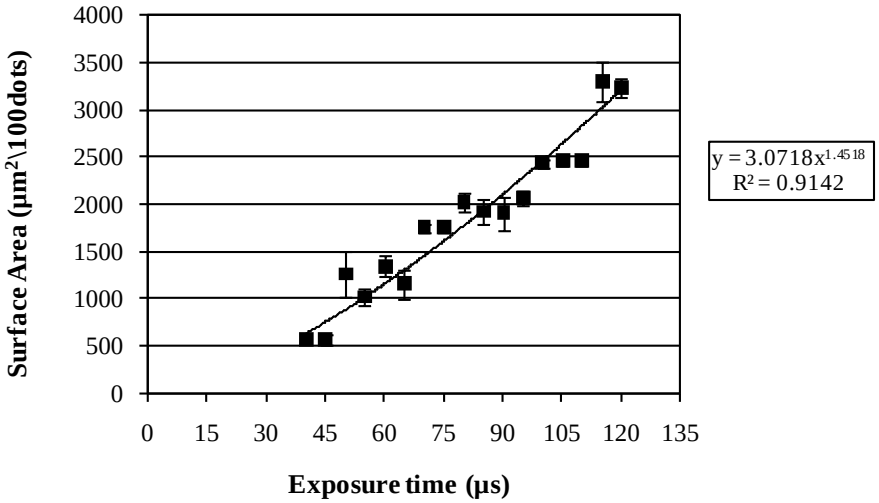



Figure 4-6. Relationship between exposure time (30 to 120 µs) and laser-labeled surface area (covered by 100 dots) of pepper fruit. Each point represents the average of five replicates labeled at ambient temperature. Vertical lines represent the standard error

### 4.3.2 Measurements of Water Loss

Water loss from laser labeled surfaces was measured as a factor of exposure time and pinhole size (Figure 4-7 and Figure 4-8). For this experiment, four different exposure times, 35 µs, 55 µs, 75 µs and 100 µs were selected. Water loss measurements were made immediately

after labeling. As shown in Figure 4-7, the rate of water loss from tomato fruit followed a nearly linear pattern while pepper fruit (Figure 4-8), showed a rapid increase in the rate of water loss between 35 and 55 µs. Afterwards, there was a negligible increase in the rate of water loss. The results for both the fruits were represented using the same scale so as to provide for a better comparison.

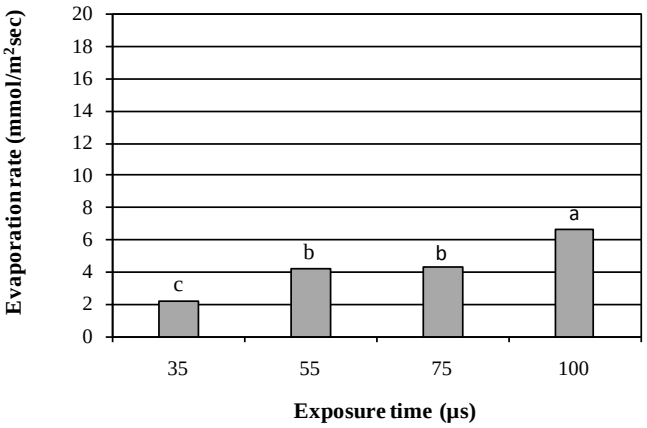



Figure 4-7. Water loss from laser-labeled tomato fruit peel using four different exposure times. Water loss from the labeled surface was measured at ambient temperature immediately after laser labeling 20 fruit per exposure time. Letters atop bars indicate statistical groupings. Bars with different letters are significantly different (P=0.05).

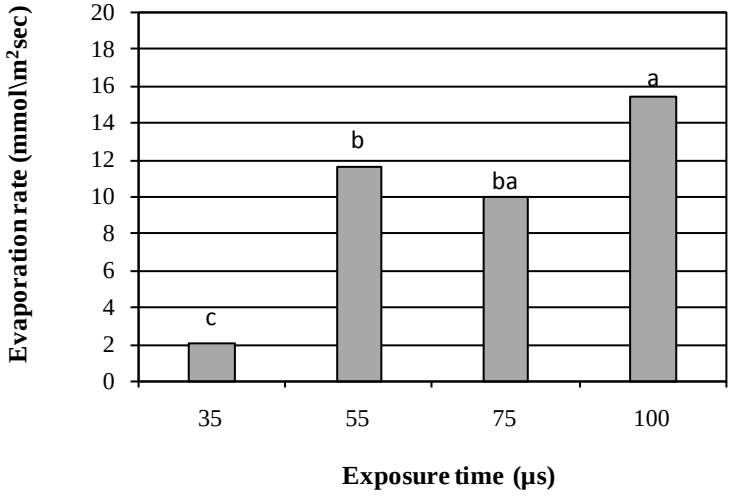



Figure 4-8. Water loss from laser-labeled pepper fruit peel using four different exposure times. Water loss from the laser labeled surface was measured at ambient temperature immediately after laser labeling 20 fruit per exposure time. Letters atop bars indicate statistical groupings. Bars with different letters are significantly different (P=0.05)

### 4.3.3 Peel Stability

The potential effect of water loss from the etched area on the label stability during storage was investigated at two levels of RH. To determine the physical effect on label stability a visual scale was created (see Materials and Methods; Figure 4-1and Figure 4-2). As expected, degree of label distortion increased with time and was inversely proportional to ambient RH (Figures 4-7 and Figure 4-8). After just a week in storage, approximately 50% of laser labels in fruit at 95% RH showed some degree of shrinkage, although most were classified as very low (Figure 4-9 and Figure 4-11). On the contrary, storage at 65% RH resulted in substantial and rapid label shrinkage with approximately 98% of the fruit affected (Figure 4-10 and Figure 4-12). The results presented do not include the decayed fruit which were discarded during the evaluation.

There was a sharp contrast in the label stability of tomato and pepper fruit when compared to citrus. Owing to the epidermal permeability of tomato and pepper fruit, there was a quick and very high level of shrinkage of the laser labels and surrounding areas as compared to citrus fruit.

Table 4-1. Peel stability of laser labeled tomato fruit stored at 12.5$^{o}$C and 65% RH

| | Percentage of total fruit | | | | |
|---|---|---|---|---|---|
| Time after labeling (days) | 1 (very low) | 2(low) | 3(medium) | 4(high) | 5(very high) |
| 2 | 94 | 6 | 0 | 0 | 0 |
| 6 | 79.2 | 8.8 | 4.8 | 0 | 0 |
| 8 | 66 | 21.6 | 10.8 | 1.2 | 0 |
| 10 | 59.6 | 27.2 | 10 | 3.6 | 0 |
| 16 | 27.6 | 54.4 | 11.6 | 3.6 | 0 |
| 23 | 19.2 | 57.2 | 10.4 | 5.2 | 0 |

Table 4-2. Peel stability of laser labeled tomato fruit stored at 12.5$^{o}$C and 95% RH

| | Percentage of total fruit | | | | |
|---|---|---|---|---|---|
| Time after labeling (days) | 1 (very low) | 2(low) | 3(medium) | 4(high) | 5(very high) |
| 2 | 74.8 | 7.2 | 0 | 0 | 0 |
| 6 | 85.6 | 10 | 3.6 | 4.4 | 0 |
| 8 | 82.4 | 13.2 | 4.4 | 0 | 0 |
| 10 | 77.2 | 14 | 6.8 | 0 | 0 |
| 16 | 64.8 | 17.6 | 10.4 | 1.2 | 0 |
| 23 | 46.8 | 26.8 | 8.4 | 0 | 0 |

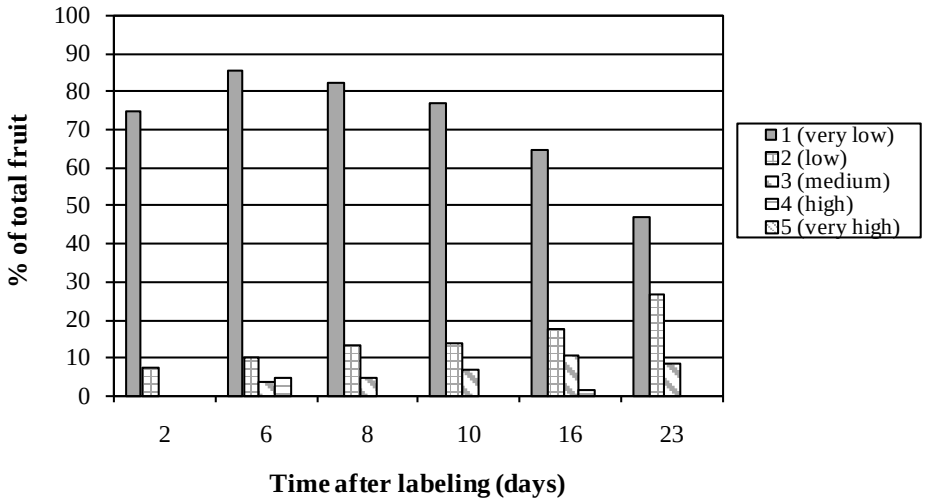



Figure 4-9. Peel shrinkage of laser labeled tomato fruit during storage at 12.5°C (55 °F) and 95% relative humidity. Shrinkage severity was determined on 250 unwaxed fruit per exposure time using a visual scale of 0 (very low or no shrinkage) to 5 (very high or total label collapse) depicted in Figure 4-1.

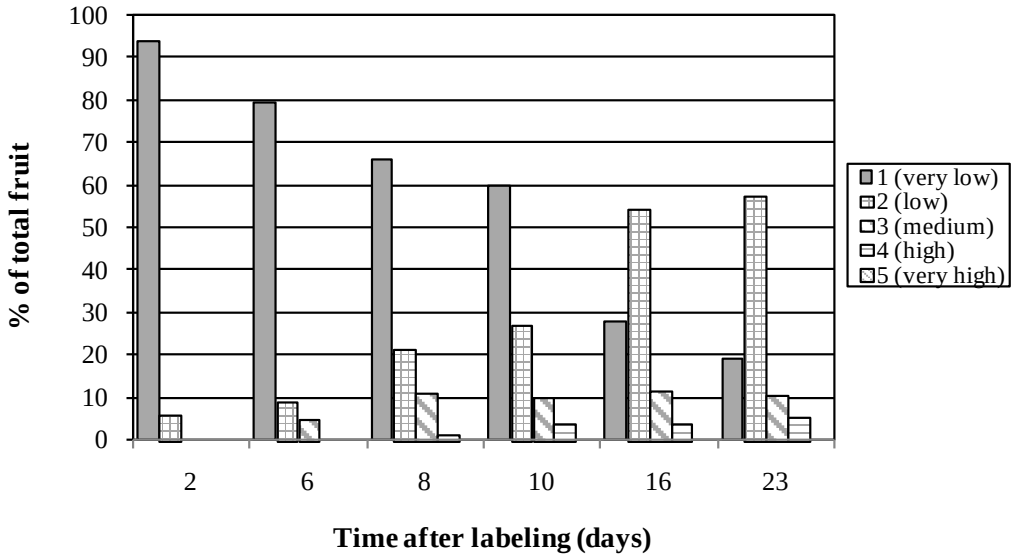



Figure 4-10. Peel shrinkage of laser labeled tomato fruit during storage at 12.5 °C (55 °F) and 65% relative humidity. Shrinkage severity was determined on 250 unwaxed fruit per exposure time using a visual scale of 0 (very low or no shrinkage) to 5 (very high or total label collapse) depicted in Figure 4-1.

Table 4-3. Peel stability of laser labeled pepper fruit stored at 7.5°C and 65% RH

| | Percentage of total fruit | | | | |
|---|---|---|---|---|---|
| Time after labeling (days) | 1 (very low) | 2(low) | 3(medium) | 4(high) | 5(very high) |
| 4 | 3.32 | 19.4 | 62.4 | 1.98 | 0 |
| 7 | 0 | 0 | 49.8 | 44.2 | 2.4 |
| 11 | 0 | 0 | 35 | 48.4 | 5.8 |
| 14 | 0 | 0 | 0 | 19.6 | 34.6 |
| 18 | 0 | 0 | 0 | 19.8 | 36.6 |
| 21 | 0 | 0 | 0 | 2.6 | 18.4 |

Table 4-4. Peel stability of laser labeled pepper fruit stored at 7.5°C and 95% RH

| | Percentage of total fruit | | | | |
|---|---|---|---|---|---|
| Time after labeling (days) | 1 (very low) | 2(low) | 3(medium) | 4(high) | 5(very high) |
| 4 | 15.8 | 54.6 | 23.8 | 0 | 0 |
| 7 | 0 | 14.2 | 72.8 | 5.2 | 0 |
| 11 | 0 | 3.2 | 46.8 | 35.6 | 1.2 |
| 14 | 0 | 1.2 | 34.4 | 41 | 6.4 |
| 18 | 0 | 1.2 | 15.8 | 37.6 | 11.8 |
| 21 | 0 | 0 | 7.2 | 34.4 | 18.4 |

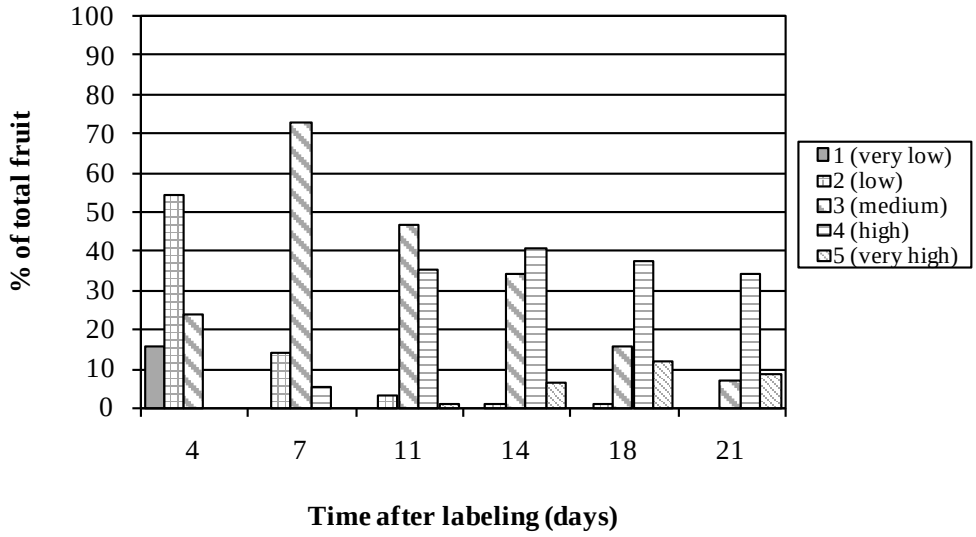



Figure 4-11. Peel shrinkage of laser labeled pepper fruit during storage at 7°C (45 °F) and 95% relative humidity. Shrinkage severity was determined on 150 unwaxed fruit per exposure time using a visual scale of 0 (very low or no shrinkage) to 5 (very high or total label collapse) depicted in Figure 4-2

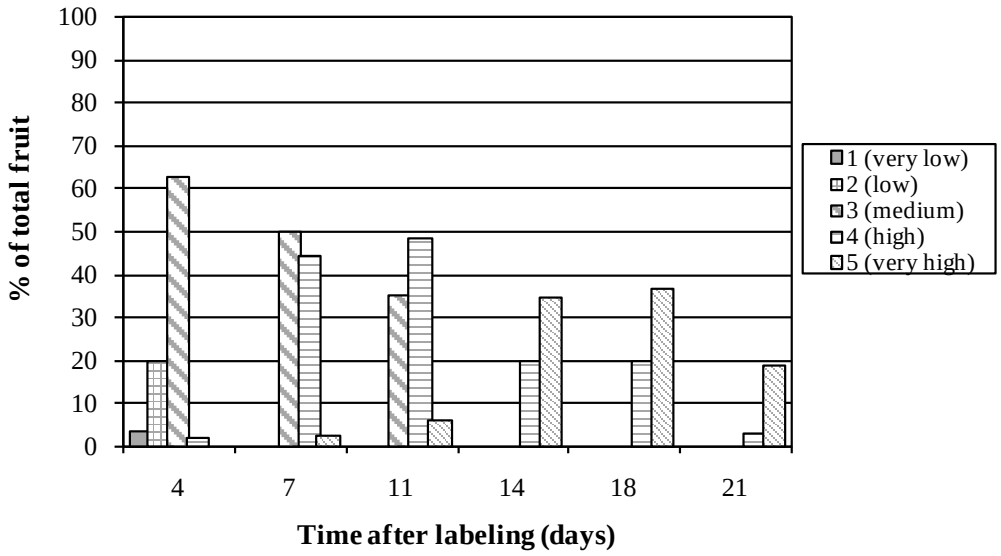



Figure 4-12. Peel shrinkage of laser labeled pepper fruit during storage at 7°C (45 °F) and 65% relative humidity. Shrinkage severity was determined on 150 unwaxed fruit per exposure time using a visual scale of 0 (very low or no shrinkage) to 5 (very high or total label collapse) depicted in Figure 4-2.


Figure 4-13. Unwaxed and waxed laser labeled tomato fruit during storage.

When stored for 7 days at 12.5 $^{o}$C and 95% RH conditions, waxed laser labels maintained the peel integrity as opposed to unwaxed labels which showed considerable shrinkage around the labeled area (Figure 4-13).

### 4.3.4 Decay Study

Fruit decay was monitored for 3 weeks in packed tomato and pepper stored at their respective optimum temperature and 95% RH. Close attention was placed to the sites of decay with special emphasis on the labeled area. Although different kinds of decay were observed, for simplification, only total decay is reported. Decay in fruits labeled with all four exposure times was similar to control non-labeled fruit (Figure 4-14 and Figure 4-15). Most of the decay during the 3-week storage period was independent from the labeled areas, the most common being soft rot (*Erwinia carotovora*) in both tomato and pepper, and sour rot (*Geotrichum candidum*) in tomato. Unlike citrus, laser labeled tomato and pepper fruit occasionally showed some decay about 2-3 % of the total decay on and around the label. However, there may be varied reasons for this observation. The high moisture content of the etched surface may provide entry sites for pathogens as compared to citrus. Despite of having few fruits with decay on label, the overall decay study results are in conformity to those of Yuk et al. (2007) who demonstrated a lack of *Salmonella* migration and survival in tomato laser labels.

Table 4-5. Decay of non laser labeled and laser labeled tomato fruit using four different exposure time

| | Total Decay (%) | | | | |
|---|---|---|---|---|---|
| Time after labeling (days) | Control | 35 µs | 55 µs | 75 µs | 100 µs |
| 2 | 0 | 0 | 0.4 | 0 | 0 |
| 6 | 0 | 1.2 | 0.8 | 0 | 0 |
| 8 | 0 | 1.2 | 0.8 | 0 | 0 |
| 10 | 0 | 1.2 | 0.8 | 0.4 | 0 |
| 16 | 0.8 | 4.8 | 14.8 | 12.8 | 18.4 |
| 23 | 1.6 | 17.2 | 36.4 | 41.2 | 34.4 |

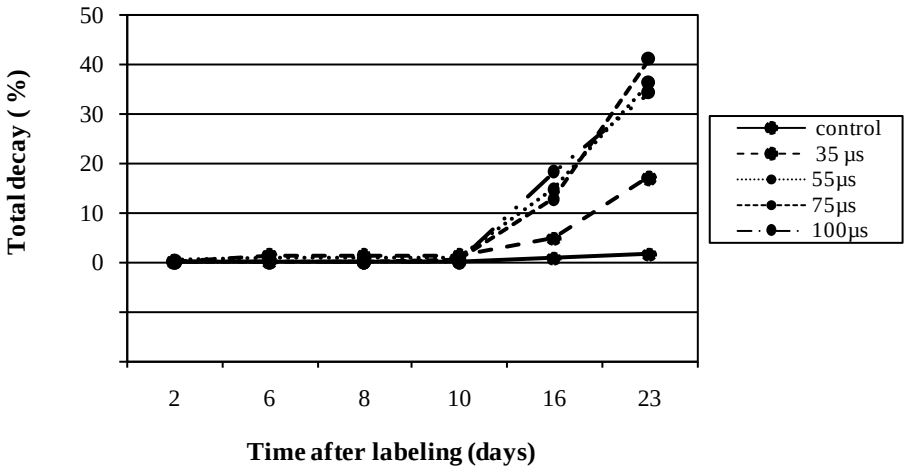



Figure 4-14. Total decay of labeled tomato fruit in storage. Fruit were labeled using 4 exposure times (35 µs, 55 µs , 75 µs and 100 µs) at ambient temperature and stored at 12.5°C (55 °F) and 95% relative humidity. Each point represents the average of five boxes, each box containing 50 fruit

Table 4-6. Decay of non laser labeled and laser labeled pepper fruit using four different exposure time

| | Total Decay (%) | | | | |
|---|---|---|---|---|---|
| Time after labeling (days) | Control | 35 µs | 55 µs | 75 µs | 100 µs |
| 2 | 1.3 | 0 | 1.3 | 0 | 0 |
| 4 | 3.9 | 5.3 | 3.9 | 0.6 | 6 |
| 7 | 13.2 | 7.3 | 5.9 | 2.6 | 9.3 |
| 11 | 16.5 | 17.3 | 11.2 | 10.6 | 12.6 |
| 14 | 21.8 | 19.9 | 15.2 | 15.9 | 21.2 |
| 18 | 57.8 | 30.5 | 31.8 | 40.5 | 31.2 |
| 21 | 62.4 | 47.8 | 58.4 | 62.5 | 72.5 |

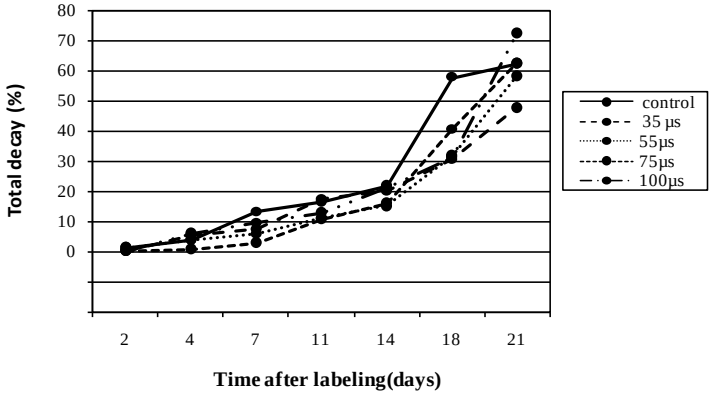



Figure 4-15. Total decay of labeled pepper fruit in storage. Fruit were labeled using 4 exposure times (35 µs, 55 µs , 75 µs and 100 µs) at ambient temperature and stored at 7°C (45 °F) and 95% relative humidity. Each point represents the average of five boxes, each box containing 30 fruit

## 4.4 Discussion

Etching the required information in form of permanent dot matrix style alphanumerical characters or letters causes the opening up of outer protective cuticular barrier. These pinhole depressions created can serve as a potential site for water loss and pathogen invasion. However, it appears that the low energy $CO_2$ beam momentarily vaporizes the natural and commercial wax accumulating in exposed cell walls, thus creating a repellent shield (Etxeberria et al, 2006).

The relationship between pinhole size and water loss was investigated to establish optimal label range in terms of maximal readability with minimal water loss. Unlike citrus, tomato and pepper peel provides a good contrast for laser labels. Therefore, no additional dyeing of the labels is required. Tomatoes were laser labeled when mature green. The laser labels were faint in the beginning due to the lack of contrast provided by green tomato peel. However, they became clear and developed visual appeal with the time as the tomato ripened. The results showed that increasing exposure time beyond 45 µs substantially increased water loss proportional to perforation size in both tomato (Figure 4-3 and Figure 4-4) and pepper (Figure 4-5 and Figure 4-6), while readability was optimal between 45 to 85 µs. Therefore, optimizing readability at the lowest range of water loss between the range 45 to 55 µs can be achieved without substantial amount of water loss.

Resistance to water movement is mainly offered by the natural cuticular layer (Ben Yehoshua, 1969; Burg and Burg, 1965; Horrocks, 1964). Water permeabilities from cuticle vary with plant species and organ (Becker et al, 1986). During packinghouse operations, commercial waxes are applied to most horticultural commodities to alleviate undesirable water loss and improve visual appeal. Furthermore, storage at high RH conditions (90-95%) helps in retaining moisture in the tissue. Water loss from the etched areas alters the appearance of the label. The present study results also showed fruit peel shrinkage on and around the label. The distortion of

the labels occurred rapidly in tomato and pepper as compared to citrus fruit. This faster and large number of label shrinkage in laser labeled tomato and pepper peel could be likely due to higher amount of water loss through the labeled areas.

Tomato is a climacteric fruit and mostly harvested in the mature green stage. Tomato fruit is quite perishable in nature, and storage at optimum temperature and relative humidity is very essential to maintain its quality postharvest. The mature green or partially ripe fruit can be stored best at 12.7 to 15.5 $^{o}$C for 2-4 weeks in contrast to ripe fruit which is best at 7.2 to 10 $^{o}$C for nearly 2 weeks. On the other hand, Pepper is best stored at 7.5 $^{o}$C for maximum shelf life of 3-5 weeks (Kader, 2002). In general, these fruits are vulnerable to various decay organisms during storage, especially *Erwinia sp*. and *Geotrichum sp*. Most pathogen requires the rupture of the produce surfaces for penetration and growth. Laser generated pinhole depressions seemingly provide the avenue for the pathogen invasion, although as noticed previously, citrus fruit held at 10 ºC and 95% RH for 5 weeks showed no decay symptoms associated with the laser labeled area. Stored laser labeled tomatoes and peppers were stored for maximum of 3 weeks to monitor decay. In the present study, we observed appreciable differences in the postharvest behavior of laser labeled between tomato and pepper fruit especially on the extent of decay associated with the label. Nearly 45-70% of tomato fruit showed decay symptoms as oppose to pepper with 25-40% decay. These decay symptoms were not associated with the etching markings. Additionally, these results were different than the citrus decay study results as the percentage of decayed tomato and pepper fruit obtained was higher than that of citrus. In very few instances, laser labeled tomato and pepper developed bacterial rot symptoms on the labels. These results were in contrast to citrus fruit where there was no decay observed on and around the label. Further, some inoculated fruits also developed bacterial ooze on the label during the storage. Despite of having

some decay fruits (2-3% of the total rots) with decay symptoms on the label; the overall decay was not statistically associated with the etched areas. The disassociation between decay and labeled area was further confirmed by exposing the fruit to their most common postharvest organism. The fruit was also subjected to the worst conditions where inoculation was performed immediately after laser labeling. As expected, there was no decay in either of the situations.

As evident from these results and the ones presented in previous chapters, this novel technology could be a viable alternative to label produce. Besides being permanent, it does not support pathogen invasion, incite decay and water loss, thereby keeping the fruit quality maintained.

# CHAPTER 5
# CONCLUSIONS

The study reported here was aimed at determining the effects of laser labeling, an emerging technology, on the overall storage quality of selected fruits and vegetables. The effects of this technique on the storage quality vary with the crop type. According to the results, the optimum laser energy for the entire selected commodities ranged between 40-55 µs. It was also noticed that etched markings on tangerine and grapefruit require dyeing by a food based color as the citrus peel does not provides a better contrast for the labels. Water loss measured from the laser labels was proportional to the exposure time of laser beam in all the selected crops. However, the rate of water loss was highest in laser labeled pepper fruit among all. Citrus, a relatively non perishable crop when laser labeled and stored at optimum temperature (10 $^{o}$C) and relative humidity (95%) conditions for 4 weeks, exhibited negligible water loss throughout the storage period. Additionally, the rate of water loss from waxed labels was no different than the control (non laser labeled) in citrus fruit after 4-7 days of storage.

The laser labels were waxed using different kinds of commercial coatings. The data showed that wax application on the labels subsequently diminishes the rate of water loss from 35% to 94 % in citrus fruit. Among all the waxes used, citrus wax shellac resulted in the highest reduction of water loss. Further it was noticed that the water loss preserves the label appearance in all selected commodities. A considerable portion of fruit with unwaxed labels showed some level of shrinkage rendering the label unreadable. The shrinkage was proportional to relative humidity and time of storage. The label distortion or shrinkage was more at low RH (65%) than the higher RH (95%) conditions. Tomato and pepper fruit showed severe and quicker label shrinkage than citrus at both the low and higher humidity conditions. However this label

distortion was negligible when wax is applied immediately after labeling as waxing helps in sealing of the pinhole depressions for further water loss.

Fruit held at 95% relative humidity, storage conditions conducive for decay and their respective optimum temperature, showed nearly no decay symptoms associated with the etched areas. Although some decayed fruit were found in the laser labeled fruit samples in all the selected crops however, were no different than the control (non-laser labeled) fruit samples. In very few instances, laser labeled tomato and pepper developed decay symptoms on the labels. Despite having some fruits with decay symptoms on the labels (2-3% of the total decay), the overall decay was not statistically associated with the etched markings.

The lack of decay associated with inoculated etched areas also confirmed that laser labeling does not facilitate decay. Whether coated with pathogen spores before laser labeling or a layer of spores spread over the freshly etched areas, no fruit decay from the labeled area was noticed in all the four selected commodities. This further corroborates the previous findings of Yuk et al (2007) who challenged tomato fruit with Salmonella and found that laser labeling does not cause sufficient damage to allow Salmonella infiltration. It can be assumed that, with time, the accumulation of lignin and wax deposits in and around etched areas also provides a repellant shield and prevents the penetration of decay organism through etched openings. Various studies have shown previously that the deposition of lignin and other cell wall components in the wound tissue provides resistance to the diseases. The fungal development was found to be arrested by phenolic compounds and callose deposits in the cell walls of young tomato fruit inoculated with *Botrytis cinerea* (Glazener, 1982).

There was indeed an appreciable difference in the postharvest behavior of laser labeled fruit namely citrus, tomato and pepper. For example the optimum exposure time of laser beam

for tomato and pepper is shorter than citrus fruit The optimum range of exposure times with minimum water loss while maintaining the readability of labels was 45-55μs in citrus fruit whereas 35-45μs in tomato and pepper fruit. Additionally, the extent of laser label shrinkage was higher in tomato and pepper as compared to citrus. All these differences are mainly attributed to the peel morphology of the above selected crops. As depicted the thin skinned fruits such as tomato and pepper suffer substantial amount of water loss at etched areas than the thick peeled citrus fruit, as the thickness of the cuticle is directly related to the protection against undesirable moisture loss, but this loss can be greatly diminished by applying wax coatings on the label. Taking into account all these above mentioned results, this study suggests that laser labeling could be a viable alternative to the current labeling system; provide individual traceability without enhancing safety concerns. It was demonstrated that the fruit quality of the laser labeled fruit remains high as the invasion of epidermis does not promote decay, provide avenue for pathogens and water loss is easily controlled.

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# BIOGRAPHICAL SKETCH

Preeti Sood was born in India. She obtained her Bachelor of Science in agriculture from Punjab Agricultural University, Ludhiana, India in 2006. In May 2007, she started her master's program in horticulture at the University of Florida and successfully completed her degree in 2009.