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Growth or penetration of *Salmonella* into citrus fruit is not facilitated by natural-light labels

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ABSTRACT

In natural-light labeling of fruits and vegetables, the desired information is etched onto the produce surface using a low-energy carbon dioxide laser beam (10,600 nm). Etched characters are formed by surface depressions in the epidermis that may facilitate entrance of decay and pathogenic organisms. The objective of this study was to determine the effects of natural-light labeling and different postharvest treatments on *Salmonella* populations' ability to survive/grow and penetrate into citrus fruit. A five-strain cocktail of *Salmonella* was spot inoculated onto Valencia orange in different application sequences with wax and natural-light etching. Samples were stored at 10, 26 °C, or combinations of both, for up to 42 days. Etched peels and corresponding juices were extracted from whole oranges following storage and enumerated for *Salmonella*. No set of conditions involving natural-light labeling promoted the growth of *Salmonella* on the fruit surface or resulted in the detection of *Salmonella* from the juice of sound fruit. Survival of *Salmonella* populations on the peel surface did not differ between any of the treatment and control samples. In all cases, *Salmonella* declined between 1.5 and 3.0 log CFU/orange after 30 days, with faster decline noted at 10 °C. Based on the data obtained from all treatments and under conditions extremely unfavorable and unrealistic in terms of fruit storage, natural-light labeling citrus fruit peels and subsequent waxing in any order did not allow for the growth or influence the natural decline of *Salmonella* populations on citrus fruit surfaces, or movement into juices, as compared to controls.

1. Introduction

Most produce items are currently labeled with Price Look-Up (PLU) stickers that contain a four number code used to identify product groupings (Produce Electronic Identification Board, 1995). PLU stickers are commonly adhered to the surface of individual fresh fruits and vegetables during the packing process, typically after washing, culling and waxing, and prior to final packing (Varon and Paddock, 1978). PLU stickers can easily detach during post-harvest handling following sticker application, eliminating any information the PLU stickers may provide related to traceability and may also leave sticky residues or damage on produce surfaces (Etxeberria, Miller, & Achor, 2006a).

Imprinting an alphanumeric code directly onto the surface of produce, at the same point in production as PLU stickers, is currently being explored as an alternative way of permanently labeling fresh fruits and vegetables. A natural-light etching device, designed for labeling citrus surfaces with a low energy carbon dioxide laser beam (10,600 nm; Drouillard & Rowland, 1997), is a promising technology. The code produced is permanent, requires no adhesive and labeling information can be easily modified prior to application to individual citrus fruits (Etxeberria, Miller, & Achor, 2006b). In previous tests, penetration depression diameter and depth due to natural-light labels were similar on tomato and avocado fruits surfaces, averaging 200 μm and 25 μm, respectively, and affecting only the outermost 2–5 epidermal cells which are much smaller than those of the underlying endocarp (Etxeberria et al., 2006a).

When examined immediately after labeling, label markings are formed by pinhole depressions (Etxeberria et al., 2006b).

Abbreviations: PLU, Price Look-Up; TBZ, thiobendazole; XLD, xylose lysine deoxycholate.

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Interruption to the natural cuticular barrier of the citrus surface potentially opens a route that could allow for the penetration of spoilage and pathogenic microorganisms. Studies evaluating the susceptibility of natural-light labeled tangerines (Sood, Ference, Narciso, & Etxeberria, 2008) and grapefruits (Sood, Ference, Narciso, & Etxeberria, 2009) to *Penicillium digitatum* spoilage failed to demonstrate the ability of decay microorganisms to colonize etched surfaces of citrus fruits. The lack of colonization of decay organisms into citrus fruit was attributed to the unique anatomical organization of the citrus peel which contains numerous oil glands and a loosely packed thick mesoderm (Bain, 1957). In addition, anatomical studies of etched citrus peel indicated the cauterization of the label markings (Etxeberria et al., 2006b; Etxeberria, Narciso, Sood, Gonzalez, & Narciso, 2009), unsuitable conditions for spore germination.

Sensitivity to food safety risks associated with *Salmonella* exists within the citrus production and processing industries due to outbreaks associated with fresh orange juice that occurred in the mid-1990s (Vojdani, Beuchat, & Tauxe, 2008). Although previously reported studies indicate *Salmonella* is unable to colonize natural-light labels on mature green (Danyluk, Interiano, Friedrich, Schneider, & Etxeberria, 2010) or mature red (Yuk, Warren, & Schneider, 2007) tomato fruits, even in the presence of soft-rot organisms (Danyluk et al., 2010), colonization of citrus fruit by enteric pathogens through natural-light labels remains undetermined.

The objective of the present study was to determine if differences in *Salmonella* survival and/or growth on orange peel surfaces exist between non-light labeled oranges and those that are natural-light labeled, and to evaluate the potential for *Salmonella* penetration through the flavedo and albedo of the citrus peel into juice vesicles, resulting in internalization of *Salmonella* and its presence in orange juice.

2. Materials and methods

2.1. Plant material

Valencia oranges (*Citrus sinensis*) were shipped overnight from Sunkist LTD (Ventura, CA, USA) to the University of Florida's research facilities located at the Citrus Research and Education Center in Lake Alfred, Florida, USA. Fruit was packed the day of shipment following standard commercial protocol, which included waxing the fruit with 5 ppm thiobendazole (TBZ). Fruit was stored at 10 °C immediately after arrival and transferred to 21 °C the day before experimental use.

2.1.1. Selection of strains and inoculum preparation

A cocktail of five *Salmonella* serovars isolated from orange juice was used. *Salmonella* serovars included: Gaminara (CDC HO662); Rubislaw (F2833); Typhimurium (ATCC14028); Hardford (CDC H0778); and Muenchen (LJH 0592).

Prior to each replication, frozen stock cultures of each strain were streaked onto nutrient agar (Difco: Becton, Dickinson and Co., Sparks, MD, USA) and incubated at 35 °C for 24 h. One isolated colony from each strain was transferred to 10 mL of nutrient broth with 1% glucose (Difco: Becton, Dickinson and Co.) and incubated at 35 °C. After 24 h, 1 mL of culture was spread over a nutrient agar + 1% glucose and incubated at 35 °C for 24 h, when a lawn of stationary cells had formed. For acid adaption reasons, nutrient broth and agar was supplemented with 1% glucose (Buchanan & Edelson, 1996). Cells from each individual strain of the cocktail were harvested from five plates using a sterile hockey stick and 10 mL of 0.1% peptone water (Difco: Becton, Dickinson and Co.). Serial dilutions were carried out in 0.1% peptone water (9 mL) to prepare final inocula concentrations (ca. 9.6 log CFU/mL), verified for each strain by enumeration on tryptic soy agar (Difco: Becton, Dickinson and Co.). Equal volumes (1 mL) of each *Salmonella* strain were combined to obtain the final inoculum. Final inoculum cocktails were stored on ice prior to and during citrus inoculation.

2.1.2. Inoculation treatments

Fruit were inoculated, punctured, labeled and/or waxed in 11 different sequence combinations (Fig. 1), including two control sets of fruit where no *Salmonella* was inoculated (treatments A and B), four controls where fruit were inoculated but not labeled (treatments C, D, E, and F), and one control where fruit were punctured (treatment G).

2.1.3. Fruit peel inoculation

Oranges were inoculated with 20 μL of inoculum distributed in 4–6 droplets over the designated inoculated area of the fruit (Fig. 2). Samples were held in a biological safety cabinet (NU4625600, Nuaire, Plymouth, MN, USA) for 20 min to allow the inoculum to dry prior to any further inoculation treatment steps.

2.1.4. Fruit peel labeling

Fruit labeling was conducted as previously described by Sood et al. (2008). Fruit were individually placed on a plastic ring with the orange surface 10 cm apart from the natural-light source. All fruit were labeled with the phrase "Citrus 35us USA" using a maximum energy level of 0.578 W per character with a time of exposure of 35 μs and a duty cycle of 25%. As a positive control, oranges were wounded by puncturing the peel. Wounds, in a similar location and area as the natural-light label, were 2 mm in diameter and 3 mm deep, and achieved by puncturing the peel with a metal puncturing device specifically designed and manufactured in house for puncturing citrus peels.

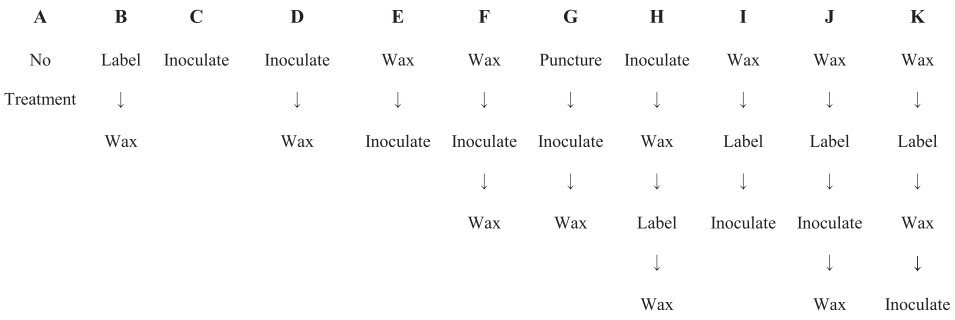

Fig. 1. Flow diagram of the inoculation treatments. Fruit were inoculated, labeled and/or waxed in different sequence combinations before storage at 10 or 26 °C. Inoculation was carried out by spotting 20 μL of inoculum in 4–6 droplets over the labeled surface or designated area of the fruit. Carnauba wax was used in those experiments requiring reapplication using a sterile swab.