Infiltration and Survival of *Salmonella* spp. on Tomato Surfaces Labeled Using a Low-energy Carbon Dioxide Laser Device

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Summary. **Infiltration and survival of a rifampicin-resistant five-serovar *Salmonella* cocktail was investigated in laser-etched tomatoes (*Lycopersicum esculentum*) and smooth (untreated) and punctured (dye solution only) tomato surfaces in storage for 14 days at 25 °C/60% relative humidity. Surviving *Salmonella* populations were enumerated on tryptic soy agar supplemented with the antibiotic rifampicin. In the first survival study (laboratory-etched tomatoes), the population of *Salmonella* spp. in wounds increased to 6.8 log cfu/fruit, whereas cells on smooth surfaces decreased to undetectable levels during 14 days of storage. For etched tomatoes, the storage reduced 2.7 log cfu/fruit after the first 3 days; however, an increase was observed at 7 days, followed by a population decreased to 2.9 log cfu/fruit at 14 days. In the second survival study (pilot plant-etched tomatoes), the populations decreased a total of 3.5 log cfu/fruit and 2.5 log cfu/fruit comparing 1 day with 14 days for smooth and etched surfaces respectively. Infiltration of the dye solution or *Salmonella* beyond the area of immediate tissue damage was not observed on any tomato surface tested.**

Labeling of individual fresh fruit and vegetables has the potential for providing product traceability, thereby strengthening food safety systems against food-borne outbreaks. Currently, each piece of fresh produce entered into the conventional retail sales chain is marked with a price look-up (PLU) sticker. The sticker contains a four-digit number developed by the Produce Electronic Identification Board (PEIB), which identifies the variety of fruit or vegetable (PEIB, 1995). The PLU sticker is most commonly placed on the fruit or vegetable surface at the packing line (Varon and Paddock, 1978). Disadvantages to the PLU sticker include the sticky residue left behind on produce surfaces when the sticker is removed, as well as damage to the produce surface by fingernails during sticker removal (Durand–Wayland, 2005). In addition, PLU stickers may be detached during postharvest handling, thereby eliminating any traceability the sticker may provide (Etxeberria et al., 2006). An alternative to using removable adhesive stickers with produce data is implanting the alphanumeric code directly on the produce surface. This can be accomplished using a newly designed laser etching device for labeling produce surfaces. The new device uses a low-energy CO_2 laser beam (10,600 nm) (Drouillard and Rowland, 1997) for etching. The alphanumeric code produced by the laser etching method is permanent, requires no additional adhesive, and labeling information can be easily modified at the packinghouse (Etxeberria et al., 2006).

Nontyphoidal *Salmonella* accounts for an estimated 1.5 million cases of food-borne illness, 16,430 hospitalizations, and 582 deaths annually in the United States. (Mead et al., 1999). In 2005, the Food-borne Diseases Active Surveillance Network of the U.S. Centers for Disease Control and Prevention Emerging Infectious Program reported 6471 laboratory-confirmed cases of *Salmonella*, the most of any food-borne pathogens under surveillance (Centers for Disease Control and Prevention, 2006). Since 1990, common vehicles in several *Salmonella* outbreaks have been tomatoes. Large multistate outbreaks associated with tomato consumption have involved the serovars *Salmonella* Javiana (Hedburg et al., 1999), *Salmonella* Montevideo (Hedburg et al., 1999), and *Salmonella* Baildon (Cummings et al., 2001). Although the exact sources of *Salmonella* have not been determined, some reports indicated that *Salmonella* may have been a contaminant in dump tank water resulting from uncontrolled or unmonitored chlorine levels allowing *Salmonella* survival (Cummings et al., 2001; Hedburg et al., 1999; Wei et al., 1995). In addition, temperature differentials between tomatoes and the wash water may allow *Salmonella* cells to internalize into tomato pulp through natural openings such as wounds and the stem scar (Bartz, 1982).

The application of an alphanumeric code directly to the surface of produce (such as tomatoes) by the laser etching system disrupts the natural cuticular barrier on produce surfaces. It must be determined whether this disruption of the natural cuticular barrier facilitates greater survival or infiltration of pathogens, such as *Salmonella*, over undisrupted produce surfaces. Therefore, the purpose of this study was to determine the survival and infiltration of *Salmonella* spp. inoculated on laser-etched, puncture wounded, and untreated tomato surfaces during 14 d of storage at 25 °C/60% relative humidity (RH).

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29,574	fl oz	µL	3.3814×10^{-5}
29.5735	fl oz	mL	0.0338
0.3048	ft	m	3.2808
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
1	ppm	$mg \cdot L^{-1}$	1
1	ppm	$\mu L \cdot L^{-1}$	1
(°F – 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

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Materials and methods

MAINTENANCE AND PREPARATION OF *SALMONELLA* CULTURES. All *Salmonella* cultures were obtained from L.J. Harris, University of California, Davis. The serovars used in this study were *Salmonella* Agona LJH618 (alfalfa sprouts isolate), *Salmonella* Gaminara LJH616 (orange juice isolate), *Salmonella* Michigan LJH615 (muskmelon isolate), *S.* Montevideo LJH614 (human isolate from tomato outbreak), and *Salmonella* Poona LJH631 (human isolate from tomato outbreak). Subcultures of each of the *Salmonella* serovars resistant to 200 ppm rifampicin (rif+; Fisher Scientific, Fair Lawn, N.J.) by spontaneous adaptation were isolated on tryptic soy agar (TSA; BD Diagnostics, Franklin Lakes, N.J.) plates supplemented with 100 ppm rif+ and maintained on TSA rif+ slants at 4 °C. Three days before each experiment, each of the five *Salmonella* cultures were cultivated (37 °C, static incubation) in 10-mL tubes of tryptic soy broth (BD Diagnostics) rif+ and overnight transfers were performed using 10-μL sterile, disposable loops (BD Diagnostics) each day. On the day of the experiment, a cocktail of the five *Salmonella* serovars was compiled by transferring 2.0 mL from each of the five 18-h cultures (late stationary phase) to a clean, sterile 15-mL centrifuge tube. The cocktail was centrifuged (3220 g_n for 10 min) and the resulting pellet was washed twice and resuspended in 10 mL 0.1% peptone.

LASER ETCHING OF TOMATO SURFACES. Two methods for etching fully ripe, beefsteak tomato surfaces with a low-energy CO_2 laser (model XY mark 10; Durand-Wayland, LaGrange, Ga.) were investigated in this study. These tomatoes were purchased at a local grocery. In the first method, tomatoes were placed in a lead ring and etched in place (hereafter referred to as the "laboratory-etched tomatoes;" Fig. 1A). In the second method, tomatoes were etched as they passed under the laser on a conveyor belt (hereafter referred to as the "pilot plant-etched tomatoes"). For both studies, the laser maximum energy level was 0.578 W per character at 30 μs with a duty cycle of 25%. Two lines of alphanumeric code were etched on the surface of each tomato as follows: "TOMATO" (line one) and "30USUSA" (line two) (Fig. 1B). For the pilot plant study, the conveyor line speed was 150 ft/min, which was equal to 7.5 tomatoes/s. The temperature of the tomatoes was ≈21 °C and the ambient air temperature was ≈24 °C. Laboratory-etched tomatoes were etched on location by Durand–Wayland personnel. Pilot plant-etched tomatoes were etched at a pilot plant facility then delivered by Durand–Wayland personnel to the laboratory for 24-h postlaser treatment analysis.

SURVIVAL OF *SALMONELLA* ON LABORATORY-ETCHED TOMATOES. Tomatoes treated with the laser etching device, tomatoes with artificial puncture wounds, and control (untreated) tomatoes were placed in sterile aluminum foil rings in fiberglass trays and inoculated with an appropriate dilution of a five-serovar *Salmonella* cocktail to obtain final inoculation levels of ≈1.0 × 10^5

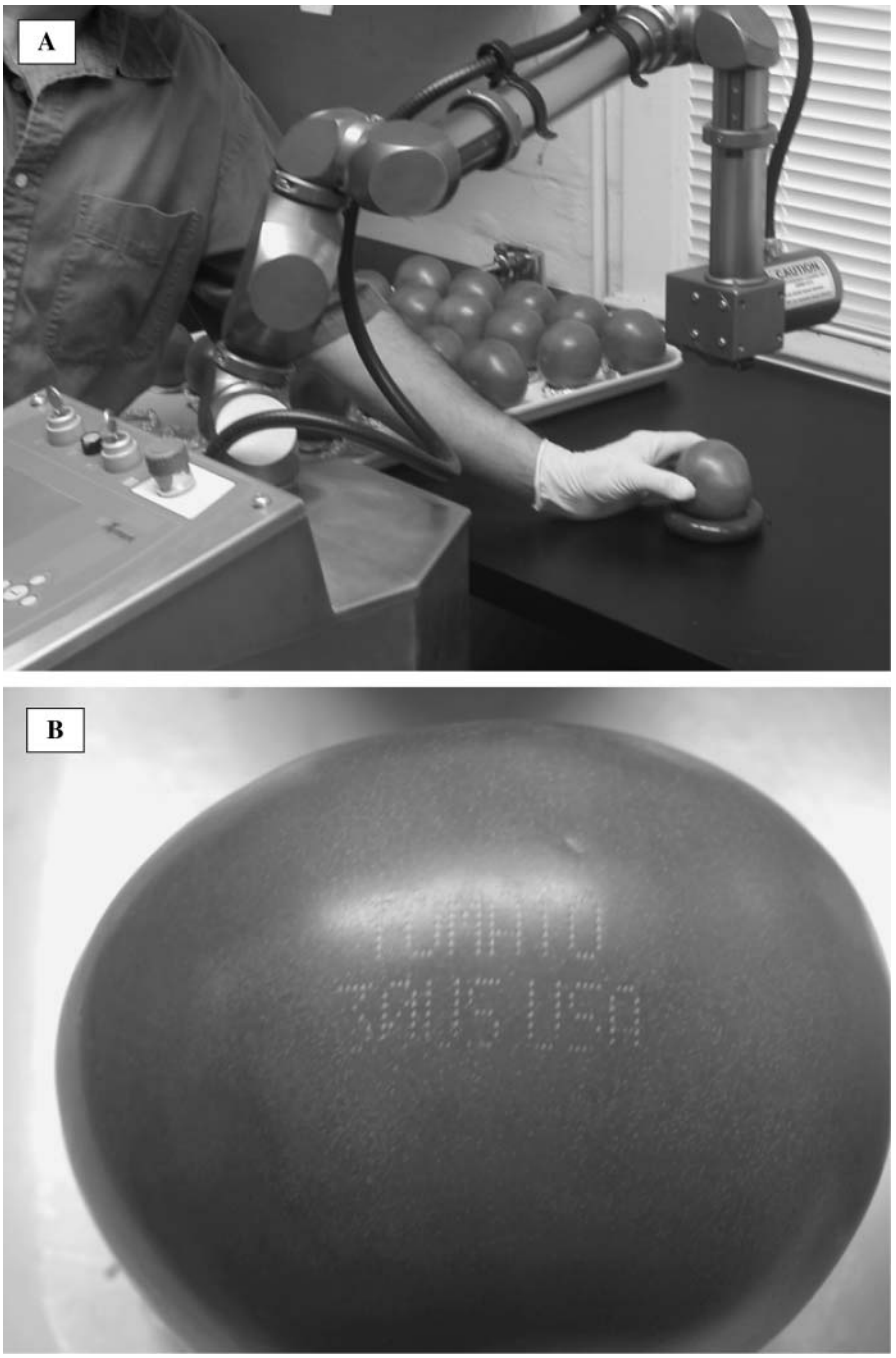

Fig. 1. Laser etching machine at laboratory (A) and tomato after labeling (B).

cfu/fruit. The inoculated surfaces remained face up. Etched tomato surfaces were inoculated within 4 h of the laser treatment using a single spot of 100 µL. Smooth tomato surfaces were inoculated at 10 sites per fruit with 10 µL per site. Three artificial puncture wounds of 1 mm diameter by 1 mm deep each were introduced to tomato surfaces using a sterile paper clip, each inoculated with 10 µL of the diluted *Salmonella* cocktail, yielding a total of 30 µL per tomato. The inocula were allowed to air dry completely (usually within 1 h) at room temperature before storage in an environmental chamber (Caron 6030; Caron Products and Service, Inc., Marietta, Ohio) at 25 °C/60% RH.

Five replicate tomatoes from each treatment were analyzed for survivors on days 0, 1, 3, 7, 10, and 14. For recovery of survivors, tomatoes were transferred to sterile stomacher bags (Secure T, Fisher Scientific) containing 100 mL 0.1% of peptone water and subjected to a 30-s vigorous shake followed by a 1-min hand massage similar to the method described by Zhuang et al. (1995). To enumerate survivors, the tomato rinse was serially diluted 1:10 using 0.1% peptone water, and appropriate dilutions were analyzed by pour-plate using TSA (rif+). Resulting colonies on TSA (rif+) were enumerated after incubation at 37 °C for 24 h. Uninoculated control tomatoes were analyzed to verify the absence of indigenous tomato microflora resistant to 100 ppm rif+.

Survival of *Salmonella* on pilot plant-etched tomatoes. All methods were performed as described earlier for survival of *Salmonella* on laboratory-etched tomatoes with the following exceptions. Pilot plant-etched tomatoes and control (untreated) tomatoes were inoculated with three 10-µL spots of an appropriate dilution of the five-serovar *Salmonella* cocktail to obtain a final inoculation level of $\approx 1.0 \times 10^5$ cfu/fruit. Tomatoes with artificial puncture wounds were not analyzed in parallel with the pilot plant-etched tomatoes. Five replicate tomatoes from each treatment were analyzed for survivors on days 1, 3, 7, and 14. Because pilot plant-etched tomatoes were received 24 h after laser treatment, no day 0 analysis was possible.

Dye infiltration of laboratory-etched tomatoes. Laser treated tomatoes were stored at 25 °C/60% RH for 14 d. At days 0, 1, 3, 7, and 14, etched tomato surfaces were inoculated with 100 µL 0.5% aniline blue (Fisher Scientific). In addition, tomatoes with artificial puncture wounds (as described earlier) and control (untreated) tomatoes were analyzed on day 0. All dye inoculations were allowed a 1-h contact time at room temperature (≈55% RH), after which the uninternalized dye was rinsed off using distilled water. The tomatoes were then cut in half using a sterile knife starting opposite the inoculation site and slicing through the tomato toward the inoculation site. Images of the cut surfaces were captured using a digital camera (Nikon MB-E5000; Nikon, Melville, N.Y.).

Infiltration of *Salmonella* in pilot plant-etched tomatoes. Laser-treated tomatoes were stored at 25 °C/60% RH for 14 d. At days 1, 3, 7, and 14, the etched surfaces of three tomatoes and the smooth surfaces of three control (untreated) tomatoes were inoculated with 100 µL of a five-serovar *Salmonella* cocktail such that the final inoculation level was $\approx 1.0 \times 10^7$ cfu/fruit. The inocula were allowed a 1-h contact time at room temperature (≈55% RH) before testing at each day. Using a sterile knife, each tomato was cut as shown in Fig. 2 and images of the resulting surfaces (line AB and line CD in Fig. 2) were captured using a digital camera. The knife was cleaned and sterilized with flame between each cut to prevent cross-contamination. The sliced surfaces were used to inoculate the surface of inverted TSA rif+ plates, the plates were incubated at 37 °C for 24 h, and images of the resulting growth were captured using a digital camera. Photographic overlays of the tomato surface and growth images were prepared using Adobe Photoshop (version 7.0; Adobe System, San Jose, Calif.).

Statistical analysis. All statistical analyses were performed using the Statview statistical software package (version 9.1; SAS Institute, Cary, N.C.) using a mixed model. Sample replications were treated as random variables within time. $P < 0.05$ was considered as a significant difference for all experiments.

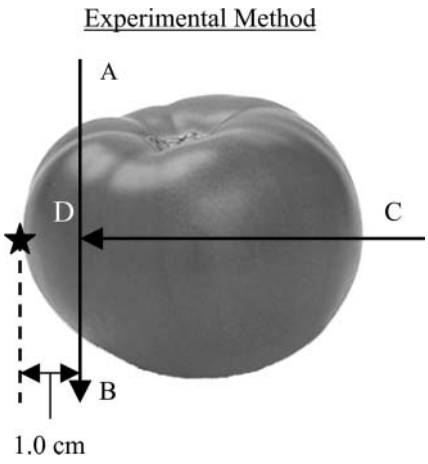

Fig. 2. Diagram of cutting pattern used in infiltration studies. The first cut was made by cutting along a line in the direction of A to B, resulting in 1.0-cm (0.39-inch) slice (AB slice). A second cut was made by cutting along a line in the direction of C to D (CD slice), cutting in the direction toward the inoculation site, to prevent inadvertently moving cells further into the fruit. The inoculation site is represented by ★.

Results and discussion

Survival of *Salmonella* on laboratory- and pilot plant-etched tomatoes. The survival of *Salmonella* spp. inoculated on smooth, laboratory-etched, and puncture-wounded tomato surfaces is shown in Fig. 3A. Initial *Salmonella* populations declined significantly ($P < 0.05$) during the 14-d storage at 25 °C/60% RH on both smooth and laser-etched surfaces of tomatoes. On day 7, an unexpected increase in the *Salmonella* population was observed for laser-etched, but not smooth, tomato surfaces; however, this spike was not likely the result of increased availability of nutrients because of tissue damage, because *Salmonella* populations continued to decline on days 10 and 14. On day 14, there was no significant difference ($P < 0.05$) between *Salmonella* populations on laser-etched and smooth tomato surfaces. In contrast, initial *Salmonella* populations increased rapidly ($P < 0.05$) in puncture-wounded tomato surfaces, which indicated the availability of nutrients as a result of tissue damage. It was noted that several of the laser-etched tomatoes experienced dehydration around the etched surfaces at days 10 and 14.

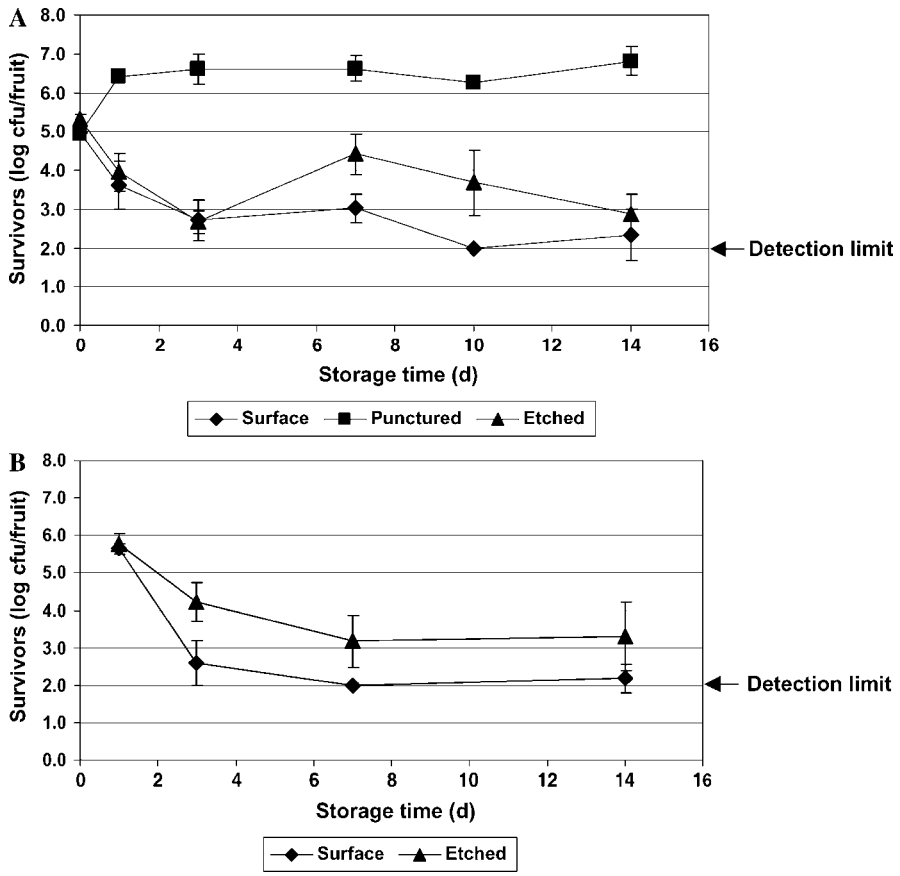

Fig. 3. Survival of a five-serovar *Salmonella* cocktail artificially inoculated on smooth surface, puncture wounds, and etched marks of tomatoes for 14 d at 25 °C (77 °F) and 60% RH for laboratory-etched tomatoes (A) and pilot plant-etched tomatoes (B).

The survival of *Salmonella* spp. inoculated on smooth and pilot plant-etched tomato surfaces is shown in Fig. 3B. Although initial *Salmonella* populations decreased significantly ($P < 0.05$) on smooth and pilot plant-etched tomato surfaces during the 14-d study, the populations were significantly higher ($P < 0.05$) on the laser-etched tomatoes on days 3, 7, and 14. Unlike the survival study conducted with laboratory-etched tomatoes, no unexpected increase in *Salmonella* was observed for pilot plant-etched tomato surfaces. Tomatoes with artificial puncture wounds were not investigated in parallel with pilot plant-etched tomatoes. It was also observed that dehydration around the etched surfaces was greatly reduced on the pilot plant-etched tomatoes compared with laboratory-etched tomatoes (data not shown). The reduced dehydration on the pilot plant-etched tomatoes may result from less heat damage to the tomato surface as a result of the cooling effect of air as the tomatoes moved along the conveyor belt compared with laboratory-etched tomatoes, which remained static.

The results on smooth and puncture-wounded tomato surfaces support previous reports of *Salmonella* survival on tomato surfaces. Allen et al. (2005) reported that *Salmonella* populations decreased on smooth tomato surfaces when stored at 20 °C/60% RH, 20 °C/90% RH, or 30 °C/70% RH over 28 d. Guo et al. (2002) reported *Salmonella* populations decreased 4 $\log_{10}$ units on smooth surfaces of mature, green tomatoes over 14 d at 20 °C/70% RH. Joy (2005) showed that puncture-wound sites on tomato surfaces supported an increase in *Salmonella* populations. Furthermore, the results on laser-etched tomato surfaces supported observations of Etxeberria et al. (2006). When analyzed microscopically, laser-etched depressions were not observed to penetrate beyond the third layer of epidermal cells and, after 4 d of storage, structural changes were observed in the cell wall directly beneath the etched depressions, thickening the third layer of the cell wall (Etxeberria et al., 2006).

DYE INFILTRATION OF LABORATORY-ETCHED TOMATOES. Dye solutions, such as aniline blue, have been used to evaluate the potential for bacterial infiltration of fruit surfaces (Eblen et al., 2004; Penteado et al., 2004). In this study, the dye solution was observed to penetrate partially the laser-etched surfaces (Fig. 4A) and puncture-wound sites on tomatoes (Fig. 4B). In all laser-etched and puncture-wounded tomatoes, the dye solution was not observed to penetrate beyond the damaged tissue area. In contrast, the dye solution was not observed to penetrate the intact smooth surface of tomatoes (data not shown). These results support the observations of Etxeberria et al. (2006), who demonstrated that tissue damage caused during the laser etching of tomatoes was limited to the first few cell layers of the tomato.

INFILTRATION OF *SALMONELLA* IN PILOT PLANT-ETCHED TOMATOES. Preliminary studies on the slice and stamp method determined that a 1.0-cm slice containing the inoculum must be removed before cross-sectioning the tomato to prevent contamination of TSA rif+ plates with cells located on the tomato surface (data not shown). For this reason, *Salmonella* cells would have to infiltrate the tomato beyond the 1.0-cm slice to be detected. In this study, no infiltration of laser-etched or smooth tomato surfaces was observed. None of the composite images exhibited any signs of infiltration (images not shown). These results were consistent with those of the dye infiltration study and indicate that laser etching of tomato surfaces, as performed in this study, does not cause sufficient tissue damage to permit *Salmonella* infiltration.

Conclusion

The results of this study demonstrate that laser-etched tomato surfaces do not support the penetration of dye solutions or *Salmonella* to internal tissues. In addition, laser-etched tomato surfaces do not

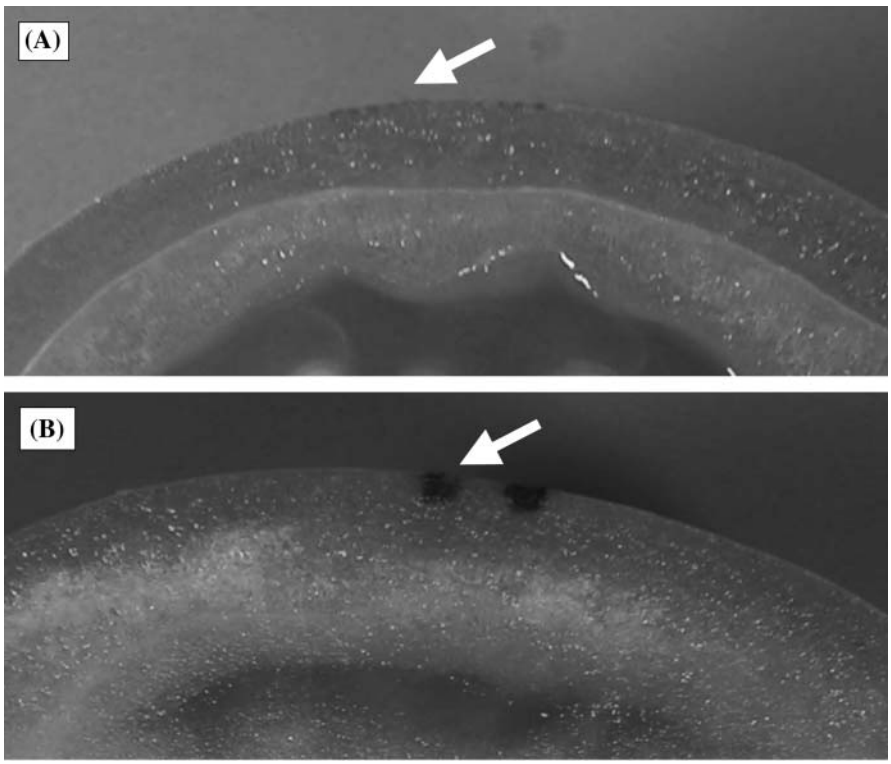

Fig. 4. Comparison of etched marks (A) and puncture wounds (B) of tomatoes after dye infiltration.

support the survival or proliferation of *Salmonella* when stored at a temperature/RH combination similar to that experienced in a typical retail environment. The results of this study suggest that laser-etched alphanumeric codes, when applied to tomato surfaces, provide individual fruit traceability without increasing food safety concerns coincident with laser etching.

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