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Survival of *Salmonella* spp. on surface-inoculated forced-air cooled and hydrocooled intact strawberries, and in strawberry puree

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ABSTRACT

Fresh-market strawberries are cooled to 1–3 °C before commercial storage and distribution; typically by forced-air cooling. Hydrocooling ensures a faster and more uniform cooling of strawberries, although its effect on reducing microbial contamination on the fruit has not been evaluated. *Salmonella* has been reported to survive on damaged strawberries, but is unable to multiply, potentially due to the low pH or other intrinsic factors associated with strawberries. This study evaluated *Salmonella* survival a) on the surface of intact hydrocooled or forced-air cooled strawberries; b) as affected by agitation and density of packing during hydrocooling and c) as affected by pH, temperature and food matrix (strawberry or tomato puree). Intact strawberries inoculated with *Salmonella* were subjected to forced-air cooling or hydrocooling in water containing 100 or 200 ppm HOCl. *Salmonella* population was enumerated 0, 7 and 8 days post-treatment. Strawberry and tomato puree (pH 3.7 and 4.6) spiked with *Salmonella* and incubated at 4, 10 or 25 °C, were evaluated at 0, 1 and 3 days post-inoculation ($n = 9$). Compared to forced-air cooling, hydrocooling significantly reduced *Salmonella* survival on inoculated intact strawberries, with levels below the enumerable limit (1.5 log CFU/berry) by day 8. Hydrocooling reduced the initial *Salmonella* levels by 1.9 log CFU/berry, while the addition of 100 or 200 ppm HOCl reduced levels by 3.5 and 4.4 log CFU/berry, respectively. Initial *Salmonella* populations (day 0) were significantly lower when the berries were agitated or loosely packed during hydrocooling. *Salmonella* survival was significantly higher at a higher pH (4.7) compared to lower intrinsic pH (3.6) of strawberry puree. Higher temperature (25 °C) was conducive for *Salmonella* survival on strawberry puree compared to lower temperatures (4 and 10 °C). The data shows that a lower pH of 3.6 or refrigeration below 10 °C are effective in controlling the survival of *Salmonella* on damaged strawberries.

1. Introduction

Florida is one of the major winter producers of fresh-market strawberries in North America, shipping to a wide geographical area. Strawberries are highly perishable, susceptible to moisture loss, bruising, and postharvest decay during shipping (Ferreira, Sargent, Brecht, & Chandler, 2008). To minimize postharvest decay, strawberries are harvested at ¾ to full ripe stage, field-packed into retail plastic clamshells, and then into corrugated fiberboard shipping cartons (eight one-pound/454 g clamshells per carton or flat). Strawberries are then transported to a central facility, where they are typically rapidly cooled to temperatures ranging from 2 to 5 °C before storage and distribution (Talbot & Chau, 1991).

Forced-air cooling is the most common method employed for rapid cooling of strawberries in processing facilities, and the typical cooling times for the pulp temperature to reach 3 °C ranges from 60 to 90 min (Ferreira, Brecht, Sargent, & Chandler, 2006). However, the final strawberry pulp temperature can vary widely due to the location within the cooling tunnel (Talbot & Chau, 1991), resulting in non-uniform cooling and a delay in achieving the desired temperature. In addition, moisture loss has been associated with forced-air cooling (Talbot, Brecht, & Sargent, 1995), contributing to reduced postharvest life of the strawberries (Mitchell, 1992a, 1992b). Hydrocooling is an alternate method that has been

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employed for cooling various produce items including snapbean, cucumber, apricot, plum, and cantaloupes (Brecht & Sargent, 1990; Dincer, Yildiz, Loker, & Gun, 1992; Gagliardi, Millner, Lester, & Ingram, 2003; Reina, Fleming, & Humphries, 1995). Compared to forced-air cooling, hydrocooling reduces the time to reach an internal strawberry pulp temperature of 3 °C from 60–90 min to 12–13 min (Ferreira et al., 2006). Additional benefits of hydrocooling are firmer and better-colored strawberries (Ferreira, Brecht, Sargent, & Aracena, 1994) and also the removal of dirt and field debris (Nunes, Brecht, Morais, & Sargent, 1995).

Previous studies have reported that a single contaminated fruit or vegetable can cross-contaminate multiple products during washing (Danyluk & Schaffner, 2011). Thus during hydrocooling, water contacting a single contaminated strawberry can potentially cross-contaminate multiple strawberries. In many operations, the hydrocooling water is recirculated to conserve energy and reduce cost. If this water becomes contaminated with microorganisms it can increase the risk of cross-contamination resulting in increased spoilage and potential foodborne illness. However, the addition of a sanitizer to the hydrocooling water may reduce the risk of cross-contamination. Chlorine sanitizers are the most commonly used treatments for postharvest cooling water (Beuchat, 1998). Several studies have shown that hydrocooling with HOCl-based chemistry reduces postharvest decay (Ferreira, Bartz, Sargent, & Brecht, 1996; Vigneault, Bartz, & Sargent, 2000), and could potentially reduce the microbial load on the surface of the strawberries, thereby enhancing food safety.

Since strawberries are currently field-packed and marketed unwashed, the risk of microbial contamination during cultivation, harvest and postharvest handling exists. One potential source of contamination most often addressed is worker hygiene, which is a critical component in the various Good Agricultural Practice (GAPs) programs implemented by the strawberry growers. Strawberries have been associated with only a single reported bacterial foodborne illness outbreak (FDA, 2011), though surveillance by FDA revealed that 1 out of 143 lots of imported strawberries into the US was contaminated with *Salmonella* (FDA, 2001). Additionally, fresh and frozen strawberries have also been implicated in three outbreaks of Hepatitis A (Dougherty, Barbour, Meyers, & Johnson, 1965; Hutin et al., 1999; Niu et al., 1992). These outbreaks, along with several studies reporting prolonged survival of human pathogens, including *Salmonella* spp. and *Escherichia coli* O157:H7 on intact and cut strawberries (Flessa, Lusk, & Harris, 2005; Knudsen, Yamamoto, & Harris, 2001), point to a potential of future outbreaks and a need for better sanitation and process control. The minimal processing of strawberries along with typically raw consumption, highlight the need for research in this area. Currently, no studies have been conducted to compare the survival of the human pathogen *Salmonella* on hydrocooled and forced-air cooled strawberries.

Rough postharvest handling of strawberries can cause punctures, cuts and other wounds resulting in the exposure of internal tissues to potential contamination. A previous study has shown that the survival of bacterial pathogens, including *Salmonella,* was significantly higher on punctured/wounded strawberries, compared to intact fruit (Knudsen et al., 2001). Although *Salmonella* was able to survive on cut strawberries for prolonged periods of time, it was unable to multiply presumably due to the low pH (3.2–4.1). Conversely, despite its relatively low pH, *Salmonella* can survive and multiply well on damaged and chopped tomatoes (Asplund & Nurmi, 1991; Knudsen et al., 2001). In addition to pH, other intrinsic factors including nutritional composition may also affect pathogen survival and growth in certain fruits and vegetables (Han & Linton, 2004; Han, Sherman, Linton, Nielsen, & Nelson, 2000; Nutt, Li, Woodward, Zabala-Diaz, & Ricke, 2003). However, limited information is available on whether the inability of *Salmonella* to multiply on cut strawberries is due to the low pH or due to other intrinsic factors. A clear understanding of the various factors affecting *Salmonella* survival is important in regulating these conditions for safe storage of fresh and processed strawberries.

The objectives of this study were to 1) compare *Salmonella* survival on inoculated, intact forced-air cooled or hydrocooled strawberries immediately after treatment, after storage for 7 d at 4 °C, followed by 24 h storage at 25 °C to simulate retail display; 2) investigate effect of 0, 100 and 200 ppm HOCl in hydrocooler water on *Salmonella* survival, and 3) evaluate the survival and growth of *Salmonella* in strawberry puree at several pH and temperature combinations.

2. Materials and methods

2.1. Salmonella strains and inoculum preparation

Five rifampicin (rif) (Fisher Scientific Springfield, NJ) resistant *Salmonella enterica* serovars, Newport ATCC 6962 (tomato outbreak), Javiana ATCC BAA-1593 (tomato outbreak), Enteritidis ATCC 4931, Typhimurium ATCC 13311, and Braenderup ATCC BAA-664 (tomato outbreak) were used as a cocktail in all experiments. The strains were stored in 15% glycerol at −80 °C and streaked on tryptic soy agar (TSA, Difco, BD, Sparks, MD) plates supplemented with 80 ppm rif. After growing the cultures at 37 °C for 24 h, a single colony from each plate was transferred to tryptic soy broth (TSB, Difco, BD) supplemented with 80 ppm rif and incubated overnight at 37 °C. A five-strain cocktail of the *Salmonella* strains was prepared by combining 5 ml of each culture, centrifuging (Sorvall RC-5B, DuPont Instruments) for 20 min at 4000 g, washing the pellets twice with buffered peptone water (BPW). The pellets were resuspended in either 25 ml of BPW for surface inoculation studies or distilled water for puree inoculation studies. Both yielded a ca. 9 log CFU/ml *Salmonella* cocktail. The cocktail was stored for up to 1 h on ice before inoculation.

2.2. Preparation of hydrocooling solution containing sodium hypochlorite

Forty gallon coolers (Igloo Products Corp, Katy, TX) were filled with 24 L of deionized water each and stored at 2 °C overnight. Required volumes of sodium hypochlorite (NaOCl) solution (5.65–6.00%; Fisher Scientific, Springfield, NJ) were added to the coolers to yield final concentrations of either 100 ± 2 or 200 ± 2 ppm active chlorine (HOCl). The concentration of HOCl in hydrocooling solutions was tested using AccuVac® DPD Free Chlorine ampules and a Hach DR/890 colorimeter (Hach Company, Loveland, CO). The pH of sanitizer solutions were adjusted to 6.8 ± 0.05 using 6 N HCl solution (Fisher Scientific, Springfield, NJ).

2.3. Inoculation of intact strawberries

Eight, one-pound (454 g) clamshells of strawberries (*Fragaria x ananassa*) were purchased from a local produce supplier on three separate days. Only unbruised and healthy fruit were used for the study. Intact strawberries were inoculated on undamaged surface with a single 10 μl spot of the *Salmonella* spp. cocktail resulting in approximately 7 log CFU/berry. Inoculated strawberries were allowed to air-dry in a biosafety hood for a minimum of 60 min, but no longer than 90 min. After drying, eight strawberries were then packed into each one-pint plastic clamshell (Highland Corp, Inc., Plant City, FL).