STUDENT BEST PRESENTATION AWARD (1ST PLACE)

Proc. Fla. State Hort. Soc. 125:280–281. 2012.

Fate of *Escherichia coli* O157:H7 and *Salmonella* on Full and Three-quarter Ripe Strawberries

THAO NGUYEN AND MICHELLE DANYLUK*

University of Florida, IFAS, Citrus Research and Education Center, Food Science and Human Nutrition, 700 Experiment Station Road, Lake Alfred, FL 33850

ADDITIONAL INDEX WORDS. *Salmonella, E. coli*, strawberries, handling, bruising

Strawberries (*Fragaria ×ananassa*) harvested at or near full ripe maturity for superior eating quality may be more susceptible to pathogen proliferation due to bruising. Little is known about pathogen behavior on strawberries. The objective of this study was to determine the fate of *Escherichia coli* O157:H7 and *Salmonella* on bruised and intact surfaces of freshly harvested strawberries of full and three-quarter red maturity. A five strain cocktail (7 log CFU/mL) of rifampicin resistant *Escherichia coli* O157:H7 or *Salmonella* was spot inoculated onto bruised and intact surfaces of strawberries and incubated at 2 °C and 15.5 °C for 24 hours and 7 days, respectively. Pathogens were enumerated on nonselective and selective media. Significant differences in pathogen decline was not found between full red and three-quarter red strawberries or between bruised and intact treatments at each time point. At 2 °C, *E. coli* O157:H7 and *Salmonella* populations declined by 0.9–1.1 log CFU/berry over 24 hours. Population declines of 1.4–2.4 log CFU/berry were observed for both pathogens when held over 7 days at 15.5 °C. Strawberry surfaces did not support growth of *E. coli* O157:H7 or *Salmonella*, regardless of maturity or bruising.

Strawberry (*Fragaria ×ananassa*) is a major crop with four to five millions in tons of production worldwide (USDA, 2010). The U.S. leads the world in strawberry production, with production values in 2011 estimated at U.S. $2.4 billion (USDA, 2010). Strawberry consumption in the U.S. has grown favorably over the last two decades, and is one of the top five fruits most consumed (USDA, 2011).

Strawberries are often consumed in a raw, unprocessed form (USDA, 2011), thus no thermal treatment is involved before consumption to kill pathogens if contaminated. Outbreaks implicating strawberries have resulted in over 300 illnesses and one death, and pathogens involved in outbreaks include parasites (*Cyclospora cayetanensis*), viruses (Heptatitis A and norovirus), and bacteria (*Escherichia coli* O157:H7 and *Salmonella*) (CDC, 2011; FDA, 2011). These outbreaks indicate that strawberries are suitable substrates for foodborne pathogens to survive and their consumption may cause infection.

Strawberries are classified as a non-climacteric fruit; berries allowed to fully ripen on the parent plant have the highest quality in terms of organoleptic properties. However, strawberry firmness is known to decrease extensively during ripening, and degree of firmness typically correlates with the maturity stage of the strawberry (Nunes et al., 2006). Postharvest losses due to strawberry bruising are a major concern (Ferreira et al., 2009), and bruising may also have adverse effects associated with pathogen proliferation. The fate of *E. coli* O157:H7 and *Salmonella* spp. on bruised surfaces of strawberries has not yet been established. The objective of the current study was to determine the fate of *E. coli* O157:H7 and *Salmonella* spp. on bruised and intact surfaces of strawberries at three-quarter red and full red maturity stages and at two temperatures, shipping (2 °C) and retail display (15.5 °C).

Materials and Methods

Escherichia coli O157:H7 strains and *Salmonella* serovars were obtained from the culture collection of Dr. Michelle Danyluk (University of Florida, Citrus Research and Education Center). Five *E. coli* O157:H7 strains were used, which were originally isolated from outbreaks involving juice, spinach, cantaloupe, lettuce, and alfalfa sprout. Five *Salmonella* serovars were used, including Agona, Poona, Montevideo, Newport, and Saint Paul. All strains were rifampicin resistant (80 µg/mL). Individual strains were streaked onto tryptic soy agar plates supplemented with rifampicin (TSAR; 80 µg/mL) and incubated overnight at 35 ± 2 °C. Isolated colonies were transferred into tryptic soy broth supplemented with rifampicin (TSBR; 80 µg/mL) for overnight incubation at 35 ± 2 °C. Two subsequent 24-h transfers were made into fresh TSBR. After harvesting and suspending cells in peptone water, equal volumes of each strain were combined to make either *E. coli* O157:H7 or *Salmonella* spp. cocktails.

Strawberries were freshly harvested from Dover, FL, sorted into two maturities stages (full and three-quarter red), and used the same day. To bruise the strawberry, a 32.6-g steel ball was dropped through a 20-cm PVC pipe, directly impacting the berry. Careful consideration was taken to bruise the strawberry without rupturing the epidermal surface. Twenty microliters of inoculum were used to inoculate bruised and intact surfaces. After 1 h drying, strawberries were transferred to whirl-pak bags and stored at 2 ± 2 °C and 15.5 ± 2 °C.

Samples stored at 2 ± 2 °C and 15.5 ± 2 °C were enumerated at time 0, 2, 5, and 24 h and on day 0, 1, 3, and 7, respectively. To enumerate, whirl-pak bags were filled with 30 mL of phosphate buffered saline (PBS; pH 7.2) and homogenized in a stomacher

Acknowledgments. The authors would like to thank the technical assistance of Lorrie Friedrich, Angela Valadez, and Brian Buzzie. This research was supported by the USDA CSREES Specialty Crop Research Initiative 2009-51181-05783.
*Corresponding author; phone: (863) 956-8654; email: mddanyluk@ufl.edu

(BAGMIXER® 400, Interscience Laboratories Inc., Weymouth, MA; 8 strokes/s) for 2 min. Appropriate serial dilutions were made in peptone water and spread plated on nonselective (TSAR) and selective media (*E. coli* O157:H7: Sorbitol MacConkey agar supplemented with rifampicin [SMACR; 80 µg/mL]; *Salmonella*: bismuth sulfite agar supplemented with rifampicin [BSAR: 80 µg/mL]). Nonselective and selective media were incubated at 35 ± °C for 24 and 48 h, respectively.

Two experiments were conducted with triplicate samples (n=6). Data were statistically analyzed using analysis of variance (ANOVA; JMP, software version 9.0.2; SAS Institute Inc., Cary, NC) to determine differences over time and among treatments. If differences were present, Tukey's Honest Significant Difference (HSD) test was performed to see which means were different. Differences were considered significant at $P \leq 0.05$.

Results and Discussion

Similar decreases were observed for *E. coli* O157:H7 and *Salmonella* populations, under all conditions, when held 24 h at 2 °C. Both *E. coli* O157:H7 and *Salmonella* had significant ($P \leq 0.05$) declines in populations of 0.9–1.1 log CFU/berry – except for the three-quarter bruised samples; where *Salmonella* populations remained stable over 24 h. Our data are in accordance with previous research conducted on pathogen fate on the surface of whole strawberries, where *E. coli* O157:H7 and *Salmonella* populations declined under refrigerated storage (5 °C) over 7 d (Knudsen et al., 2001).

Populations of *E. coli* O157:H7 and *Salmonella* on bruised and intact strawberries held at 15.5 ± 2 °C significantly declined ($P \leq 0.05$) by 1.5–1.8 log CFU/berry and 1.4–2.4 log CFU/berry, respectively, under all conditions, over 7 d of storage. Similar findings have been reported for pineapples, which are also considered a high acid fruit (pH < 4.0), where *E. coli* O157:H7 and *Salmonella* populations declined by 1.4 and 3.3 log CFU/g on fresh-cut surfaces (Strawn and Danyluk, 2010).

There were no significant ($P \geq 0.05$) differences in *E. coli* O157:H7 and *Salmonella* population declines between full and three-quarter red strawberries and between bruised and intact samples within treatments, at each time point, when held at 2 ± 2 °C and 15.5 ± 2 °C—except for *Salmonella* populations on day 3 when held at 15.5 °C, where three-quarter intact samples had significantly ($P \leq 0.05$) lower populations than on full red bruised samples. Strawberry firmness decreases as ripening progresses, however, firmness remains relatively unchanged from the three-quarter to the full red stage (Menager et al., 2004). No differences in population dynamics for both pathogens were expected between full and three-quarter samples, regardless of bruising. Although we saw some differences with *Salmonella* populations when held at 15.5 °C, these differences were not found throughout all experiments; these differences may be attributed to differences in strawberry to strawberry characteristics (e.g., surface characteristics and degree of firmness). Bruising that results in exposure of produce tissue has been documented to better support survival and sometimes growth of *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* (Dingman, 2000; Huff et al., 2012; Wei et al., 1995); however, no differences ($P \geq 0.05$) were found between bruised and intact strawberries in our study. This difference in results from previous studies is likely due to strawberry epidermal surfaces not rupturing during bruising of the strawberry.

The intact surface of fresh produce is an adverse environment for foodborne pathogens to proliferate, likely due to the lack of nutrients and moisture (Rodriguez-Romo and Yousef, 2006). *Escherichia coli* O157:H7 and *Salmonella* did not proliferate on strawberry surfaces, regardless of bruising; however, pathogen fate may be different if strawberries are bruised to a level that releases strawberry exudates thus should be investigated. No significant differences ($P \geq 0.05$) in pathogen dynamics on full or three-quarter red strawberries were observed; the current practice of harvesting strawberries with three-quarter red color can be amended to harvesting strawberries with full red color, to ensure maximum quality and flavor, and if bruising occurs, no additional food safety risks are imposed.

Literature Cited

Centers for Disease and Prevention (CDC). 2011. Foodborne outbreak online database (FOOD). 26 May 2012. <http://wwwn.cdc.gov/foodborneoutbreaks/>.

Dingman, D.W. 2000. Growth of *Escherichia coli* O157:H7 in bruised apple (*Malus domestica*) tissue as influenced by cultivar, date of harvest, and source. Appl. Enviro. Microbiol. 66:1077–1083.

Food and Drug Administration (FDA). 2011. Fresh strawberries from Washington County farm implicated in *E. coli* O157:H7 outbreak in NW Oregon. 6 Sept. 2012. <http://www.fda.gov/Safety/Recalls/ucm267667.htm>.

Ferreira, M.D., S.A. Sargent, J.K. Brecht, and C.K. Chandler. 2009. Strawberry bruising sensitivity depends on the type of force applied, cooling method, and pulp temperature. HortScience 44:1953–1956.

Huff, K., R. Boyer, C. Denbow, S. O'Keefe, and R. Williams. 2012. Effect of storage temperature on survival and growth of foodborne pathogens on whole, damaged, and internally inoculated jalapenos (*Capsicum annuum* var. *annuum*). J. Food Prot.75:382–388.

Knudsen, D.M., S.A. Yamamoto, and L.J. Harris. 2001. Survival of *Salmonella* spp. and *Escherichia coli* O157:H7 on fresh and frozen strawberries. J. Food Prot. 64:1483–1488.

Menager, I., M. Jost, and C. Aubert. 2004. Changes in physicochemical characteristics and volatile constituents of strawberry (cv. Cigaline) during maturation. J. Agr. Food Chem. 52:1248–1254.

Nunes, M.C.N., J.K. Brecht, A. Morais, and S.A. Sargent. 2006. Physicochemical changes during strawberry development in the field compared to those that occur in harvested fruit during storage. J. Sci. Food Agr. 86:180–190.

Rodriguez-Romo, L.A. and A.E. Yousef. 2006. Microbial stress adaptation and safety of produce, p. 95–114. In: G.M. Sapers, J.R. Gorny, and A.E. Yousef (eds.). Microbiology of fruits and vegetables. CRC Press, Boca Raton, FL.

Strawn, L.K. and M.D. Danyluk. 2010. Fate of *Escherichia coli* O157:H7 and *Salmonella* on fresh and frozen cut pineapples. J. Food Prot. 73:418–424.

United States Department of Agriculture–Agriculture and Marketing Resource Center (USDA–AgMRC). 2011. Commodity strawberry profile. 28 May 2012. <http://www.agmrc.org/commodities__products/fruits/strawberries/commodity_strawberry_profile.cfm>.

United States Department of Agriculture–Economic Research Services (USDA–ERS). 2010. U.S. strawberry industry (95003). 27 Mar. 2012. <http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1381>.

Wei, C.I., T.S. Huang, J.M. Kim, W.F. Lin, M.L. Tamplin, and J.A. Bartz. 1995. Growth and survival of *Salmonella* Montevideo on tomatoes and disinfection with chlorinated water. J. Food Prot. 58:829–836.