Handling & Processing Section

Proc. Fla. State Hort. Soc. 125:258–259. 2012.

Thermal Inactivation of Stationary Phase, Acid and Non-acid Adapted *Salmonella* Gaminara in Single-strength Orange Juice

Zeynal Topalcengiz and Michelle D. 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*, thermal inactivation, orange juice

Thermal death time parameters of stationary phase, acid and non-acid adapted pathogens, primarily as cocktails of multiple strains, have been studied in various food products. The objective of this study was to evaluate the thermal tolerance of stationary phase and acid adapted *Salmonella* Gaminara, associated with an orange juice outbreak, in orange juice. The strain was grown in tryptic soy broth, supplemented with 1% glucose for acid adaption, and inoculated (≈109 CFU/mL) into single-strength pasteurized orange juice without pulp. Juice was sealed into microcapillary tubes. Tubes were immersed into water baths at 55, 58, and 60 °C, removed at predetermined time intervals, and placed immediately onto ice. Survived *Salmonella* populations were enumerated on a tryptic soy agar supplemented with 0.1% sodium pyruvate. *D*-values for acid adapted and non-adapted *Salmonella* Gaminara at 55, 58, and 60 °C were 0.89 ± 0.06 and 0.80 ± 0.10 min, 0.32 ± 0.05 and 0.33 ± 0.09 min, 0.15 ± 0.08 and 0.14 ± 0.08 min, respectively. No statistically significant differences were seen between acid adapted and non-adapted strains at all temperatures. Acid adaptation did not provide an increase of thermal tolerance. Evaluation of individual strains using the microcapillary tubes will allow the impact of strain to strain variability on public health to be evaluated.

Foodborne pathogens remain a significant food safety concern in food production and processing. To inactivate these pathogens, thermal pasteurization is one of the most commonly applied and effective techniques (MacGregor and Farish, 2000). In thermal fruit juice pasteurization, the success of the process depends on the establishment of appropriate heat application times and temperatures. Validation of these parameters through elaborated studies with appropriate methodologies is essential.

Non-typhoidal *Salmonella* infections cause salmonellosis, whose symptoms include diarrhea, fever, vomiting, nausea, and abdominal pain. *Salmonella* can be introduced to orange juice in several ways during processing. Valero et al. (2010) indicated that high initial microbial population level (105 to 106 CFU/mL) in citrus juice may occur as a consequence of insufficient sanitation, poor hygiene practices, deteriorated fruits, and poor equipment sanitation, potentially increasing the risks of foodborne pathogens in juice. Danyluk et al. (2010) concluded that under typical postharvest handling practices, *Salmonella* population on the orange peel surface did not grow or penetrate into juice vesicles but could survive on the peel surface more than 45 d. The long-term survival of *Salmonella* on fruit surfaces may be a potential contamination risk for processing equipment.

Current juice pasteurization parameters describe "pertinent microorganisms" or the most heat resistant microorganisms of public health significance that are likely to occur in juice (FDA, 2001). Fruit juice processors target 5-log reduction of pertinent microorganisms during pasteurization, as described in the Juice HACCP rule (FDA, 2001); *Salmonella* is recognized as pertinent microorganism by FDA (2001). The thermal inactivation parameters of *Salmonella* serotypes have been studied in fruit juices, primarily as cocktails (Mak et al., 2001; Mazotta, 2001). The objective of this study was to evaluate the heat resistance of stationary phase and acid adapted *Salmonella* Gaminara in orange juice.

Material and Methods

One brand of single-strength, pasteurized orange juice without preservatives and pulp was used. *Salmonella* serotype Gaminara isolated from an orange juice outbreak in 1995 was obtained from the culture collection of Dr. Michelle D. Danyluk (University of Florida, Citrus Research and Education Center) (Harris et al., 2003). *Salmonella* Gaminara cells, stored at –80 °C, were streaked onto non-selective tryptic soy agar (TSA; Becton, Dickinson and Company, Sparks, MD). Agar plates were incubated at 35 ± 2 °C for 24 ± 2 h. One isolated colony of *S.* Gaminara was transferred into tryptic soy broth (TSB; Becton, Dickinson and Company) and incubated at 35 ± 2 °C for 18 h. One loopful of overnight growth was transferred into an additional tube of TSB and incubated at 35 ± 2 °C for 18 h. Acid adaptation of *S.* Gaminara cells was achieved by the addition of 1% glucose (10 g/L) to TSB (TSBG; Buchanan and Edelson, 1996), which induces the acid production of gluconic acid and the development of acid adaptation. Following incubation, cells were collected by centrifugation at 3000 × g for 10 min (Allegra X-12, Beckman Coulter, Fullerton, CA). The supernatant was removed and 10 mL of 0.1% peptone (Becton, Dickinson and Company) water was vortexed with the pellet to wash cells. *Salmonella* Gaminara cells were washed for three times, and resuspended in 5 mL orange juice to obtain the desired concentration of cells (10^8–10^{10} CFU/mL).

Acknowledgments. The authors would like to thank Loretta Friedrich, Gwen Lundy, Louis Martinez, and Brian Buzzie for technical assistance.

*Corresponding author; phone: (863) 956-8654; email: mddanyluk@ufl.edu

Sterile microcapillary tubes [1.5–1.8 (ID), 90 mm long; Kimble-Kontes, Vineland, NJ] with one sealed end were injected with 50 µL of inoculated orange juice aseptically via a sterile 20 gauge 4-inch deflected-point needle (Popper and Sons, Inc., Hyde Park, NJ), and a 1-mL syringe (Luer-Lok Tip, Franklin Lakes, NJ). The open end of the microcapillary tube was sealed with a Bunsen burner flame. Inoculated, sealed, microcapillary tubes were immersed into water baths (ECO-Line RE120; LAUDA Brinkmann, Lauda-Königshofen, Germany) at desired temperatures. At least seven time intervals were used to determine the thermal destruction time (*D*-values) of *S.* Gaminara at three temperatures, 55, 58, and 60 °C. Thermally treated microtubes were poured onto ice for immediate cooling before sterilization in 70% ethanol at room temperature for 1 min. Microtubes were crushed in 0.1% peptone water, resulting in an initial 1:100 dilution, for microbiological analysis. Six replicates were used for each time interval at all temperatures; duplicate samples of each time interval were examined in triplicate. Populations of *Salmonella* in thermally treated juice were determined by spread plating on TSA supplemented with 0.1% sodium pyruvate (Sigma-Aldrich, St. Louis, MO; TSAP).

Linear regression equations were used to calculate *D* and *z*-values. Statistical significances between *D*-values, and between non-adapted and acid adapted cells, were evaluated by analysis of covariance (ANCOVA; $P < 0.05$) in JMP software (Version 9.0.2 SAS® Institute Inc., Cary, NC).

Results and Discussion

To estimate *D*-values, population of survived cells (log CFU/mL) from thermal destruction treatment versus time (min) were plotted to obtain linear curve at tested temperatures. *D* and *z*-values of acid and non-acid adapted *S.* Gaminara, heated at 55, 58, and 60 °C are shown in Table 1. At all temperatures tested, non-adapted and acid adapted *S.* Gaminara have similar responses to thermal treatment. *D*-values for acid adapted and non-adapted cells at 55, 58, and 60 °C are 0.89 ± 0.06 and 0.80 ± 0.10 min, 0.36 ± 0.09 and 0.36 ± 0.07, and 0.20 ± 0.08 and 0.17 ± 0.017 min, respectively. *D*-values for acid adapted and non-adapted *S.* Gaminara at all temperatures are not statistically significantly different ($P > 0.05$). The *z*-values were calculated using the linear regression equations of plots of log *D*-values vs. temperature (°C) for each strain; *z*-values for acid adapted and non-adapted *S.* Gaminara are 7.5 and 7.1 with 0.99 R^2.

Salmonella have been linked to outbreaks from orange juice, and are considered the "pertinent microorganism" in orange juice (FDA, 2001); the elimination of *Salmonella* from orange juice is essential. The *D*-values reported here are lower than those reported in Mazotta (2001) at all temperatures; *z*-values reported in here are higher, indicating that *S.* Gaminara has higher thermal tolerance as temperature increases. Methodology differences, serotype variability, and use of cocktails are possible reasons for different thermal tolerance results among the studies.

Table 1. *D* and *z*-values of *Salmonella* Gaminara from linear regression equations. Non-adapted pathogens were grown in tryptic soy broth (TSB) and acid adapted pathogens were grown in TSB supplemented with 1% glucose (TSBG).

	D-value ± SD (min)			*z*-value
Adaptation	55 °C	58 °C	60 °C	(°C)
Non	0.80 ± 0.10[z]	0.33 ± 0.09[z]	0.14 ± 0.08[z]	7.1
Acid	0.89 ± 0.06[z]	0.32 ± 0.05[z]	0.15 ± 0.08[z]	7.5

[z]Different letters in rows indicate significant difference in *D*-values between acid adapted and non-adapted cells within each temperature and strain ($P < 0.05$)

Acid adaptation is generally believed to enhance the thermal tolerance of pathogens in fruit juice studies on strain cocktails, and has been demonstrated for *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in apple, orange, white grape juices (Mazotta, 2001), for *E. coli* O157:H7 in orange juice and apple cider (Ryu and Beuchat, 1998), and in cantaloupe and watermelon juice with individual strains (Sharma et al., 2005). Here, acid-adapted *S.* Gaminara were not more thermally tolerant at all temperatures in orange juice (pH 3.87 ± 0.01; Brix° corrected 12.24). At 58 °C, non-adapted cells were more, but not statistically significantly ($P > 0.05$), heat resistant than adapted cells. Similarly, acid adaptation did not improve *S.* Newport and *S.* Saintpaul thermal tolerance at 56 °C in mango or pineapple juice (Yang et al., 2012). The use of individual *Salmonella* serotypes provides us with a better understanding of thermal inactivation response differences among *Salmonella* serotypes. Evaluation of additional *Salmonella* serovars should be studied in orange and other juice types.

Literature Cited

Buchanan, R.L. and S.G. Edelson. 1996. Culturing enterohemorrhagic *Escherichia coli* O157:H7 in the presence and absence of glucose as a simple means of evaluating the acid tolerance of stationary-phase cells. Appl. Environ. Microbiol. 52:4009–4013.

Danyluk, M.D., P. Sood, L. Proano, L.M. Friedrich, and E. Exteberria. 2010. *Salmonella* does not penetrate citrus peel through natural light labels. Proc. Fla. State Hort. Soc. 123:217–219.

Harris, L.J., J.N.Farber, L.R. Beuchat, M.E. Parish, T.V. Suslow, E.H. Garrett, and F.F. Busta. 2003. Outbreaks associated with fresh produce: Incidence, growth, and survival of pathogens in fresh and fresh-cut produce. Compr. Rev. Food Sci. F. 2:78–141.

MacGregor, S.J. and O. Farish. 2000. Inactivation of pathogenic and spoilage microorganisms in a test liquid using pulsed electric fields. IEEE T. Plasma Sci. 28:144–149.

Mak, P.P., B.H. Ingham, and S.C. Ingham. 2001. Validation of apple cider pasteurization treatments against *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes*. J. Food Prot. 64:1679–1689.

Mazzotta, A.S. 2001. Thermal inactivation of stationary-phase and acid-adapted *Escherichia coli* O157:H7, *Salmonella*, and *Listeria* monocytogenes in fruit juices. J. Food Prot. 64:315–320.

Ryu, J.H. and L.R. Beuchat. 1998. Influence of acid tolerance responses on survival, growth, and thermal cross-protection of *Escherichia coli* O157H7 in acidified media and fruit juices. Intl. J. Food Microbiol. 45:185–193.

Sharma, M., B.B. Adler, M.D. Harrison, and L.R. Beuchat. 2005. Thermal tolerance of acid-adapted and unadapted *Salmonella, Escherichia coli* O157H7, and *Listeria monocytogenes* in cantaloupe juice and watermelon juice. Lett. Appl. Microbiol. 41:448–453.

U.S. Food and Drug Administration. 2001. Federal Register Final Rule – 66 FR 6137, 19 Jan. 2001: Hazard analysis and critical control point (HACCP); Procedures for the safe and sanitary processing and importing of juice. Accessed 29 Sept. 2011. <http://www.fda.gov/Food/FoodSafety/HazardAnalysisCriticalControlPointsHACCP/JuiceHACCP/ucm073594.htm>.

Valero, M., V. Micol, D. Saura, N. Munoz, J. Lorente, and N. Marti. 2010. Comparison of antimicrobial treatments for citrus juices. p. 63-74. In: H. David (ed.). Plant Sci. Rev. 2010, S.R. Nova Pvt. Ltd., Bangalore, India.

Yang, Y., J.H. Teo, W.S. Bang, and H.G. Yuk. 2012. Changes in acid and heat resistance of *Salmonella* Newport and *Salmonella* Saintpaul stored in mango and pineapple juices. J. Food Safety 32(3):311–317.