

## Research Note

# Effects of Combined Heat and Acetic Acid on Natural Microflora Reduction on Cantaloupe Melons

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### ABSTRACT

Produce is an important source of nutrients and phytochemicals, which is important in a healthy diet. However, perishable fresh produce has caused recent outbreaks of foodborne diseases. High level of nutrients and water activity, direct contact with soil, and lack of thermal procedures during primary processing make fresh produce a potential food safety hazard. Fruits and vegetables with rough surfaces can harbor microorganisms and support their multiplication, increasing the risk of this hazard. This study evaluated the effects of extreme thermal processes combined with acetic acid on natural microflora reduction on cantaloupe melons. Melons from a local supermarket were assigned into five treatment groups: control, water at 25°C, water at 95°C, 5% acetic acid at 25°C, and 5% acetic acid at 95°C. Four skin samples were obtained from each melon, separately stomached for 2 min with 0.1% peptone water, and serially diluted. Aerobic plate counts (APC) of dilutions were determined. Statistical analysis (least significant difference–based analysis of variance) showed that there were no significant ( $P > 0.05$ ) differences in APC among control, water at 25°C, and 5% acetic acid at 25°C. Thermal treatments with water at 95°C, and 5% acetic acid at 95°C, were both significantly ( $P < 0.05$ ) more effective in APC reduction than were nonthermal treatments, but were not significantly different from each other. Results indicated that a thermal water immersion intervention in primary processing of fresh melons can result in a 3-log reduction of natural microflora surface contamination, but 5% acetic acid will not significantly augment this reduction.

Fresh produce is an important source of vitamins, minerals, dietary fiber, and various phytochemicals that play an important role in a healthy diet. Fruits and vegetables are generally recognized as a high source of nutrients, and low in fat, calories, and sodium (24). The U.S. Department of Agriculture dietary guidelines recommend 2.5 to 6.5 cups of fresh produce per day, based on different caloric needs (12). However, the perishable nature of fresh produce and the fact that most fresh produce is consumed primarily without thermal processing procedures have raised increasing concerns of foodborne diseases in recent years. At the time of arrival to packaging sheds, fresh produce on average have a microbial load of  $10^4$  to  $10^6$  CFU/cm<sup>2</sup> (10). From 1993 to 2002, a number of reported fresh produce–related outbreaks have increased from 12 to 53 per year in the United States (20). Among various fresh produce, cantaloupe melons have been associated with some recent outbreaks. The 2008 outbreak of salmonellosis from cantaloupe melons that were distributed nationwide in the United States and Canada is a recent example (5).

Cantaloupe melons have a rough surface that can harbor the growth of microorganisms (26), have direct contact with soil during agricultural production, and are consumed commonly without thermal processing. Although

cantaloupe rinds are not edible, bacterial transfer from contaminated rind to fresh-cut pieces can easily occur (31). Cantaloupe flesh is also rich in sugar and other nutrients, and has nearly neutral pH that can support the growth of a wide array of microorganisms including the pathogens of public health concern (15). Washing contaminated melons with water is not a sufficient approach for reduction of microbial load. Ukuku and Sapers (31) showed that washing the contaminated melons with water results in no significant reduction of microbial load. Similar results were obtained in the study of Parnell et al. (18); effects of washing practices were compared in honeydew melons (smooth rind) and cantaloupe melons (complex rind), where it was shown that washing and scrubbing was less effective for reduction of inoculated pathogens on cantaloupes relative to honeydew melons. In addition, Alvarado-Casillas et al. (2) showed that while water wash reduces the microbial load of bell peppers to a significant level, it does not yield similar results for cantaloupe melons. Low efficacy of commercially available sanitizers of cantaloupe melons, such as chlorine and hydrogen peroxide, has also been demonstrated in recent studies (29, 31). This was even more noticeable when intervention was targeted for reduction of natural microflora of cantaloupes or when bacterial inoculation occurred more than 24 h prior to washing interventions (14, 31). In the present study, heat and acetic acid interventions were applied as alternative approaches for reduction of microbial

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load of cantaloupe melons. It has been known for a long time that pathogenic bacteria are more fastidious in their relationships to low pH environments than are most spoilage bacteria, molds, and yeasts (16). Food grade acetic acid is generally recognized as safe as a food ingredient; a 5% aqueous acetic acid solution is commonly known as vinegar, which has been used for decontamination of various products. Acetic acid interventions for inactivation and/or detachment of natural microflora of chicken and beef carcasses, as examples, have been suggested as a primary processing application (7, 9, 22). Thermal interventions of animal origin products (6, 8, 25) and fresh produce (3, 23, 26, 30) have also been extensively studied in commercial food processing.

The objective of the present study was to determine the effectiveness of postharvest processing interventions in reduction of natural surface microflora of cantaloupe melons. For this purpose, water and acetic acid immersion treatments at two temperatures of 25 and 95°C (total of four treatments) were compared with a control treatment. The hypothesis tested was that the acetic acid and/or heat can enhance microbial reduction on skin surface of cantaloupe melons. The study was designed to approximate realistic commercial processing applications.

## MATERIALS AND METHODS

**Experimental design.** The study was designed to evaluate the effects of hot water and dilute acetic acid immersion interventions on reduction of natural microflora of cantaloupe melons by the following treatments: (i) immersion in water at 25°C for 1 min, (ii) immersion in boiling water (95°C) for 1 min, (iii) immersion in 5% acetic acid at 25°C for 1 min, (iv) immersion in 5% acetic acid (95°C) for 1 min, and (v) no immersion intervention (control). After treatments, four samples were aseptically obtained from the following sites: site 1, the location of connection of stem (stem scar); site 2, the least visibly contaminated area; site 3, the rounder, thicker rind at the bottom; and site 4, the most visibly contaminated area of each melon. Each rind sample was separately prepared for microbial evaluations. Effects of each treatment intervention were determined in three (for treatments i, iii, and v) and four (for treatments ii and iv) separate experiments with duplicate analysis for each treatment. Statistical power for this experiment, based on within group standard deviations, and number of samples per experiment was 96.2%. With inclusion of four excised sample rinds from each melon, the statistical power was 99.7%.

**Sampling procedure.** Cantaloupe melons (*Cucumis melo* L. var. *reticulatus*) were purchased from a local supermarket, individually wrapped in paper bags, and left at 25°C for no more than 24 h before the intervention. Melons were uniform in size and were free of visible spoilage. After each treatment, circular rind samples (diameter of 3.6 cm, surface area of 10.2 cm<sup>2</sup>) were excised with a sterile brass cutting cylinder and aseptically removed with sterile forceps and scalpel. This sampling procedure was applied to investigate the evenness of contamination on surface of cantaloupe melons and for reporting the contamination per calculated centimeter squared of cantaloupe rind.

**Treatment interventions.** Five liters of immersion fluid in a stainless steel pot was used for treatments at 25°C. The fluid was heated to boil (95°C, at an elevation of 1.6 km), measured with a

mercury thermometer, and maintained boiling on a hot plate for heat treatments. With sterile metal tongs, the whole melons were individually immersed in the fluid for 1 min, removed, and drained rapidly. To approximate the commercial application, commercial unflavored 5% acetic acid (Kroger Co., Cincinnati, OH), at pH 2.8 ± 0.4 and deionized water at pH 7.1 ± 0.1, was used for treatment fluids.

**Microbiological analyses.** Excised rind samples were individually placed in a bag with 100 ml of sterile 0.1% peptone (Fisher Scientific, Fairlawn, NJ) diluent, stomached for 2 min at 230 rpm (Stomacher 400, Seward, NY), serially diluted to 10<sup>-5</sup>, and pour plated in duplicate. Microbial enumeration was performed with Trypticase soy agar (Difco, Becton Dickinson, Sparks, MD) with 0.6% yeast extract (Difco, Becton Dickinson). Plates were incubated at 35°C for 72 to 96 h. After incubation, aerobic plate counts (APC) of each sample were reported as log transformation of number of CFU per square centimeter of cantaloupe surface rind. Detection limit of these experiments was below 10 and above 25,000,000 CFU/cm<sup>2</sup> of cantaloupe rind.

**Statistical analyses.** Excel 2003 (Microsoft Corp., Redmond, WA) and SAS 9.2 (SAS, Inc., Chicago, IL) were used for data collection, power analysis, transformations, and descriptive and inferential statistical analyses. The main analysis of the study was the least significant difference-based analysis of variance (ANOVA) test to determine the effects of treatments (independent variable) on colony counts (dependent variable). Dunnett's procedure and linear contrast methods were used to determine the differences among control treatment and immersion interventions, and to compare the effects of heated and unheated treatments, respectively. *P* values less than 0.05 were considered as a significant difference.

## RESULTS

APC of the control, water at 25°C, acetic acid at 25°C, water at 95°C, and acetic acid 95°C samples were 6.7, 6.3, 6.0, 3.7, and 3.3, respectively. Results of least significant difference-based ANOVA paired comparisons (Table 1) showed that CFU of samples immersed in water and acetic acid at boiling temperature were significantly (*P* < 0.05) lower than the control, water at 25°C, and acetic acid at 25°C were. However, there were no significant (*P* > 0.05) differences among microbial load of control samples, water at 25°C, and acetic acid at 25°C, as well as between water at 95°C and acetic acid at 95°C. This indicates that the reduction in natural microflora on cantaloupe surfaces was due mainly to thermal treatments rather than 5% acetic acid.

Similar results were concluded based on Dunnett's procedure. Results of this test, which yield a more conservative comparison of treatment interventions with control samples, showed that while there is no significant (*P* > 0.05) difference among control samples and samples treated by water and acetic acid at 25°C, the difference is significant (*P* < 0.05) among the control samples and samples treated by water and acetic acid at 95°C. Results of pooling data from water at 25°C with water at 95°C and comparing them with pooled acetic acid treatments at 25°C and 95°C showed no significant (*P* = 0.61) differences between two pooled groups, indicating that the reduction in microbial load is due mainly to thermal treatments. Also, comparison of samples treated at 95°C by both water and

TABLE 1. Influences of thermal and acetic acid interventions on surface natural microflora of cantaloupe melons

Condition	Rind APC (log CFU/cm <sup>2</sup> ) <sup>a</sup>				
	Sampling site 1	Sampling site 2	Sampling site 3	Sampling site 4	Avg
Control	6.8 ± 2.1 a A <sup>b</sup>	6.3 ± 2.3 a A	6.8 ± 1.7 a A	7.0 ± 1.5 a A	6.7 ± 1.9 a A
Water at 25°C	7.1 ± 0.6 a A	5.2 ± 0.7 a B	6.2 ± 0.8 a A	6.7 ± 0.7 a A	6.3 ± 0.7 a A
Water at 95°C	4.2 ± 0.9 b A	3.3 ± 1.3 b A	3.7 ± 0.7 b A	3.5 ± 1.0 b A	3.7 ± 1.0 b A
Acetic acid at 25°C	6.5 ± 0.4 a A	5.9 ± 0.6 a A	5.1 ± 0.4 a B	6.3 ± 0.4 a A	6.0 ± 0.5 a A
Acetic acid at 95°C	3.1 ± 0.6 b A	2.7 ± 0.7 b A	3.7 ± 0.5 b A	3.7 ± 0.4 b A	3.3 ± 0.6 b A

<sup>a</sup> Values represent means ± standard deviations of three or four separate experiments with duplicate analysis for each treatment. Sampling sites: 1, location of stem connection (stem scar); 2, least visibly contaminated area; 3, rounder, thicker rind at the bottom; 4, most visibly contaminated area of each melon.

<sup>b</sup> Values within a column followed by different lowercase letters, and values within a row followed by different uppercase letters are significantly ( $P < 0.05$ ) different.

acetic acid with samples treated at 25°C by both water and acetic acid indicated that microbial load in the pooled group of 95°C is significantly ( $P = 0.02$ ) lower than the pooled group of 25°C is.

Microbial counts of four excised samples of each melon, in most cases, were not significantly ( $P > 0.05$ ) different from each other. However, with water at 25°C and acetic acid at 25°C, samples excised from least visibly contaminated area (sampling site 2), and rounder, thicker rind at the bottom (sampling site 3) showed lower microbial counts, respectively ( $P < 0.05$ ).

## DISCUSSION

Primary processing of cantaloupe melons in the United States is different in each state. Californian grown cantaloupes are field packed without any aqueous processing procedures, whereas Georgian grown melons are brought to a shed, washed, and packed (1, 11). Several recent studies have shown a variety of common sanitizers in primary processing of fresh produce were not capable of reduction of microbial load on the melons to an acceptable level (4). Hydrogen peroxide and chlorine, common commercial sanitizers of fresh produce (29), have shown to have low efficacy in reduction of natural and inoculated microorganisms on the surface of cantaloupe melons, particularly when bacterial attachment occurs more than 24 h prior to washing intervention. Ukuku and Sapers (31) demonstrated that efficacy of 1,000 mg/liter solution of chlorine and 5% hydrogen peroxide solutions for detachment and/or inactivation of inoculated *Escherichia coli* considerably decreased as the interval between inoculation and treatment increased. Similar results were observed by Solomon et al. (23), wherein reductions in efficacy of sanitizer on melons were reported when the interval between inoculation and treatment increased. In study of Fan et al. (14), it is reported that washing the melons in chlorinated water (180 mg/liter) was not a sufficient intervention for detachment and/or inactivation of inoculated *Salmonella* on cantaloupe rinds. In addition, Akins et al. (1) showed that as concentration of organic material in washing fluid increases, efficacy of chlorinated water decreases. In the present study, heat and acetic acid interventions were applied as alternative approaches for reduction of microbial load on melons. Five

percent acetic acid at both 25 and 95°C showed no additional reduction of natural microflora of the samples, compared with 25 and 95°C water treatments, respectively. This low efficacy of acetic acid interventions was similar to low effectiveness of other chemical sanitization methods of cantaloupes in reported studies in which intervention of chlorine and hydrogen peroxide showed low ability for sanitization of natural microflora and inoculated pathogens when bacterial inoculation occurred more than 24 h prior to washing intervention. Immersion of the melons in 25°C fluid for 1 min in our study also did not result in reduction of microbial load on the melons. Similar results were reported in study by Sapers et al. (19), which showed washing in ambient and 50°C water was ineffective for reduction of microbial load on cantaloupe melons. Immersion of melons in 95°C water in our study showed a significant reduction in natural microbial load of the melons. This treatment resulted in a 3-log reduction of natural surface microflora of the melons. Similarly, Annous et al. (4) demonstrated that surface pasteurization of cantaloupe melons in water can result in greater than 5-log reductions of inoculated pathogens on surface of melons. Ukuku et al. (25, 26, 30) and Solomon et al. (23) also showed significant reduction of inoculated pathogens and natural microflora on cantaloupe melons, due to hot water interventions. Effects of hot water interventions on quality attributes of treated melons have also been investigated in recent studies. Solomon et al. (23), by experimental and simulation approaches, showed that internal temperatures of hot water-treated melons did not increase rapidly, compared with surfaces of the melons. Fan et al. (13) also reported no adverse sensory and quality effects of fresh-cut samples prepared from hot water-treated melons. Similarly, Selma et al. (21) and Lamikanra et al. (17) reported no adverse sensory and quality loss due to hot water treatment of melons.

Immersing cantaloupe melons in water at elevated temperature appears to be an effective primary processing method, compared with acetic acid and other reported chemical alternatives. Reduction of microbial load due to a 1-min immersion in heated water in present study was more than immersion of melons for 1 min in unheated 70% ethanol, as reported in previous studies (27, 28). However, application of heat for pasteurization of melons requires

more consideration. If the hot water-treated melons are not processed or consumed immediately after process, sanitized melons can easily become recontaminated with pathogens of public health concern, due to a reduced level of natural microflora. In case of prolonged refrigeration of hot water-treated melons, psychrotrophic pathogens such as *Listeria monocytogenes* and *Yersinia enterocolitica* could multiply to levels of concern, due to reduced natural microflora of hot water-treated melons.

## REFERENCES

- Akins, E. D., M. A. Harrison, and W. Hurst. 2008. Washing practices on the microflora on Georgia-grown cantaloupes. *J. Food Prot.* 71: 46–51.
- Alvarado-Casillas, S., S. L. Sanchez, O. R. Garcia, N. M. Gonzales, and A. Catillo. 2007. Comparison of rinsing and sanitizing procedures for reducing bacterial pathogens on fresh cantaloupes and bell peppers. *J. Food Prot.* 70:655–660.
- Annous, B. A., A. Burke, and J. E. Sites. 2004. Surface pasteurization of whole fresh cantaloupes inoculated with *Salmonella* Poona or *Escherichia coli*. *J. Food Prot.* 67:1876–1885.
- Annous, B. A., E. B. Solomon, P. H. Cooke, and A. Burke. 2005. Biofilm formation by *Salmonella* spp. on cantaloupe melons. *J. Food Saf.* 25:276–287.
- Anonymous. 2008. Enforcement report, U.S. Food and Drug Administration. Available at: <http://www.fda.gov/bbs/topics/ENFORCE/2008/ENF01065.html>. Accessed 14 February 2009.
- Avens, J. S., S. N. Albright, A. S. Morton, B. E. Prewitt, P. A. Kendall, and J. N. Sofos. 2002. Destruction of microorganisms on chicken carcasses by steam and boiling water immersion. *J. Food Control* 13:445–450.
- Avens, J. S., P. Clayton, D. K. Jones, R. Bolin, W. Lloyd, and D. Jankow. 1996. Acetic acid spray ineffective on beef carcasses with low bacteria counts. *Lebensm.-Wiss. Technol.* 29:28–32.
- Avens, J. S., and A. S. Morton. 1999. Virtual eradication of psychrophilic and mesophilic bacteria on turkey carcass tails by steam and boiling water immersion. *J. Appl. Poultry Sci.* 8: 205–213.
- Bell, M. F., R. T. Marshall, and M. E. Anderson. 1986. Microbial and sensory tests of beef treated with acetic and formic acids. *J. Food Prot.* 49:207–210.
- Beuchat, L. R. 1996. Pathogenic microorganisms associated with fresh produce. *J. Food Prot.* 59:204–216.
- Deann, A. E., M. A. Harrison, and W. Hurst. 2008. Washing practices on the microflora on Georgia-grown cantaloupes. *J. Food Prot.* 71:46–51.
- Department of Health and Human Services, U.S. Department of Agriculture. 2005. Dietary guidelines for Americans: Department of Health and Human Services and U.S. Department of Agriculture. Available at: <http://www.usda.gov>. Accessed 14 February 2009.
- Fan, X., A. B. Annous, J. C. Beaulieu, and J. E. Sites. 2008. Effect of hot water surface pasteurization of whole fruit on shelf life and quality of fresh-cut cantaloupe. *J. Food Microbiol. Saf.* 73:91–98.
- Fan, X., B. A. Annous, L. Keskinen, H. Zhuang, and J. M. Mattheis. 2009. Effect of chemical sanitizers of whole fruit on microbial population and quality of fresh-cut cantaloupe, abstr. 204-04. Abstr. 2009 Annu. Meet. Inst. Food Technol., Anaheim, CA, 6 to 9 June.
- Hanning, I. B., J. D. Nutt, and S. C. Ricke. 2009. Salmonellosis outbreaks in the United States due to fresh produce: sources and potential intervention measures. *Foodborne Pathog. Dis.* 6:635–648.
- Jay, M. J. 1992. Modern food microbiology, p. 37–44. Chapman and Hall, Inc., New York.
- Lamikandra, O., and M. A. Watson. 2007. Mild heat and calcium treatment effects on fresh-cut cantaloupe melons during storage. *Food Chem.* 102:1383–1388.
- Pamell, T. L., L. J. Harris, and T. V. Suslow. 2005. Reducing *Salmonella* on cantaloupes and honeydew melons using wash practices applicable to postharvest handling, food service, and consumer preparation. *Int. J. Food Microbiol.* 99:59–70.
- Sapers, G. M., R. L. Miller, V. Pilizota, and A. M. Matrazzo. 2001. Antimicrobial treatments for minimally processed cantaloupe melon. *J. Food Microbiol. Saf.* 66:345–349.
- Sapers, G. M., E. B. Solomon, and K. R. Mathews. 2009. The produce contamination problem, causes and solutions, p. 5–6. Elsevier, Inc., Burlington, MA.
- Selma, M. V., A. M. Ibanez, A. Allende, M. Cantwell, and T. Suslow. 2008. Effect of gaseous ozone and hot water on microbial and sensory quality of cantaloupe and potential transference of *Escherichia coli* O157:H7 during cutting. *J. Food Microbiol.* 25:162–168.
- Smulders, F. J. M., and G. G. Greer. 1998. Integrating microbial decontamination with organic acids in HACCP program for muscle foods: prospects and controversies. *Int. J. Food Microbiol.* 44:149–169.
- Solomon, E. B., L. Huang, J. E. Sites, and B. A. Annous. 2006. Thermal inactivation of *Salmonella* on cantaloupes using hot water. *J. Food Microbiol. Saf.* 71:25–30.
- Su, L. J., and L. Arab. 2006. Salad and raw vegetable consumption and nutritional status in the adult US population: results from the third national health and nutrition examination survey. *J. Am. Diet. Assoc.* 106:1394–1404.
- Tompkins, N. M., J. S. Avens, P. A. Kendall, and M. D. Salman. 2008. Effects of boiling water carcass immersion on aerobic bacteria counts of poultry skin and processed ground poultry meat. *Zoonoses Public Health* 55:235–241.
- Ukuku, D. O. 2006. Effect of sanitizing treatments on removal of bacteria from cantaloupe surface, and re-contamination with *Salmonella*. *J. Food Microbiol.* 23:289–293.
- Ukuku, D. O., and W. Fett. 2002. Behavior of *Listeria monocytogenes* inoculated on cantaloupe surfaces and efficacy of washing treatments to reduce transfer from rind to fresh-cut pieces. *J. Food Prot.* 65:924–930.
- Ukuku, D. O., W. Fett, and G. M. Sapers. 2004. Inhibition of *Listeria monocytogenes* by native microflora of whole cantaloupe. *J. Food Saf.* 24:129–146.
- Ukuku, D. O., V. Pilizota, and G. M. Sapers. 2001. Influence of washing treatment on native microflora and *Escherichia coli* population of inoculated cantaloupes. *J. Food Saf.* 21:31–34.
- Ukuku, D. O., V. Pilizota, and G. M. Sapers. 2004. Effect of hot water and hydrogen peroxide treatments on survival of *Salmonella* and microbial quality of whole and fresh-cut cantaloupes. *J. Food Prot.* 67:432–437.
- Ukuku, D. O., and G. M. Sapers. 2001. Effects of sanitizer treatments on *Salmonella* Stanley attached to the surface of cantaloupe and cell transfer to fresh-cut tissues during cutting practices. *J. Food Prot.* 64: 1286–1291.