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Reducing Transfer of *Salmonella* and Aerobic Mesophilic Bacteria on Melon Rinds Surfaces to Fresh Juice by Washing With Chlorine: Effect of Waiting Period Before Refrigeration of Prepared Juice

Dike O. Ukuku[1]*, *Sudarsan Mukhopadhyay*[2] *and Modesto Olanya*[1]

[1] *Food Safety Intervention Technologies Research Unit, U.S. Department of Agriculture, Wyndmoor, PA, United States,*
[2] *Residue Chemistry and Predictive Microbiological Research Unit. U.S. Department of Agriculture, Agriculture Research Service, Eastern Regional Research Center, Wyndmoor, PA, United States*

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***Correspondence:**
Dike O. Ukuku
dike.ukuku@ars.usda.gov

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Cantaloupes, honeydew melons and watermelons inoculated with *Salmonella* cocktail at 4.5, 3.8, and 3.2 $\log_{10}$ CFU/cm^2, respectively, were sanitized with 200 ppm chlorine before rinds removal, cutting, and juice preparation. Efficacy of 200 ppm chlorine in reducing transfer of *Salmonella*, aerobic mesophilic bacteria, yeast and mold, and *Pseudomonas* from the melon surfaces to freshly prepared fruit juice was investigated including the effect of waiting period before refrigeration of the juices. The melon juice filtrates were refrigerated immediately or stored at room temperature (~22°C) for 3 and 5 h before refrigeration. Average *Salmonella* bacteria recovered in fresh melon juice prepared from unwashed whole cantaloupes, watermelon and honeydew melons was 1.4, 0.5, and 0.4 $\log_{10}$ CFU/ml, respectively. Juices from unwashed inoculated melons had the highest bacterial populations and storage at an abusive temperature of 10°C led to proliferation. Holding these juices at room temperature for 5 h before refrigeration allowed *Salmonella* bacteria to increase by 0.5–0.8 log in cantaloupe juice and 0.3–0.5 log in watermelon and honeydew juices. No *Salmonella* bacteria was determined in fresh juices prepared from melons washed with chlorinated water. The results of this study showed that washing melons with 200 ppm chlorine before juice preparation and immediately refrigerating the juice will minimize the chances of *Salmonella* proliferation.

Keywords: storage, temperature, watermelon, honeydew, cantaloupe, juices, *Salmonella*

INTRODUCTION

Consumers are becoming health conscious and are demanding food products that are fresh and safe, and fresh cut fruits and juices are among the foods that are in high demand. Produce processors, and food manufacturers are responding to consumers' demand for safe juice or fruits which may be improperly processed and/or contaminated with human bacterial pathogens. Prepared fresh-cut melon in the supermarket is becoming very popular with the US consumer due to the benefits of a diet rich in fruits and vegetables (Ukuku et al., 2017). Foodborne illnesses due to consumption of fresh fruit and vegetable produce contaminated with bacteria is food

safety concern in the United States. Several researchers have documented incidences of foodborne illnesses associated with consumption of fresh fruit and vegetable produce (Batz et al., 2004; CDC, 2011). The authors reported that consumption of bacteria contaminated fresh fruit and vegetable produce leads to 1.2 million illness per year, leading to 7,100 hospitalizations, and 134 human deaths. The authors also concluded that the cost associated with these illnesses was $1.4 billion per year (Batz et al., 2004; CDC, 2011). The reason for bacterial contamination of produce surfaces are mostly due to frequent contact with soil, insects, animals, or humans during growing or harvesting and in the processing plant (Castillo et al., 2004; Heaton and Jones, 2007; Ukuku et al., 2012). Therefore, surface microflora of fruits and vegetables is very important to fresh juice producers, processors and consumers when considering microbial food safety of fresh-cut fruits and or fruit juices. In a survey of imported 151 cantaloupe melons from Mexico, Costa Rica and Guatemala, FDA indicated that 5.3% of these melons was positives for *Salmonella* and 2% for *Shigella* (FDA, 2016) and several salmonellosis outbreaks has been associated with consumption of contaminated fresh-cut melon pieces (Ukuku, 2004) and fresh fruits and vegetable juices (Danyluk et al., 2012). Survival and growth of bacteria on fresh-cut melons was attributed to the nutrient composition of fresh-cut melons (FDA, 2015).

Methods of washing treatments for reducing bacterial populations on melon rind surfaces have been reported (Ukuku and Fett, 2004; Parnell et al., 2005) and the amount of chlorine in the wash water is usually up to 200 ppm as suggested in the Federal regulations (21 CFR-Part 173 and 21 CFR Part 178 permit). The chlorine wash treatments do not inactivate all bacterial populations on produce surfaces and can only achieve ∼2–3 log reductions depending on type and method of application. Also, there is a concern of potential formation of harmful by-products by chlorine (CDC, 2016) prompting the need for alternative antimicrobial wash treatments. Prepared fresh-melon juice is becoming very popular with the U.S. due to the health benefits associated with drinking unpasteurized raw fresh juices. To date, most of the fresh-juices are prepared at home, bars, supermarkets and or by regional distributors.

Fresh juices prepared at home may be left at room temperature for several hours before consumption or refrigeration for later use. Bacterial populations on fruits surfaces that transferred to the juice during juice preparation can survive and grow when such juice is left standing at room temperature for hours before consumption. Previously, we reported transfer, survival and growth of *Salmonella* Poona from cantaloupe rind surfaces to fresh-cut pieces during fresh-cut preparation and later reported survival of this pathogen in fresh-cut pieces during storage at room or refrigeration temperatures (Ukuku, 2004). Similarly, there is limited information on the effect of soluble solids of fruit pulps or juices on the survival and growth of yeast and mold, aerobic mesophilic bacteria including human pathogenic bacteria. In this study, the impact of soluble solids of freshly prepared melon juice and the effect of time after preparation and before refrigeration and storage temperature at 5, 10, and 25°C on bacterial populations associated with each juice was investigated. The information on transfer and survival of spoilage and *Salmonella* bacteria in fresh cantaloupe, watermelon and honeydew juices and the effect of waiting period before refrigeration as presented in this study is aimed to provide the regulatory industry a base line data that should guide consumers, fresh juice industry and consumers in good manufacturing practices (GMP's) procedures for enhancing the microbial food safety of freshly prepared fruit juice.

MATERIALS AND METHODS

Preparation of Bacterial Inoculum

Salmonella strains: *Salmonella* Newport 02-216, sprout related outbreak; *Salmonella* Poona 418, meat; *Salmonella* Hidalgo 02-517-2, cantaloupe; *Salmonella* Typhimurium 45, cantaloupe; *Salmonella* St. Paul FSIS 039, cantaloupe used in this study were maintained on Brain Heart Infusion Agar (BHIA, BBL/Difco, Sparks, MD) slants at 4°C. Each culture was grown on 5 ml Brain Heart Infusion Broth (BHIB, BBL/Difco) with two successive transfers by loop inocula to a new 5 ml BHIB. A final transfer of 0.2 ml from each strain was made into 20 ml BHIB with incubation at 36°C for 18 h under static conditions. The bacterial cells were centrifuged (10,000 × g, 10 min) at 4°C, and the cell pellets were harvested and washed in phosphate buffer saline (PBS) (BBL/Difco). The final bacterial concentration of each *Salmonella* strain was 2.5 × 10^9 CFU/ml, and were used to prepare a cocktail of 6.8 log CFU/ml *Salmonella* inoculum in 3 L of deionize distill water (ddH_2O).

Inoculation of Melons With *Salmonella* Bacteria

Cantaloupes (Western shippers, 1,648–1,672 g), honeydew (Cucumis melo, 1,663–1,678 g), and watermelons (Citrullus lantus, 1,925–1,976 g) purchased from a local distributor were placed inside refrigerator at 4°C until used. Melons were removed from refrigerator and left at room temperature (22°C) for 18–20 h before inoculation with *Salmonella* bacteria prepared above. For inoculation treatment, two melons each were submerged in 3 L of 6.8 log CFU/ml bacterial inoculum stated above and agitated by stirring with a glove-covered hand for 5 min to ensure uniform inoculation (Ukuku et al., 2016). A total of ten melons each were inoculated with *Salmonella* bacteria. For washing treatment, a 200 ± 3 ppm chlorine was prepared from diluting Clorox [5.25% sodium hypochlorite (NaOCl), Clorox Company, Oakland CA] with sterile water. Total available chlorine in the solution was tested with a chlorine test kit (Hach Co., Ames, IA, a U.S. Environmental Protection Agency approved test kit). During washing treatments, two melons each were submerged in 3 L of ∼200 ppm chlorine to minimize organic loading in the wash water and melons were washed inside a biosafety cabinet (Nuaire, Class II, Type A2, Plymouth, MN, USA) according to Ukuku and Fett (2004). All washed melons were air dried for 30 min inside the biosafety cabinet before rind removal and fresh-juice preparation.

Microbial Analysis of Whole Melon

Rind plugs from watermelon, honeydew and cantaloupe were randomly cut from the melon surfaces with sterilized stainless-steel cork-borer (22 mm in diameter). A total of rind plugs (70, with a total surface area of 266 cm^2) per melon weighing ∼25 g were homogenized in a waring commercial blendor (Dynamic Corp, New Hartford, CT, speed set at level 5, for 1 min) with 75 ml of 0.1% peptone water according to Ukuku and Fett (2004). Homogenized samples were diluted with 0.1% PW and a 0.1 ml aliquot was plated in duplicate on plate count agar (PCA, BBL/Difco Becton Dickinson Sparks, MD), with incubation at 30°C for 3 days for enumeration of aerobic mesophilic bacteria (Messer et al., 1984). Yeast and mold was enumerated according to Ukuku et al. (2012) on potato dextrose agar (PDA, BBL/Difco, Detroit, MI), amended with 10% tartaric acid to pH 3.5 (PDAA) and incubated at 25°C for 5 days. Similarly, *Pseudomonas* spp. were enumerated by plating 0.1 ml on *Pseudomonas* isolation agar (PIA, Difco/BBL) with incubation at 27°C for 3 days. *Salmonella* bacteria was enumerated on Xylose Lysine Sodium Tetradecylsufate (XLT4, BBL/Difco, Sparks, MD) agar incubated at 37°C for 24 h according to Ukuku et al. (2015). Colonies presumed to be *Salmonella* was confirmed by serological assays using latex agglutination (Oxoid, Ogdensburg, New York) and conventional biochemical methods of Andrews et al. (2018) in FDA Bacteriological Analytical Manual. The populations of *Salmonella* bacteria recovered on cantaloupes, honeydew and watermelons rind surfaces averaged 4.5, 3.8, and 3.2 $\log_{10}$ CFU/cm^2, respectively.

Preparation of Melon Juices

Prior to fresh juice preparation, cutting boards and knives were sanitized in 200 ppm chlorine solution. Sanitized and un-sanitized whole cantaloupe, watermelon and honeydew melons were individually cut into four sections using sterile knives. The rinds were removed, and the interior flesh were further cut to generate average melon pieces of ∼ 3 cm. The flesh of each melon's pieces was pooled (∼500 g), and the pieces were put inside sterile filtered stomacher bag (Filtra, Fisher Scientific, Pittsburgh, PA, USA) containing 100 ml sterile water and pummeled for 2 min at medium speed with Stomacher model 400 (Dynatech Laboratories, Alexandria, VA). Filtrate per type of melon was poured into sterile 250 ml conical flasks (Fisher Scientific, Pittsburgh, PA, USA).

Microbiological Analysis of Melon Juice

Aliquots (0.1 ml) from the juices including the supernatants were plated on non-selective (PCA), and selective (PDAA, PIA, and XLT4) media depending on the microorganism of interest. Plating was done in duplicate and after appropriate incubations, CFU on each plate were counted and firmed as stated above.

Soluble Solids and PH

The pH of all melon juices and the soluble solids (°Brix) was measured using a Basic pH meter (Denver Instruments Inc., Arvada, CO, USA) and Abbe 3L refractometer (Spectronic Instruments, Rochester, NY, USA), respectively, as described by Sharma et al. (2005). Juice from each melon was split into two parts and one part was centrifuged at 1,000 g for 5 min at 5°C to sediment the soluble solids. The supernatants were collected and used as part of this study.

Effect of Waiting Period Before Refrigeration of Prepared Juices

All fresh juice was divided into different categories based on storage condition (5 or 10°C for 15 days). Some of the juices were placed inside the refrigerator immediately after preparation while some were left at room temperature (22°C) for 3 and 5 h before refrigeration and microbial determination. In another study, a serial dilution of *Salmonella* inoculum prepared above was added (50 μl) to cantaloupe, honeydew and water melon juices to achieve an average population of 2.1 $\log_{10}$ CFU/ml. All samples were taken, and 0.1 ml plated on XLT4 agar (Difco) with incubation as stated above to enumerate the colony forming unit (CFU).

Analysis of Data

Experiments were performed three times with duplicate analysis of samples during sampling time. Colony forming units was converted to $\log_{10}$ CFU/cm^2 and $\log_{10}$ CFU/ml. All data generated were analyzed for analysis of variance (ANOVA) using Statistical Analysis System Program (SAS Institute, Cary, NC, USA), and each significant ($p < 0.05$) mean values for cells determined at specific waiting time before storage were calculated using Bonferroni LSD method (Miller, 1981).

RESULTS AND DISCUSSION

Aerobic mesophilic bacteria, yeast and mold and *Pseudomonas* spp. on untreated whole cantaloupe, watermelon and honeydew rind surfaces and the populations transferred to fresh-cut pieces is shown in **Table 1.** Whole cantaloupe surfaces and cantaloupe fresh-cut pieces had the highest populations of aerobic mesophilic bacteria and the populations for each class of these organism was significantly ($p < 0.05$) higher in cantaloupe surfaces and fresh-cut pieces than whole watermelon and honeydew including their respective fresh-cut pieces (**Table 1**). The same trend for higher populations of yeast and mold and *Pseudomonas* spp. was observed on cantaloupes and fresh-cut pieces. Yeast and mold and *Pseudomonas* populations determined on watermelon and honeydew surfaces were not significantly ($p > 0.05$) different. Ukuku and Fett (2004) reported higher microbial populations on whole cantaloupe than honeydew melon rind surfaces and the results of this study agrees with our earlier report. The microbial populations determined on watermelons were significantly ($p < 0.05$) lower than cantaloupe and honeydew melons despite the lager surface area of the watermelon (**Table 1**).

Effect of 200 ppm chlorine wash on aerobic mesophilic population of each melons before fresh-cut preparation is shown in **Table 2**. The average surviving populations of aerobic mesophilic bacteria and yeast and mold and *Pseudomonas* were on cantaloupes, watermelons and honeydew melons after washing with 200 ppm chlorine was 4.4, 1.9, and 1.7 $\log_{10}$ CFU/cm^2, respectively. Populations for aerobic mesophilic

TABLE 1 | Populations of aerobic mesophilic bacteria, yeast, and mold and *pseudomonas* spp. on unwashed cantaloupe, watermelon and honeydew rind surfaces and fresh-cut pieces.

	Population (Log_{10} CFU)[a]					
Microflora	**Cantaloupe**		**Watermelon**		**Honeydew**	
	Whole (CFU/cm^2)	**Fresh-cut (CFU/g)**	**Whole (CFU/cm^2)**	**Fresh-cut (CFU/g)**	**Whole (CFU/cm^2)**	**Fresh-cut (CFU/g)**
Aerobic mesophilic bacteria	6.8 ± 0.2 A	3.5 ± 0.1 A	4.4 ± 0.1 B	1.3 ± 0.2 B	3.2 ± 0.2 C	1.4 ± 0.3 B
Yeast and mold	3.2 ± 0.2 A	1.2 ± 0.1 A	0.9 ± 0.2 B	BD[b]	0.8 ± 0.2 B	BD
Pseudomonas spp.	3.1 ± 0.2 A	0.8 ± 0.2 A	0.6 ± 0.2 B	BD	0.6 ± 0.3 B	BD

[a] *Values represent means ± SD for data from three experiments with duplicate determinations per experiment. Means for each class of organism in the same row for whole or fresh-cut melon not followed by the same letter are significantly ($p < 0.05$) different.*
[b] *BD, Below limit of detection (1 CFU/g).*

TABLE 2 | Effect of 200 ppm chlorine wash on populations of aerobic mesophilic bacteria, yeast, and mold and *pseudomonas* spp. on cantaloupe, watermelon and honeydew rind surfaces and fresh-cut pieces.

	Population (Log_{10} CFU)[a]					
Microflora	**Cantaloupe**		**Watermelon**		**Honeydew**	
	Whole (CFU/cm^2)	**Fresh-cut (CFU/g)**	**Whole (CFU/cm^2)**	**Fresh-cut (CFU/g)**	**Whole (CFU/cm^2)**	**Fresh-cut (CFU/g)**
Aerobic mesophilic bacteria	4.4 ± 0.2^{A}	2.3 ± 0.1^{x}	2.1 ± 0.1^{B}	0.6 ± 0.2^{B}	1.9 ± 0.2^{C}	0.7 ± 0.3^{B}
Yeast and mold	1.9 ± 0.2^{A}	0.7 ± 0.1^{x}	BD^{Z}	BD^{Z}	0.3 ± 0.2^{Y}	BD^{Z}
Pseudomonas spp.	1.7 ± 0.2^{A}	0.3 ± 0.2^{x}	BD^{Z}	BD^{Z}	0.2 ± 0.1^{Y}	BD^{Z}

[a] *Values represent means ± SD for data from three experiments with duplicate determinations per experiment. Means for each class of organism in the same row for whole or fresh-cut melon not followed by the same letter are significantly ($p < 0.05$) different.*
BD, Below limit of detection (1 CFU/g).

bacteria on watermelons treated with 200 ppm chlorine was 2.1 log_{10} CFU/cm^2 while yeast and mold and *Pseudomonas* populations were below detection (<1 CFU/cm^2). The average surviving populations of aerobic mesophilic bacteria and yeast and mold and *Pseudomonas* on honeydew melons surfaces washed with 200 ppm chlorine averaged 1.9, 0.3, and 0.2 log_{10} CFU/cm^2, respectively.

Effect of Waiting Period on pH and Soluble Solids Before Refrigeration of Juice

The pH of cantaloupe juice and the soluble solids (°BRIX) determined immediately after preparation was higher than watermelon and honeydew melons (**Table 3**). Immediately after juice preparation, cantaloupe's pH and °BRIX values averaged 6.4 and 10.6, respectively, and was significantly different ($p < 0.05$) than watermelon and honeydew juices. There were no significant ($p < 0.05$) changes in pH and °BRIX values when the fresh juice was placed inside the refrigerator immediately after preparation and left refrigerated (5°C) for 5 h. However, holding melon juices at room temperature for 5 h before refrigeration led to significant ($p < 0.05$) changes in pH and °BRIX. The pH of cantaloupe and watermelon juices decreased slightly compared to day 0 while the °BRIX in all juices increased slightly. Similarly, the populations of aerobic mesophilic bacteria increased within this waiting period and was significantly ($p < 0.05$) higher than values in juices placed immediately inside refrigerator and those left at room temperature for 3 h before refrigeration. There were no significant ($p > 0.05$) changes in population of aerobic mesophilic bacteria in all juices that were placed inside the refrigerator immediately after preparation and determined after 3 h of refrigerated storage (**Table 4**).

Effect of Waiting Period on *Salmonella* Bacteria in Juice

Populations of *Salmonella* in all fresh-melon juices recovered immediately after preparation and those determined in samples after waiting for 5 h at room temperature before refrigeration is shown in **Table 5**. All melon juice was prepared from control melons without 200 ppm chlorine wash treatments. Cantaloupe juice had the highest population of *Salmonella* bacteria compared to watermelon and honeydew juices. *Salmonella* bacteria in all juices left at room temperature for 3 and 5 h showed a slight increase and were significantly higher than juices stored immediately inside a refrigerator (5°C) or 3 h at room temperature after preparation. Holding cantaloupe juice at room temperature for 3 and 5 h before refrigeration led to 0.3 and 0.8 log increase of *Salmonella* bacteria (**Table 4**), respectively. A similar increase in the soluble solids of cantaloupe, honeydew, and watermelon juices was observed within the waiting period at room temperature before refrigeration (**Table 3**). It is appropriate

TABLE 3 | Soluble solid (°brix) and pH of washed cantaloupe, watermelon, and honeydew melon juices immediately after preparation and storage at room temperature for 5 h before refrigeration[a].

Melon juice	0 h		5 h at 5°C		5 h at 22°C before refrigeration	
	pH	°Brix	pH	°Brix	pH	°Brix
Water melon	5.5 ± 0.1^{BC}	8.1 ± 0.1^{Z}	5.5 ± 0.1^{BC}	8.1 ± 0.1^{Z}	5.3 ± 0.2^{BC}	8.4 ± 0.2^{Z}
Honeydew	5.7 ± 0.2^{B}	9.5 ± 0.2^{Y}	5.7 ± 0.2^{B}	9.5 ± 0.2^{Y}	5.9 ± 0.2^{A}	9.8 ± 0.2^{Y}
Cantaloupe	6.4 ± 0.1^{A}	10.6 ± 1.2^{X}	6.4 ± 0.1^{A}	10.6 ± 1.2^{X}	6.1 ± 0.2^{AB}	10.8 ± 1.4^{XY}

[a] *Values represent means ± SD for data from three experiments with duplicate determinations per experiment. Means for each class of pH and °brix in the same column per melon juice and row for storage time before refrigeration not followed by the same letter are significantly ($p < 0.05$) different.*

TABLE 4 | Effect of waiting period on population of aerobic mesophilic bacteria of fresh- melon juice stored immediately at 5°C for 3 h or left at 22°C for 3 h before refrigeration[b].

	Population (Log_{10} CFU/ml)[a]			
Melon Juice	Stored at 5°C immediately after preparation	Held at 22°C for 3 h before storage at 5°C	Held at 22°C for 5 h before storage at 5°C	Held at 5°C for 3 h, after preparation
Watermelon	0.4 ± 0.1^{BZ}	0.9 ± 0.3^{BY}	1.2 ± 0.1^{CX}	0.2 ± 0.10^{BZ}
Honeydew	0.5 ± 0.2^{BZ}	0.8 ± 0.1^{BY}	1.3 ± 0.2^{BX}	0.3 ± 0.1^{BZ}
Cantaloupe	1.4 ± 0.3^{AZ}	1.9 ± 0.2^{AY}	2.2 ± 0.2^{AX}	1.1 ± 0.2^{AZ}

[a] *Values represent means ± SD for data from three experiments with duplicate determinations per experiment. Means in the same column and row for each fresh-melon juice per holding time and storage not followed by the same letter are significantly ($p < 0.05$) different.*
[b] *Juices were prepared from unwashed whole melons.*

to point out that after inoculation of all melons with *Salmonella* bacteria, cantaloupes fresh-cut pieces and juice had the highest population of *Salmonella* bacteria followed by honeydew and watermelon, respectively. In our earlier study, we reported transfer of *Salmonella* from melon rind surfaces to the interior flesh during fresh-cut preparation (Ukuku and Sapers, 2007).

Ukuku (2004) reported growth of *Salmonella* spp. in melon pieces stored at 23°C and Walsh et al. (2014) indicated that interior watermelon tissues support the growth of *Salmonella* bacteria during storage at 23°C. The authors did not report or make suggestions as to the influence of melons soluble solids may have had on the survival of *Salmonella* bacteria on the fresh-cut fruits. In our study, the 200 ppm chlorine wash reduced transfer of *Salmonella* populations on all melon rind surfaces to fresh-cut pieces. The population of *Salmonella* recovered from these fresh-cut pieces used in fresh juice preparation was <4 CFU/g for cantaloupes pieces and <1 CFU/g for honeydew and watermelon pieces, respectively. When these fresh-cut pieces were used for preparing the juices, the pathogen was not detected on XLT4 plates (**Table 5**). It is possible that the residual *Salmonella* bacteria detected in fresh-cut pieces were diluted out during the juice preparation. The juice from these melons were similarly left at room temperature and or placed immediately inside refrigerator after preparation and still, no *Salmonella* bacteria was enumerated on XLT4 plates within the 5 h of waiting period at room temperature before refrigeration.

In another study, we deliberately contaminated the juices with *Salmonella* bacteria at 2.2 log CFU/ml**.** The 2.2 log CFU/ml *Salmonella* bacteria was chosen arbitrarily to allow us monitor the behavior of *Salmonella* bacteria in these juices during storage at 5°C (**Figure 1**). Aerobic mesophilic bacteria in cantaloupes juices was significantly ($p < 0.05$) different than populations in honeydew and watermelon juices. Populations for this native microflora grew in all juice during refrigerated storage at 5°C while the inoculated *Salmonella* bacteria survived with slightly higher numbers determined in in cantaloupe and honeydew juice at day 9. In juices stored at abusive temperature (10°C), growth populations for aerobic mesophilic bacteria in all juices were significantly ($p < 0.05$) higher than juices stored at 5°C for the same number of days (**Figure 2**). *Salmonella* bacteria in juices stored at abusive temperature (10°C) for 3 days slightly declined but increased in all juices after day 6. El-Safey (2013) reported variation in the survival of *Salmonella* spp. in fruit juices and concluded that survival was depended on pH, the type of strain, the type of juices and the incubation temperature. The author reported that *Salmonella* heidelberg survived up to 18 d in mango, guava, pineapple, and cocktail juices, 15 d in orange juice and 12 d in apple juice stored at 10°C. The author concluded that acid foods, especially if kept at refrigeration temperatures, support survival of *Salmonella* Heidelberg and may cause *Salmonella* heidelberg food poisoning and our current data agrees. The pH of cantaloupe, honeydew and watermelon juices in our study was 6.4, 5.7, and 5.5, respectively. The pH of cantaloupe and watermelon juice decreased by 0.2 point while honeydew melon juice increased by 0.2 point during 5 h waiting period before refrigeration. The juice storage time in El-Safey (2013) study and the type of juices studied was quite different from the juices in our study. However, El-Safey (2013) study and our current study reached a similar conclusion about survival of *Salmonella* in juices stored at 5 or 10°C and because of

TABLE 5 | Population of *salmonella* inoculated in fresh- melon juice immediately after preparation and storage at 5°C, and 22°C for 3 and 5 h before refrigeration.

	Population (Log_{10} CFU/ml)[a]			
Melon Juice	**Stored at 5°C immediately after preparation**	**Held at 22°C for 3 h before storage at 5°C**	**Held at 22°C for 5 h before storage at 5°C**	**Held at 5°C for 3 h, after preparation**
JUICE FROM UNWASHED MELONS				
Watermelon	0.3 ± 0.1^{AY}	0.5 ± 0.2^{ABY}	1.2 ± 0.3^{ABX}	BD^{A}
Honeydew	0.6 ± 0.1^{AY}	0.8 ± 0.1^{CY}	1.3 ± 0.2^{BX}	0.4 ± 0.1^{BZ}
Cantaloupe	1.4 ± 0.1^{AZ}	1.7 ± 0.1^{AY}	2.2 ± 0.2^{AX}	1.1 ± 0.1^{AZ}
JUICE FROM WASHED MELONS				
Watermelon	BD*	BD	BD	BD
Honeydew	BD	BD	BD	BD
Cantaloupe	BD	BD	BD	BD

Juices were prepared from unwashed whole melons.

[a]*Values represent means ± SD for data from three experiments with duplicate determinations per experiment. Means in the same column and row for each fresh- melon juice per holding time and storage not followed by the same letter are significantly ($p < 0.05$) different.*

**BD, Below detection (no colony on XLT4 plates).*

these small increases in *Salmonella* bacteria in fresh-melon juices, it is appropriate to follow the recommendation for immediate refrigeration of fresh-cut pieces and or juice after preparation (Ukuku and Sapers, 2007; FDA, 2008).

Salmonella bacteria in fruits accounted for 838 illnesses and 227 hospitalizations in United States from 2009 to 2015 (Dewey-Mattia et al., 2018). In a fruit and vegetable study on juice foodborne disease outbreak from 1922 to 2010, unpasteurized apple juice accounted for more outbreaks (Danyluk et al., 2010). The authors also, reported incidences of similar outbreaks associated with mixed fruit juice and watermelon juice, and the bacterial pathogens isolated from the apple juices were mostly *E. coli* O157:H7 bacteria while *Salmonella* spp. was reported in 18 cases of watermelon juice. Restaurants and sit-down dining accounted for 61 and 48% of foodborne outbreaks-associated illness in United States (Dewey-Mattia et al., 2018). The authors also, reported 636 and 561 outbreaks with 18,141 and 8,080 associated illnesses at catering/banquet facilities and private home, respectively while illnesses associated with fruits and vegetables accounted for 2,420 and 1,972. Consumers are becoming more health conscious thereby eating more foods described as "fresh" and tends to prepare such foods at home. Fruits and vegetables are mostly eaten raw or juiced and then drink therefore bacterial contamination and spoilage of melon juices was investigated. There is limited information on foodborne disease outbreaks related to cantaloupe and honeydew melon juices, respectively, and information presented in this study is intended to expound the knowledge base on what to do and or not do when preparing cantaloupe, honeydew and watermelon juices especially at home. It is true that Hazard Analysis and Critical Control Point (HACCP) regulation applies to domestic and imported juice products, still incidences of foodborne outbreaks related to fruits and vegetable juice occur probably due to inappropriate GMPs related to sanitation standard operating procedures (SSOPs).

Despite the washing treatment with ∼200 ppm chlorine, the presence of aerobic mesophilic bacteria and *Salmonella* bacteria were detected on cantaloupe fresh-cut pieces than honeydew melon or watermelon. The inability of the washing treatment to inactivate all categories of microbes tested may be due to the rough surface of the cantaloupe rind compared to the waxy nature of honeydew and watermelon melon surfaces. Ukuku and Fett (2004) and Ukuku et al. (2017) reported that the extensive raised netting on the surface of cantaloupe melon provided more microbial attachments sites which helps to protect attached microbes from being washed off during chemical washing treatments concluding that that was the basis that led higher transfer of bacteria to fresh-cut during fresh-cut preparation. In a different study on fresh-cut cantaloupe pieces inoculated with *Salmonella* at 2 log CFU/g with storage at 23°C for 24 h, the bacterium increased to 5 log CFU/g of the fresh-cut (Parnell et al., 2005). Similarly, Jackson et al. (2013) reported a similar growth of *Salmonella* spp. on the edible portions of fruit. Spoilage of fresh-cut fruits are mostly associated with changes in color which can be attributed to oxidative browning and or microbial contamination (Barrett et al., 2010) and we are suggesting that similar oxidative and microbial contamination can be extended to explain the spoilage of fresh-juice. Barrett et al. (2010) suggested further evaluation of microflora of fresh-cut fruit pieces in other to set appropriate criteria for quality assessment. In our study, we investigated the efficacy of 200 ppm chlorine in reducing transfer of surface microflora of whole cantaloupe, honeydew melon and watermelon and the inoculated population of *Salmonella* bacteria on melons rind surfaces designated for fresh juice preparation. The 200 ppm chlorine used in this study was within the range reported in the literature (McGlynn, 2004, fapc-116, www.fapc.okstate.edu).

Melon rind surfaces are usually removed before fresh-cut and juice preparation and if proper washing treatments are not employed, microbial populations associated with the fruit surfaces or rinds (Fleming et al., 2005; Parnell et al., 2005; FDA, 2008; Ukuku et al., 2016) can end up in the juice. In our current study, we reported minimal transfer of residual surviving populations of aerobic mesophilic bacteria

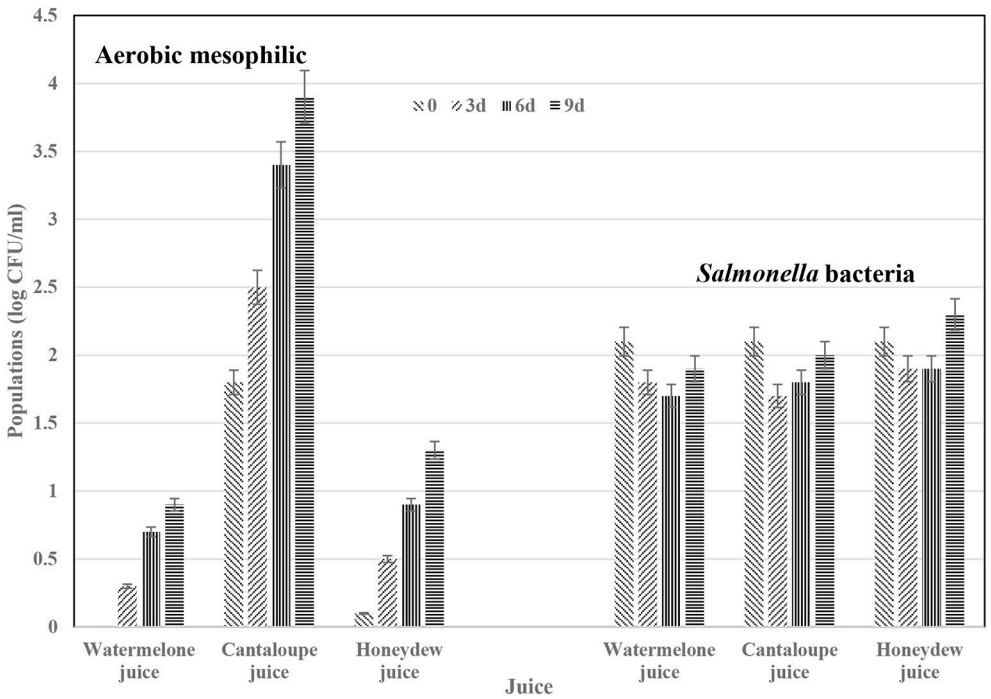

FIGURE 1 | Survival of transferred aerobic mesophilic bacteria from melon rind surfaces during fresh-juice preparation with added *Salmonella* bacteria during storage at 5°C for 9 days. Values represent means ± SD for data from three experiments with duplicate determinations per experiment.

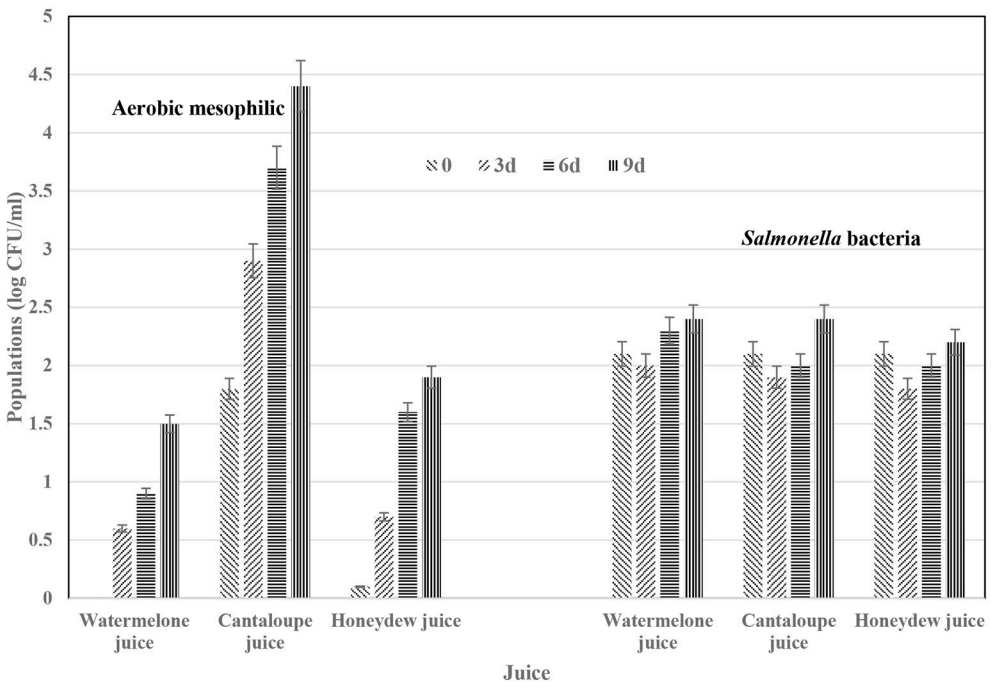

FIGURE 2 | Survival of transferred aerobic mesophilic bacteria from melon rind surfaces during fresh-juice preparation with added *Salmonella* bacteria during storage at 10°C for 9 days. Values represent means ± SD for data from three experiments with duplicate determinations per experiment.

and inoculated *Salmonella* in the prepared melon juices and recommended immediate refrigeration after preparation to slow and or suppressed growth of bacteria. We recommended similar immediate refrigeration of apple cider juice amended with nisin-EDTA where we found that cold temperature inhibited growth of *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes*

during refrigerated storage (Ukuku et al., 2009). In conclusion, immediate refrigeration of freshly prepared fresh-cut fruits and or juices prepared from cantaloupes, watermelon and honeydew fresh-cut pieces was able to inhibit growth of aerobic mesophilic bacteria, yeast and mold and *Pseudomonas* spp. including *Salmonella* bacteria. Washing all melons with 200 ppm chlorine before fresh-cut or juice preparation is highly recommended. Also, hot water (Ukuku et al., 2005; Solomon et al., 2006) and chlorine dioxide (Rodriguez et al., 2017) has been used to treat produces including whole melons before fresh-cut preparation. It is true that the 200 ppm chlorine did not inactivate all bacterial populations on melon surfaces, but the treatment was able to decrease transfer of bacterial populations from melon rind surfaces to the juice during melon juices preparation. Holding freshly prepared melon juices at 22°C for more than 3 h before refrigeration storage led to increase of the residual *Salmonella* population in the juice. More studies are needed to fully understand the relationship between the slight increase in soluble solids and the bacterial populations observed in juices held at room temperature for 3 to 5 h before refrigeration.

AUTHOR'S NOTE

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

AUTHOR CONTRIBUTIONS

DU initiated the study based on prior work. MO and SM were involved in the experimental design and review of data. The study was performed in DU's Laboratory.

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