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Comparison of the effect of saturated and superheated steam on the inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* on cantaloupe and watermelon surfaces

Sun-Ah Kwon [a, b], Won-Jae Song [a, b], Dong-Hyun Kang [a, b, *]

[a] Department of Agricultural Biotechnology, Center for Food and Bioconvergence, Research Institute of Agricultural and Life Sciences, Seoul National University, Seoul, Republic of Korea
[b] Institutes of Green Bio Science & Technology, Seoul National University, Pyeongchang-gun, Gangwon-do, Republic of Korea

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ABSTRACT

The purpose of this study was evaluation of the effectiveness of superheated steam (SHS) on inactivation of foodborne pathogens on cantaloupes and watermelons. Saturated steam (SS) treatment was performed at 100 °C and that of SHS at 150 and 200 °C. *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes*-inoculated cantaloupes and watermelons were exposed for a maximum of 30 s and 10 s, respectively. Populations of the three pathogens on cantaloupes and watermelons were reduced by more than 5 log after 200 °C steam treatment for 30 s and 10 s, respectively. After SHS treatment of cantaloupes and watermelons for each maximum treatment time, color and maximum load values were not significantly different from those of untreated controls. By using a noncontact 3D surface profiler, we found that surface characteristics, especially surface roughness, is the main reason for differences in microbial inactivation between cantaloupes and watermelons. The results of this study suggest that SHS treatment can be used as an antimicrobial intervention for cantaloupes and watermelons without inducing quality deterioration.

1. Introduction

Cantaloupes (*Cucumis melo* L. var. *reticulatus* NAUD) and watermelons (*Citrullus lanatus* (Thunb.) var. *lanatus*) are popular fruits enjoyed worldwide (Lamikanra et al., 2005; Tian et al., 2007). People consume melons for various reasons. Melons are an excellent source of beta-carotene, vitamin C, and potassium. Melons, moreover, have no cholesterol, and are low in fat and sodium (Lester, 1997), while watermelons are an excellent source of the phytochemical lycopene (Perkins-Veazie and Collins, 2004). Of importance is that cantaloupes and watermelons are frequently associated with foodborne pathogen infections. Since melons develop on contact with soil, their outer surfaces can easily become contaminated with foodborne pathogens during production and processing (Bowen et al., 2006). Melon-associated outbreaks increased from 0.5 outbreaks per year during 1973–1991 to 1.3 during 1992–2011, and during 1973–2011, cantaloupes were the most common melon type implicated in outbreaks (19 outbreaks, 56% of the total), followed by watermelons (13 outbreaks, 38% of the total) (Walsh et al., 2014).

Foodborne pathogens of high public health concern, including *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes*, are able to grow on the outside of intact cantaloupes and watermelons. *Salmonella* has been the pathogen most frequently implicated in melon-associated outbreaks (Walsh et al., 2014). In 2012, a multistate outbreak of *S.* Typhimurium and *S.* Newport infections linked to cantaloupes resulted in 94 illnesses and 3 deaths (CDC, 2012a). Also, *L. monocytogenes* and *E. coli* O157:H7 outbreaks are frequently associated with consumption of melons. In 2011, there was a multistate outbreak of *L. monocytogenes* in the US resulting in 147 illnesses and 33 deaths (CDC, 2012b). In 1993, there was an outbreak linked to cantaloupes contaminated with *E. coli* O157:H7 in Oregon (Del Rosario and Beuchat, 1995). A watermelon-borne *E. coli* O157:H7 outbreak in 2000 in Wisconsin was attributed to cross-contamination with a raw meat product (Walsh et al., 2014). Thus, controlling these

* Corresponding author. Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute of Agricultural and Life Sciences, Seoul National University, Seoul, Republic of Korea.
E-mail address: kang7820@snu.ac.kr (D.-H. Kang).

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foodborne pathogens is one of the most important challenges facing the food industry.

A previous study involving thermal treatment of whole cantaloupe at 76 °C for 3 min resulted in a reduction of *E. coli* populations by about 5 log CFU/cm^2 (Annous et al., 2004). But conventional heating is an energy intensive intervention which consumes large amount of water. Nonthermal pasteurization technologies have been studied extensively because of the need to minimize quality loss. UV-C illumination at 4.1 kJ/cm^2 produced 1–1.5 log reduction in bacterial populations on fresh-cut watermelon (Fonseca and Rushing, 2006). Intense light pulse treatments with an overall full spectrum energy of 12 J/cm^2 reduced *E. coli* populations on inoculated fresh-cut watermelon by 3.01 log (Ramos-Villarroel et al., 2012). But these results were not adequate to ensure safety of the product. The United States Food and Drug Administration (US FDA) requires decontamination steps for fruits and vegetables sufficient to achieve a 5 log reduction, so investigations targeting 5-log reduction are needed (Han et al., 2001).

Superheated steam (SHS) is steam which has been given additional sensible heat to raise its temperature above the saturation temperature at a constant pressure. Unless the temperature of SHS exceeds the saturation point at the processing pressure, a drop in temperature will not result in condensation of steam (Cenkowski et al., 2007). SHS pasteurization is an emerging technology that has the potential to replace commonly used heat treatments (Bari et al., 2010). SHS pasteurization is a time- and energy-saving and also environmentally-friendly technology in terms of avoiding the use of chemical compounds (Pronyk et al., 2004; van Deventer and Heijmans, 2001). Still, inactivation of foodborne pathogens by SHS has rarely been studied, especially for fresh food.

The objectives of this work were to compare the effect of saturated steam (SS) and SHS on the inactivation of foodborne pathogens on cantaloupe and watermelon surfaces and quality changes during treatment and also to investigate the main reason for differences of microbial inactivation by SHS between cantaloupes and watermelons.

2. Materials and methods

2.1. Stock cultures

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 43971, ATCC 700408), and *L. monocytogenes* (ATCC 15315, ATCC 19114, ATCC 19115) obtained from the bacterial culture collection of Seoul National University (Seoul, Republic of Korea) were used in this experiment. Stock cultures were maintained at −80 °C in 0.7 ml of tryptic soy broth (TSB; Difco, Becton, Dickinson, Sparks, MD, USA) and 0.3 ml of 50% glycerol for a final glycerol concentration of 15% (v:v). Working cultures were streaked onto tryptic soy agar (TSA; Difco), incubated at 37 °C for 24 h, and stored at 4 °C.

2.2. Preparation of pathogen inoculum

Each strain of *E. coli* O157:H7, *S.* Typhimurium and L. *monocytogenes* was incubated in 5 ml of TSB at 37 °C for 24 h, harvested by centrifugation at 4000×*g* at 4 °C for 20 min and washed twice with sterile 0.2% peptone (Bacto, Sparks, MD) water (PW). The final pellets were resuspended in sterile 0.2% PW to a concentration of approximately 10^7–10^8 CFU/ml. Suspended pellets of each strain of *E. coli* O157:H7, *S.* Typhimurium and L. *monocytogenes* were combined to produce a mixed species culture cocktail.

2.3. Sample preparation and inoculation

Cantaloupes and watermelons were purchased at a local grocery store (Seoul, Republic of Korea) the day before each experiment and stored at 4 °C. Cantaloupes and watermelons were then washed by dipping them in distilled water for 2 min to remove dust and dried at room temperature for 60 min in a laminar flow biological safety hood with the fan running to remove excess moisture. Samples were cut into cubes (2 cm × 5 cm × 1 cm length) including the rind using a sterile knife. A spot-inoculation method was used to inoculate *E. coli* O157:H7, *S.* Typhimurium and L. *monocytogenes* onto samples. Two hundred μl of previously described culture cocktail was inoculated onto the rind of each sample piece by distributing this volume between 20 droplets deposited at randomly selected locations with a micropipette. All inoculated samples were dried for 1 h in the laminar flow biological safety hood before treatment at room temperature (22 ± 2 °C).

2.4. Saturated steam (SS) and superheated steam (SHS) treatment

A steam generator apparatus described previously by Ban and Kang (2016) was used (Fig. 1). Water was converted into steam by heating with an electrical resistance heater. During SS or SHS treatments, temperature was controlled automatically by a temperature sensor and an intelligent power module in the steam generator. Dried inoculated cantaloupes and watermelons were spread into a single layer on a stainless steel treatment grid inside a stainless steel basket and placed in an insulated steam treatment chamber (external diameter 23 cm; external height, 32 cm; internal diameter, 17 cm; internal height, 22.5 cm). Steam passed through a flexible hose and into the chamber by opening a steam valve. Cubed cantaloupes were exposed to SS or SHS on the rind surface (2 cm × 5 cm surface) for 5, 10, 15, 20, 25 or 30 s, and watermelon cubes were exposed for 1, 3, 5, 7 or 10 s. SS treatments were conducted at 100 °C, and SHS treatments were performed at 150 and 200 °C. The basket containing treated samples was immediately removed from the chamber after each treatment.

2.5. Bacterial enumeration

At pre-selected treatment times, each treated sample was immediately transferred into a sterile stomacher bag (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 100 ml of 0.2% PW and homogenized for 2 min with a stomacher (Easy mix; AES Chemunex, Rennes, France). After homogenization, 1 ml of sample was 10-fold serially diluted in 9 ml of 0.2% PW, and 0.1 ml of sample or diluent was spread-plated onto Sorbitol MacConkey agar (SMAC; Difco), Xylose Lysine Desoxycholate agar (XLD; Difco), and Oxford Agar Base with antimicrobial supplement (OAB; MB Cell) for enumeration of *E. coli* O157:H7, *S.* Typhimurium and L. *monocytogenes*, respectively. Where low populations of surviving cells were anticipated, 1 ml aliquots of the original homogenate were equally distributed onto four plates (0.25 ml x 4) of each respective medium and spread-plated. The detection limit was 1.0 log CFU/g. All plates were incubated at 37 °C for 24 h, and then colonies enumerated.

2.6. Enumeration of injured cells

Injured cells of *S.* Typhimurium and L. monocytogenes were enumerated using the overlay (OV) method. One hundred μl of sample or diluent were spread-plated onto TSA and incubated at 37 °C for 2 h to allow injured cells to resuscitate before overlaying with 7 ml of XLD (OV-XLD) or OAB (OV-OAB) for recovery of *S.* Typhimurium or L. *monocytogenes*, respectively. The plates were