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Quality attributes and microbial survival on whole cantaloupes with antimicrobial coatings containing chitosan, lauric arginate, cinnamon oil and ethylenediaminetetraacetic acid

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ABSTRACT

Cantaloupes are susceptible to microbiological contamination in pre- or postharvest environments. Novel intervention strategies, such as antimicrobial coatings, are needed to improve the microbiological safety of cantaloupes. The objective of this study was to prepare whole cantaloupes coated with mixtures containing chitosan, lauric arginate (LAE), cinnamon oil (CO), and ethylenediaminetetraacetic acid (EDTA) and determine survival characteristics of inoculated foodborne pathogens during storage as well as cantaloupe quality attributes. Chitosan coating with 0.1% LAE, 0.1% EDTA, and 1% CO was the most effective for inactivating foodborne pathogens inoculated on cantaloupes. This coating caused a >3 log CFU/cm^2 reduction of *Escherichia coli* O157:H7 and *Listeria monocytogenes* immediately after coating and reduced *Salmonella enterica* to below the detection limit during a 14-day storage. Total molds and yeasts also were reduced to the detection limit by the coating. The redness and yellowness of uncoated cantaloupes were significantly higher than coated ones from day 6. The firmness of uncoated cantaloupes and those coated with chitosan only was significantly lower than other treatments from day 10. No significant differences were found in total soluble solids content or weight loss between coated and uncoated cantaloupes. Results showed the potential benefits of applying the coating mixtures to improve the quality and microbiological safety of cantaloupes.

1. Introduction

Cantaloupes are perishable and susceptible to microbiological contamination in pre- or postharvest environments. Since cantaloupes are grown on the ground, pre-harvest safety concerns come from contamination with foodborne pathogens by irrigation water, manure fertilizers, and wild or domestic animals (Bowen et al., 2006). Post-harvest threats include field workers or handlers where poor hygiene and unsanitary procedures can lead to cross-contamination of cantaloupes (Bowen et al., 2006). Cross-contamination can also occur during cutting of cantaloupes (Ukuku and Sapers, 2001). A contributory feature of cantaloupes is their rough surface which can favor the attachment of bacteria (Bowen et al., 2006), as was evidenced by the positive linear correlation between the adhesion rate of *Escherichia coli* O157:H7 and the surface roughness of fruits (Wang et al., 2009). Surface roughness was also negatively linearly correlated to the inactivation efficacy of *E. coli* O157:H7 by acidic electrolyzed water and peroxyacetic acid (Wang et al., 2009). Cantaloupe was more resistant to effective sanitization treatments than other fruits (apple, avocado and orange) with smoother surfaces (Wang et al., 2009). These pre- and post-harvest safety factors have directly or indirectly contributed to more than 25 outbreaks of foodborne illnesses associated with the consumption of cantaloupes between 1973 and 2003 in the United States and Canada (Bowen et al., 2006). A large scale outbreak of listeriosis in 2011 was linked to whole cantaloupes from Jensen Farms in Colorado, USA and resulted in 147 infections, 33 deaths, and 1 miscarriage (CDC, 2012). Therefore, strategies are needed to improve the safety of cantaloupes.

Antimicrobial coatings have been widely investigated to improve the safety of food products (Li et al., 2013), such as broccoli (Alvarez et al., 2013), strawberries (Hernández-Muñoz et al., 2006) and roast beef (Wang et al., 2015). Chitosan, derived from deacetylation of chitin (Hajji et al., 2014), is an excellent film forming material (Domard and Domard, 2001). Chitosan-based coatings with incorporated antimicrobials or bioactive compounds have been extensively studied to improve the safety and quality of food products (Elsabee and Abdou, 2013). For example, a coating with 1% chitosan and 2% acetic acid resulted in a 5.4 log CFU/g reduction of *Listeria monocytogenes* on ready-to-eat shrimps after 16-day storage at 4 °C (Li et al., 2013). Spraying a coating solution with 1% w/v modified chitosan and 0.05% w/v carvacrol nanoemulsion on green beans resulted in a 1.7-log CFU/g reduction of *E. coli* O157:H7 after 7-day storage at 4 °C (Severino et al., 2015). Chitosan itself also has antimicrobial activities (Kong et al., 2010).

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Thus, chitosan-based antimicrobial coatings may have the potential to improve the safety of whole cantaloupes during storage.

Lauric arginate (LAE) is a generally-recognized-as-safe (GRAS) antimicrobial (USDA, 2005) and effectively inhibits a broad spectrum of foodborne pathogens (Ma et al., 2013). Essential oils (EOs) are another group of effective GRAS antimicrobials (Pan et al., 2014; Shah et al., 2013). In our recent study, combining LAE and EOs resulted in a synergistic antilisterial activity, however the same combination was antagonistic against the Gram-negative bacteria, *E. coli* O157:H7 and *Salmonella* (Ma et al., 2013). Ethylenediaminetetraacetic acid (EDTA) is a chelator that can bind divalent calcium ions that are important to bacteria structures (Vaara, 1992). It has been shown to enhance the activities of various antimicrobials, such as lysozyme, that are normally effective against Gram-positive but not Gram-negative bacteria (Branen and Davidson, 2004; Proctor et al., 1988). In a separate study, EDTA significantly enhanced an LAE- cinnamon oil (CO) combination against *L. monocytogenes*, *Salmonella enterica* and *E.coli* O157: H7 (Ma et al., 2016a, 2016b, 2016c). Moreover, chitosan-based film discs containing LAE, CO and EDTA showed large inhibition zones against the above microorganisms when tested on agar plates (Ma et al., 2016a).

Therefore, the objective of the present study was to evaluate antimicrobial effects of chitosan-based coatings containing LAE, CO and EDTA on whole cantaloupes as well as their influence on quality attributes. *L. monocytogenes*, *S. enterica* and *E. coli* O157:H7 were the test microorganisms for the cantaloupes because these foodborne pathogens have been linked to outbreaks of foodborne illnesses associated with fresh produce. Coatings were also studied for their antimicrobial effectiveness against molds and yeasts on whole cantaloupes. Color, weight loss, firmness and total soluble solids content of cantaloupes during storage were studied as quality parameters.

2. Materials and methods

2.1. Materials

Chitosan (low molecular weight, 75–85% deacetylated), EDTA and CO (from *Cinnamomum zeylanicum*, purity 80.00–88.00%) were purchased from Sigma-Aldrich Corp. (St. Louis, MO). Commercial LAE product (CytoGuard™ LA 20) containing 10% LAE and 90% propylene glycol was kindly provided by A&B Ingredients (Fairfield, NJ). Non-selective media tryptic soy broth (TSB) was purchased from Thermo Fisher Scientific, Inc. (Waltham, MA).

Cantaloupes were bought from a local supermarket on the day of arrival and were immediately washed for microbiological tests or stored overnight at room temperature (21 °C) for quality tests.

2.2. Bacteria culture

Cocktails with equal populations of 5 strains/serovars were used for each bacterium in the microbial study, as described in other studies (Ma et al., 2016b; Ma et al., 2016a; Zhang et al., 2015). *E. coli* O157:H7 cocktail consisted of strains H1730, F4546, K3995, 658 and 932. *S. enterica* cocktail contained Agona, Montevideo, Gaminara, Michigan and Saint Paul serovars. *L. monocytogenes* cocktail was comprised of LM1, LM2, 310, Scott A and V7 strains. Each of the strains used in the cocktails was cultured in TSB or TSB supplemented with yeast extract (TSBYE, for *L. monocytogenes*) and transferred at least twice at intervals of 24 h. The incubation temperature was 32 °C for *L. monocytogenes* and 37 °C for *S. enterica* and *E. coli* O157:H7. Cocktails were prepared by mixing 2 mL of each strain.

2.3. Preparation of coating solutions

As described previously (Ma et al., 2016a), chitosan stock solution was prepared by dissolving 2% *w*/w chitosan powder in 1% *w*/w aqueous acetic acid solution and stirring overnight at room temperature (21 °C). Undissolved material was removed by filtering through a microcloth (Calbiochem-Novabiochem Corp., San Diego, CA). Coating solutions were prepared by adding LAE, EDTA, CO, and deionized water to the 2% w/w chitosan stock solution. The final coating solutions contained 1% w/w chitosan, 0.5% w/w acetic acid, 0.1% w/w LAE, 0.1% w/w EDTA, and 0, 0.5 or 1% w/w CO. Hereafter, unless otherwise stated, all percentages are weight percentages,.

2.4. Inoculation and treatment of whole cantaloupes

Treatment of cantaloupes was done according to the method of Chen et al. (Chen et al., 2012). Cantaloupes were washed using deionized water containing 0.5% w/v Tween 80 and rinsed with tap water. The washed cantaloupes were placed on a laboratory bench and dried overnight at room temperature (21 °C). 100 μL culture with about 10^8 CFU/mL bacteria was inoculated on pre-marked 6.25 cm^2 squares on the cantaloupes. Two squares on each of 2 cantaloupes were inoculated for each bacterium and each coating treatment. After inoculation, cantaloupes were dried for 6 h at room temperature (21 °C) to allow the bacteria attach to the surface of cantaloupes before treatment.

For the coating treatment, 400 μL of each following coating solution was spread on the inoculated squares with a small paintbrush: A) 1% chitosan + 0.1% LAE + 0.1% EDTA; B) coating "A" + 0.5% CO; C) coating "A" + 1% CO; and D) 1% chitosan only. Cantaloupes without coating were used as a control. Cantaloupes were then stored at room temperature (21 °C) for up to 14 days.

2.5. Enumeration of foodborne pathogens

Selective media were used to reduce or eliminate the interference of background microorganisms. Cefixime-tellurite sorbitol MacConkey (CT-SMAC), xylose lysine tergitol 4 agar (XLT4), and modified oxford agar (MOX) were used for *E. coli* O157:H7, *S. enterica,* and *L. monocytogenes*, respectively. Treated areas of cantaloupe rind squares were excised using a sterile knife on day 1, 3, 7, 10 and 14. The squares were placed into sterile blender bags (Thermo Fisher Scientific, Inc., Waltham, MA) containing 25 mL sterile 10 mM phosphate buffered saline (PBS, pH 7.4) and 0.2% Tween 80 and hand-massaged for 1 min. The rinsate was then serially diluted in 0.1% w/v peptone water and surface plated on CT-SMAC plates for *E.coli* O157:H7, XLT4 plates for *S. enterica*, or MOX plates for *L. monocytogenes*. Counting of colonies was carried out after 24-h incubation at 37 °C for *E.coli* O157:H7 and *S. enterica*, or 48-h incubation at 32 °C for *L. monocytogenes*.

2.6. Effects of chitosan-based coatings on the quality characteristics of whole cantaloupes

Cantaloupes with similar size, color and degree of visual ripeness were immersed into 2 L of the above coating solutions for 30 s. After draining the excess, cantaloupes were incubated at room temperature (21 °C) for up to 14 days. Weight, color, firmness, and total soluble solids (TSS) content of cantaloupes were measured using the methods described below on days 2, 6, 10 and 14. The total populations of molds and yeasts were enumerated on day 2. Uncoated cantaloupes were used as a control.

2.6.1. Weight and color measurement

Four cantaloupes were assigned to each treatment, and color and weight of cantaloupes were measured during storage for up to 14 days. For color measurements, three spots at different locations on each cantaloupe were measured during storage. The same three spots were measured at each sampling time. The instrument was a MiniScan XE Plus Hunter colorimeter (Hunter Associates Laboratory, Inc., Reston, VA). Lightness (L^*) and chromaticity parameters a^* (green to red) and b^* (blue to yellow) in the CIELab scale were reported.