**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 $> 3 \log \text{CFU}/\text{cm}^2$ 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.

Keywords: Cantaloupe, chitosan, lauric arginate, cinnamon oil, EDTA, coating.

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). 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., 2016). Moreover, chitosan-based film discs containing LAE, CO and EDTA

showed large inhibition zones against the above microorganisms when tested on agar plates (Ma, Zhang and Zhong, 2016).

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, Zhang, Critzer, et al., 2016; Ma, Zhang and Zhong, 2016; 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, Zhang and Zhong, 2016), 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

126 room temperature (21°C). 100 µL culture with about 10⁸ CFU/mL bacteria was inoculated on
127 pre-marked 6.25 cm² squares on the cantaloupes. Two squares on each of 2 cantaloupes were
128 inoculated for each bacterium and each coating treatment. After inoculation, cantaloupes were
129 dried for 6 h at room temperature (21°C) to allow the bacteria attach to the surface of
130 cantaloupes before treatment.

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

136 ***2.5. Enumeration of foodborne pathogens***

137 Selective media were used to reduce or eliminate the interference of background microorganisms.
138 Cefixime-tellurite sorbital MacConkey (CT-SMAC), xylose lysine tergitol 4 agar (XLT4), and
139 modified oxford agar (MOX) were used for *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes*,
140 respectively. Treated areas of cantaloupe rind squares were excised using a sterile knife on day 1,
141 3, 7, 10 and 14. The squares were placed into sterile blender bags (Thermo Fisher Scientific, Inc.,
142 Waltham, MA) containing 25 mL sterile 10 mM phosphate buffered saline (PBS, pH 7.4) and 0.2%
143 Tween 80 and hand-massaged for 1 min. The rinsate was then serially diluted in 0.1% w/v
144 peptone water and surface plated on CT-SMAC plates for *E. coli* O157:H7, XLT4 plates for *S.*
145 *enterica*, or MOX plates for *L. monocytogenes*. Counting of colonies was carried out after 24-h
146 incubation at 37°C for *E. coli* O157:H7 and *S. enterica*, or 48-h incubation at 32°C for *L.*
147 *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.

2.6.2. Firmness and total soluble solids (TSS) measurement

Three cantaloupes in each treatment were used to measure firmness and TSS content. Each cantaloupe was longitudinally cut into four parts and each part was sampled with a sterile cylindrical borer (diameter = 22 mm) in the center. Then discs were cut to a thickness of 10 mm by cutting the cylindrical flesh near the rind of cantaloupe. Firmness was measured using a TA.XTplus Texture Analyzer in the compression mode (Texture Technologies Corp., Scarsdale, N.Y.). A flat head stainless steel cylindrical probe with a diameter of 7 mm was used to puncture the flesh discs vertically at a speed of 50 mm/min. Firmness was defined as the force (N)

required to puncture the flesh disc (Mahmoud, 2012). TSS of each flesh disc was measured after squeezing one drop of juice from the flesh disc onto a digital refractometer mirror (Thermo Fisher Scientific, Inc., Waltham, MA).

2.7. Enumeration of total molds and yeast

Rind discs (diameter = 22 mm) from section 2.6.2 were used to enumerate the total molds and yeast on cantaloupes. Four rind discs of each cantaloupe were put into sterile blender bags containing 25 mL sterile 10 mM PBS (pH 7.4) and 0.2% Tween 80 and hand-massaged for 1 min as described in section 2.5. The total populations of molds and yeast of uncoated and coated cantaloupes were enumerated on dichloran rose bengal chloramphenicol agar (DRBC) after a 5-day incubation at room temperature (21°C). In addition, 24 cantaloupes (divided into 3 groups) in each treatment were observed for visible mold growth during ambient storage for up to 14 days, and the percentages of cantaloupes with visible mold were reported for different coating treatments.

2.8. Statistical analysis

Experiment data was analyzed using Tukey's test in SPSS 20 (IBM, Armonk, NY) at a 5% significance level.

3. Results

3.1. Coating effect on microbial growth on cantaloupes

As shown in Fig. 1A, coating treatments significantly reduced the viable cell counts of *E. coli* O157:H7 on day 1. However, only the coating treatment with 0.1% LAE, 0.1% EDTA and 1%

CO effectively inhibited the recovery of *E. coli* O157:H7 after day 3 and resulted in a more than 3 log CFU/cm² reduction of *E. coli* O157:H7 after 14 days. For *S. enterica* (Fig. 1B), coating treatments with LAE and EDTA, with and without CO, reduced the viable cell counts to the detection limit after day 1, and no recovery was observed during storage. Conversely, some slight recovery of *S. enterica* was observed in the treatment of chitosan only on day 7 and day 14. For *L. monocytogenes* (Fig. 1C), viable cell counts were significantly reduced after coating treatments (day 1), with about 3 to 4-log CFU/cm² reduction. The treatment with 0.1% LAE, 0.1% EDTA and 1% CO showed the greatest inhibition of *L. monocytogenes* during storage, followed by treatment with 0.1% LAE, 0.1% EDTA and 0.5% CO. Overall, the chitosan coating with 0.1% LAE, 0.1% EDTA and 1% CO was the most effective in inhibiting the growth of all three tested pathogens on cantaloupes.

Fig. 2 shows that coating treatments reduced the molds and yeast to below the detection limit on day 2. In contrast, molds and yeast counts on the uncoated cantaloupes reached 3.8 log CFU/cm². Molds were visible on uncoated cantaloupes from day 2. They appeared on day 4 and day 5 for treatments with chitosan only and those containing 0.1% LAE, 0.1% EDTA and 1% CO (Fig. 3). On day 5, uncoated cantaloupes and cantaloupes coated with chitosan only had much higher percentage of cantaloupes with visible mold (56.3% and 50%, respectively) than cantaloupes in the other treatments (25%). The chitosan coating containing 0.1% LAE, 0.1% EDTA and 1% CO delayed the growth of native molds and yeasts on cantaloupes compared to other treatments (Fig. 3).

3.2. Quality properties of cantaloupes

To study the effect of coating treatments on quality properties of cantaloupes, color, weight loss, firmness, and TSS content of cantaloupes were measured during storage. As shown in Fig. 4A,

redness of uncoated cantaloupes was significantly higher than that of coated cantaloupes after day 6, and no significant differences in redness were found among coating treatments. Similarly, after day 6, yellowness of uncoated cantaloupes was much higher than that of cantaloupes coated with chitosan containing antimicrobials, but no difference was found for uncoated cantaloupes and those coated with chitosan only (Fig. 4B). Lightness of uncoated cantaloupes was significantly higher than that of coated ones after day 2 (Fig. 4C). Color change results indicated the coating treatments, especially coatings containing the antimicrobials tested, slowed the ripening process of whole cantaloupes.

Correspondingly, firmness of uncoated cantaloupes was lower than that of coated cantaloupes on day 6 and the differences became significant on day 10 ($p < 0.05$) (Fig. 5). Cantaloupes coated with chitosan only were significantly softer than those with additional LAE, EDTA, and/or CO from day 10. However, no significant difference was found in weight loss (Fig. 6) and total solids content (Fig. 7) of cantaloupes among all treatments during storage.

4. Discussion

Chitosan-based coatings containing 0.1% LAE, 0.1% EDTA and 1% CO effectively inhibited the growth or inactivated foodborne pathogens tested (Fig. 1). *S. enterica* was particularly sensitive during 14-day storage at room temperature (21°C). Cocktails of *E. coli* O157: H7 and *L. monocytogenes* were more resistant to coating treatments, although in our previous study, inhibition zones of chitosan-based films incorporated with LAE, EDTA and CO against *L. monocytogenes* were detected to be larger than those of *E. coli* O157:H7 and *S. enterica* on agar plates (Ma, Zhang and Zhong, 2016). Microbial attachment, colonization and survival on the

surface of fresh produce can be the factors causing different inactivation rates (Trinetta et al., 2013). *E. coli* O157: H7 and *L. monocytogenes* were reported to be more capable of attaching on the surface of lettuce leaves than *S. Typhimurium* (Takeuchi et al., 2000). In another study, *S. Typhimurium* was found to produce fewer microcolonies and had poorer survivability on peach and plum than *E. coli* O157: H7 and *L. monocytogenes* (Collignon and Korsten, 2010). In our study, higher populations of *E. coli* O157: H7 and *L. monocytogenes* than *S. enterica* were detected on the surface of untreated cantaloupes during storage (Fig. 1), which indicates differences in the abilities of these bacteria to attach and survive on cantaloupes. Additionally, Gorski et al. (Gorski et al., 2003) reported that attachment of *L. monocytogenes* 10403 on radish tissue was better at 20°C than at 37°C; thus, the test temperature (21°C) used in our study may favor attachment of *L. monocytogenes* as well. The inoculated bacteria also declined on uncoated cantaloupes during storage; the limited nutrients and competitive growth of native microflora on the surface of cantaloupes may have contributed to the decline during storage (Fig. 1). A similar phenomenon was observed in another study (Ukuku et al., 2001).

The coating treatments significantly reduced total molds and yeasts on the surface of cantaloupes (Fig. 2) and delayed the appearance of visible molds (Fig. 3) indicating the potential of the coating treatments to extend the shelf life of cantaloupes. However, visible mold growth did occur on coated cantaloupe after 4 days of storage (Fig. 3). One possible reason for visible surface mold growth is that the coating may not totally block access to oxygen which is required for mold growth. Similar recovery of molds and yeast was also observed after treatment of cantaloupes using chlorine gas (Trinetta et al., 2013) and X-ray (Mahmoud, 2012). Incomplete inhibition of yeasts was also observed after treating cantaloupes with 0.7 and 1.5 kGy electron

beam (Palekar et al., 2015). This indicates a need for other strategies to effectively inhibit molds and yeasts on cantaloupes throughout normal shelf life.

Ripening of cantaloupes during storage, in terms of color and firmness changes, was significantly delayed by the coating treatments, especially that with 0.1% LAE, 0.1% EDTA and 1% CO (Figures 4-5). Similar results have been reported by others. A coating comprised of 10% gum arabic and 0.4% CO maintained the firmness of banana and papaya during storage at $13 \pm 1^\circ\text{C}$ and $12 \pm 1^\circ\text{C}$ for 28 d (Maqbool et al., 2011). Coatings with 1% w/v hydroxypropylmethylcellulose or chitosan with and without 2% bergamot EO maintained the firmness of grapes stored at $1\text{--}2^\circ\text{C}$ for 22 days (Sánchez-González et al., 2011). The reason for the delayed ripening was unclear; however, lower respiration rate or decreased enzyme activity which are responsible for cantaloupe ripening could be caused by coating treatments (González - Aguilar et al., 2009; Hernández-Muñoz et al., 2006; Jitareerat et al., 2007). Another contributing factor may involve ethylene production. Ethylene plays a critical role in the regulation of the ripening process including de-greening of cantaloupe rind and softening of the pulp (Flores et al., 2008; Pech et al., 2008). Microorganisms, such as *Botrytis cinerea* can facilitate ethylene production of fresh produce through plant-microorganism interaction (Cristescu et al., 2002; Toppan and Esquerré-Tugayé, 1984). In one study, a reduction of the growth of spoilage microorganisms by EO was correlated with reduced ethylene production in table grapes (Valverde et al., 2005). In our study, inhibition of total molds and yeasts was observed in all coating treatments with antimicrobials (Fig. 2), which may contribute to the reduced ethylene production and delayed ripening of cantaloupes. However, the specific role of the antimicrobials must still be elucidated. Lastly, no significant differences of weight loss and total soluble solids content were found between coated and uncoated cantaloupes indicating the

thin coating was not able to affect these parameters, corresponding to the high water vapor permeability of chitosan-based films containing LAE, CO, and EDTA in our previous study (Ma, Zhang and Zhong, 2016).

5. Conclusions

Chitosan-based coatings with LAE, EDTA, and CO significantly inhibited the growth of *E. coli* O157:H7, *L. monocytogenes* and *S. enterica* cocktails on whole cantaloupes during 14-day storage at ambient temperature (21°C). Coatings also significantly reduced total mold and yeast counts on whole cantaloupes. Chitosan-based coating with 0.1% LAE, 0.1% EDTA, and 1% CO was observed to be the most effective in inhibiting pathogenic and spoilage microorganisms during the 14-day storage. The antimicrobial coatings also delayed the changes of color and firmness of cantaloupes during storage. These observations suggest that these novel coating formulations have potential to improve the safety and quality of whole cantaloupes.

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Figure captions

Fig. 1. Growth kinetics of cocktails of *E. coli* O157:H7 (A), *S. enterica* (B), and *L. monocytogenes* (C) on cantaloupes during storage at room temperature (21°C) up to 14 days. The inoculated cantaloupes were coated with 1% chitosan only or with additional 0.1% w/w LAE, 0.1% w/w EDTA, and 0, 0.5 or 1% w/w CO. The detection limit was 1.6 log CFU/cm². LAE+EDTA and LAE+EDTA+0.5% CO treatments after day 1 were at the detection limit in Figure B. Error bars are standard deviations from two squares obtained from each of two cantaloupes (n = 4).

Fig. 2. Populations of total molds and yeast on cantaloupe surfaces after coating (day 2) with chitosan only or with additional 0.1% w/w LAE, 0.1% w/w EDTA, and 0, 0.5 or 1% w/w CO. The detection limit was 1.2 log CFU/cm². Error bars are standard deviations from four rind discs obtained from each of three cantaloupes (n = 3). Different letters above bars indicate significant differences in the mean ($p < 0.05$).

Fig. 3. Percentages of cantaloupes with visible mold during storage at room temperature (21°C). Cantaloupes were coated with chitosan only or with additional 0.1% w/w LAE, 0.1% w/w EDTA, and 0, 0.5 or 1% w/w CO. Each treatment had 3 groups of cantaloupes with 8 cantaloupes in each group. Error bars are standard deviations from 3 groups of cantaloupes (n = 3).

Fig. 4. Changes of cantaloupe colors during storage at room temperature (21°C). Cantaloupes were coated with chitosan only or with additional 0.1% w/w LAE, 0.1% w/w EDTA, and 0, 0.5 or 1% w/w CO. Error bars are standard deviations from three measures on each of four cantaloupes (n = 12). Different letters above bars indicate significant differences in the mean ($p < 0.05$).

Fig. 5. Changes of cantaloupe firmness during storage at room temperature (21°C). Cantaloupes were coated with chitosan only or with additional 0.1% w/w LAE, 0.1% w/w EDTA, and 0, 0.5 or 1% w/w CO. Error bars are standard deviations from four flesh discs obtained from each of three cantaloupes (n = 12). Different letters above bars indicate significant differences in the mean ($p < 0.05$).

Fig. 6. Changes of total soluble solids contents of cantaloupe flesh during storage at room temperature (21°C). Cantaloupes were coated with chitosan only or with additional 0.1% w/w LAE, 0.1% w/w EDTA, and 0, 0.5 or 1% w/w CO. Error bars are standard deviations from four flesh discs obtained from each of three cantaloupes (n = 12). Different letters above bars indicate significant differences in the mean ($p < 0.05$).

Fig. 7. Percentage weight loss of cantaloupes during storage at room temperature (21°C). Cantaloupes were coated with chitosan only or with additional 0.1% w/w LAE, 0.1% w/w EDTA, and 0, 0.5 or 1% w/w CO. Error bars are standard deviations from four cantaloupes (n = 4). Different letters above bars indicate significant differences in the mean ($p < 0.05$).

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