Muskmelon Fruit Quality in Response to Postharvest Essential Oil and Whey Protein Sprays

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Abstract. The consumption of fresh muskmelons (Cucumis melo reticulatus L.) has been linked to severe illness outbreaks due to contamination with bacterial pathogens. Antimicrobial essential oils (EOs) were incorporated into wash water sprays and evaluated as potential agents for postharvest disinfection of ‘Athena’ muskmelons. Freshly harvested fruits were sprayed with 0.5% EOs from cinnamon leaf, thyme, or clove bud emulsified in a whey protein emulsion (WP) as potential washing disinfectants, together with deionized water, water with 200 μL·L\(^{-1}\) free chlorine (pH 7, free turbidity), or oil-free WP as controls. Melons were treated, stored at 4 °C and then evaluated weekly for weight loss, rind color, mesocarp firmness and the compositional quality traits soluble solids content (SSC), pH, β-carotene content, and total ascorbic acid (AsA) for up to 21 days. Essential oil–treated melons were not different from controls in fruit quality and composition with the exception of fruits treated with thyme oil, which were statistically lower in SSC (0.8°Brix) than those treated with water or cinnamon oil treatment. Internal carbon dioxide was statistically higher (>0.1% higher in value, equal to a 25% increase) in muskmelons receiving whey protein–based treatments after storage for at least 7 days. Overall, our results suggest that EOs as disinfectants have little effect on quality or composition of muskmelon fruit.

The ripening rate was slower, as indicated by firmer fruit and lower color indices (a and b) in whole muskmelon 15 d after treating with a coating containing 2% cinnamon bark oil compared with the use of a coating that did not contain the oil. In contrast, Raybaudi-Massilia et al. (2008) reported that flesh firmness decreased when cut melons were treated with an edible coating containing 0.7% lemongrass oil in comparison with melons without EOs. No phytotoxicity to muskmelons in response to these EOs was reported. One challenge in using EOs as postharvest washing disinfectants is the incorporation of the hydrophobic oils into aqueous solutions. A whey protein–based emulsion has been used to successfully disperse 0.5% clove bud oil (CBO) into water. This emulsion has been shown to reduce contaminants in wash water by 2–3 log cfu/g and prevent cross-contamination of S. enterica Enteritidis. E. coli O157:H7, and L. monocytogenes (Luo et al., 2014), offering a potential for use in postharvest wash water. The objectives of the following experiments were to determine postharvest effects of EO emulsions on muskmelon external and internal attributes to determine their suitability for larger packhouse wash systems.

Materials and Methods

Treatments. Three EOs and three controls were used for muskmelon treatments. The EOs (Sigma-Aldrich, St. Louis, MO) were thyme oil (TO, from Thymus vulgaris, Thymus zygis, or both, W306509), cinnamon leaf oil (CN, Ceylon type, Cinnamomum zeylanicum, W229202), and CBO (Eugenia spp., W232300). The three EOs were chosen based on their high antimicrobial efficacy on the most common foodborne bacterial pathogens (such as E. coli O157:H7, L. monocytogenes, but the requirement for on-site permitting, handling, and usage of this gas limits its application in small-scale operations (Gómez-López et al., 2009).

Many EOs, such as oregano, rosemary, cinnamon, clove, and thyme, have strong antimicrobial properties (Burt, 2004). Studies with lettuce (Ponce et al., 2011), cherry tomatoes (Yun et al., 2013), grape (Tripathi et al., 2008), and banana and papaya (Maqbool et al., 2011) have been conducted to determine the efficacy of EOs for postharvest microbial control and indicated promise for the use of EOs as antimicrobial agents on fresh produce.

EOs can influence microbial concentrations in postharvest muskmelons and on the quality of the fruits. A >5 log cfu/g reduction of L. monocytogenes was achieved when fruits were washed with 2% thymol at 65 °C for 5 min (Upadhyay et al., 2014), but the quality of fruit after this treatment was not reported. Zhang et al. (2015) showed that applying an alginate coating with 2% cinnamon bark oil to whole fruits achieved a >4 log cfu/g reduction of S. enterica and E. coli O157:H7 and a >5 log cfu/g reduction of L. monocytogenes. The ripening rate was slower, as indicated by firmer fruit and lower color indices (a and b) in whole muskmelon 15 d after treating with a coating containing 2% cinnamon bark oil compared with the use of a coating that did not contain the oil. In contrast, Raybaudi-Massilia et al. (2008) reported that flesh firmness decreased when cut melons were treated with an edible coating containing 0.7% lemongrass oil in comparison with melons without EOs. No phytotoxicity to muskmelons in response to these EOs was reported.

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and S. enterica). Compounds in each of these EOs were verified by gas chromatography–mass spectrometry (Jiang, 2016) with the most abundant compounds being thymol (42.1%) in TO, eugenol (54.2%) in CN, and eugenol (80.5%) in CBO. Each of these EOs were dissolved into a whey protein–based emulsion containing 1% v/v oil and 1% w/w whey protein following the method of Luo et al. (2014). These stock emulsions were adjusted to pH = 6.7 with citric acid and were used to prepare final treatment concentrations of 0.5% (pH 6.9) for each of these EOs, and each solution contained 0.5% of whey protein emulsion.

The three control treatments were deionized water (DI), pH 7.0; WP prepared as previously described but without the addition of EOs and then diluted to 0.5% with deionized water, pH 6.9; and CL with 200 ppm chlorine (Clorox Company, Oakland, CA), pH 7.0.

Two experiments were conducted with EOs on muskmelons. In the first experiment, we used three EOs and two controls (CL and DI) to compare the new disinfectants with the existing industry standard. In the second experiment, we used two EOs (TO and CBO) and three controls (WP, CL, and DI), based on preliminary data indicating that WP may have an effect on the gas permeability of melons.

Plant material and handling. Commercially grown ‘Athena’ muskmelons were harvested from Woodleaf, NC at the full-slip stage, selected for the uniformity of size and absence of blemish, rinsed with tap water to remove soil, air-dried for 15 min, and sprayed with treatment and control solutions using backpack sprayers (Solo USA, NewPort News, VA) within 2 h after harvest. Each melon was manually rotated throughout the whole 20 s spray application at 207 kPa and was then allowed to air-dry for 15 min. Melons were then separated by treatment and storage period, and five or six fruits (depending on the experiment) were stored in 220-L plastic containers. Containers had loosely fitted lids to which pet training pads (Pet All Star; All Star Pet Care Inc., Chicago, IL) moistened with distilled water were fastened to maintain high relative humidity (RH). An environmental data logger (Hobo U12-011; Onset Computer Corporation, Bourne, MA) was placed inside each container to monitor temperature and RH. Containers were held at constant 4 °C, and RH inside the containers was 80% to 95%.

Fruit quality and composition (Experiment 1). Musk melons were harvested (weight 1520 g on average) and treated on 7 and 14 July 2014 according to previously described harvest and treatment protocols. Of the 111 melons used from each of the two harvests, 11 untreated were sampled for mesocarp composition on the day of harvest, and the remaining 100 were separated randomly into five groups of 20 melons to receive five treatments (TO/WP 0.5%, CN/ WP 0.5%, CBO/WP 0.5%, CL control, and DI control). Five of the 20 melons from each treatment were selected at random for nondestructive testing of weight loss and rind color change during 21 d of storage, with a 7-d interval. Of the remaining 15 fruits/treatment, five were selected for destructive mesocarp sampling at each of 7, 14, and 21 d of storage.

Weight loss and rind color ratings were determined at harvest and after 7, 14, and 21 d of storage. Weight loss was monitored by weighing individual melons on an electronic balance (3200 PL; Mettler Toledo, Columbus, OH). Subjective ratings of rind color were done visually in two areas on the fruit surface, the ground spot (bottom) and the surface away from the ground spot (top), using a 1 to 6 scale with 1 being extremely green and 6 being extremely yellow/brown. Color ratings from the top and bottom of each melon were averaged and noted as “rind color.”

Mesocarp analyses, performed at harvest and after 7, 14, and 21 d of storage, included mesocarp firmness, pH, SSC, β-carotene content, and total AsA content. Each melon was cut longitudinally through four locations of a melon (blossom end, stem end, top, and bottom) to obtain a 3-cm thick cut slice from the center. Fruit flesh firmness (penetration) was measured at four locations on the 3-cm slice (blossom end, stem end, top, and bottom) using a force gauge (FDX5 Force One; Wagner Instruments, Greenwich, CT) with a force cell module of 25 × 0.02 N and a stainless steel probe with a flat 0.8-cm diameter surface. A 0.5-cm diameter cork borer was used to create six cylinders of mesocarp samples from each slice, at each of the four locations. These samples were then separated into two subsamples. One subsample was stored at −20 °C for 3–4 weeks before analysis of pH, SSC, and β-carotene, and the other subsample was held at −80 °C up to 3 weeks for total AsA analysis.

Frozen mesocarp samples were thawed and pureed using a homogenizer (PT 10-35 GT; Kinematica Inc., Bohemia, NY). For each puree sample, β-carotene content was measured spectrophotometrically using the method of Fish et al. (2002). β-Carotene (μg·g⁻¹ fresh weight) was determined using the absorbance at 453 nm, a molecular weight of 537 g·mol⁻¹, and a molar extinction coefficient of 13.9 · 10⁴/M/cm for β-carotene in hexane (Zeichmeister and Polgar, 1943). After the analysis of β-carotene, puree samples were warmed to room temperature; pH was measured using a pH meter (HI260G; Hach, Loveland, CO) equipped with a stainless steel rounded electrode (PH77-SS; Hach); and SSC (°Brix) was measured using a digital refractometer (Atago PAL-1; Atago Inc., Bellevue, WA).

Total AsA was extracted with 3% metaphosphoric acid (MPA) and quantified following the method of Chebrolu et al. (2012) with modifications. Samples stored at −80 °C were thawed and pureed as previously described, and 0.2 g puree was mixed with 1 mL of MPA. The sample was sonicated for 5 min at room temperature (Ultrasonic Cleaner 3510 DTH; Branson, Danbury, CT) and centrifuged for 15 min at 18,292 g at 4 °C in a micro centrifuge (5417R, Eppendorf®; Fisher Scientific, Pittsburgh, PA). The supernatant was collected and filtered through a 0.2 μm, 17-mm nylon syringe filter (F2513-2; Thermo Scientific, Grand Island, NY). Freshly made 10-mv tris (2-carboxyethyl) phosphine hydrochloride was added to the extraction in a 1:1 ratio, and the final solution was mixed well and incubated in the dark at room temperature for 35 min to convert dehydroascorbic acid into L-ascorbic acid.

Total AsA was measured using high performance liquid chromatography (HPLC). Filtered samples (20 μL) were injected into a Hitachi Elite LaChrom (Hitachi Ltd., San Jose, CA), equipped with a ultraviolet-LS diode array detector (DAD), controlled temperature auto sampler (4 °C), and column compartment (30 °C). D-2000 software (Hitachi Ltd.) was used as the system controller and for data processing. Ascorbic acid detection and quantification were performed using a reversed phase C18 column (Synergi 4 μm Hydro-RP; Phenomenex Inc., Torrance, CA). The mobile phase consisted of 0.0065 N H₂SO₄ with a flow rate of 1 mL·min⁻¹. Total AsA content was calculated from standard curves generated by injecting 20 μL of L-ascorbic acid (A7506; Sigma-Aldrich) and reported as mg/100 g fresh weight.

Musk melon internal carbon dioxide (Experiment 2). A preliminary experiment showed that melons treated with EOs in the WP appeared to have elevated internal carbon dioxide (CO₂) after storage for 10 d at 4 °C (Jiang, 2016), so muskmelon internal CO₂ was investigated further in 2015. On each of the two harvest dates (24 July and 27 July), thirty-six muskmelons with an average weight of 2245 g were harvested and sprayed with treatments following protocols in “Plant material and handling.” Internal gas samples were taken from six untreated melons immediately on each harvest, using the protocol described below. The remaining 30 fruits at each harvest were divided randomly into groups of six to receive one of the five following treatments: TO/WP 0.5%, CBO/WP 0.5%, WP control, CL control, and DI control. Treatment application and storage were as described previously, with six fruits of the same treatment placed per 220-L plastic container. Internal gas was measured after 7 and 14 d storage at 4 °C, 80% to 95% RH.

Gas was sampled from melon cavities by inserting a 23 gauge × 3.8 cm needle into the stem end. A 10 mL gas sample was pulled into a 10-mL plastic syringe and then transferred to a 15-mL evacuated airtight test tube through a rubber septum to avoid direct introduction of moisture from the melon to the gas chromatography (GC) column. This transfer of gas resulted in a 1.5 time dilution of the original sample. The diluted gas was mixed well by inverting the tube three times, before injecting 1 mL of the gas into a gas
chromatograph (Shimadzu Model 1400; Shimadzu, Durham, NC) using a 1-mL gas-tight syringe (Hamilton 1001 LTN, 22 ga, 50.8 mm, point style 2; Hamilton Laboratory Products, Reno, NY). The GC was equipped with a 2 m × 6 mm concentric packed column (CTR I; Alltech, Fisher Scientific) connected in series to a flame ionization detector and a thermal conductivity detector, with He carrier gas flowing at 25 mL·min⁻¹ to measure CO₂ and ethylene (C₂H₄). The injector and detector temperatures were 150 °C, and the column temperature was 100 °C. External CO₂ and C₂H₄ standards were used to quantify gasses.

**Experimental design and statistical analysis.** A completely randomized design was used in both experiments, each consisting of two harvests. Repeated measurements were collected throughout the storage periods of each experiment and each harvest. All data were subjected to mixed model analysis using SAS 9.4 (SAS Institute, Cary, NC). The nondestructive measurements, weight loss and rind color, were analyzed using Restricted Maximum Likelihood and Repeated Measure options in the mixed model, where disinfectant treatment (D), storage time (S), and the D × S interaction were fixed effects, and harvest (H), H × D, H × S, and Melon ID (tracked ID for each melon) were random effects. The composition measurements were analyzed with Type III Analysis of Variance (ANOVA) option in the mixed model, where disinfectant treatment (D), storage time (S), and the D × S interaction were fixed effects, and harvest (H), H × D, H × S, and Melon ID (tracking ID for each melon) were random effects. The composition measurements were analyzed with Type III Analysis of Variance (ANOVA) option in the mixed model, where fixed effects were D, S, and D × S, and random effects were H, H × D, H × S, and H × D × S. Internal CO₂ data were also analyzed with Type III ANOVA. Because the data for 7-d storage from harvest I were missing because of handling mistakes, the best model was obtained by including only D as the fixed effect and H × S as a random effect. For all the variables, multiple pairwise comparisons among treatments were made using Tukey’s honestly significant difference (P ≤ 0.05).

**Results and Discussion.** During 21 d storage, muskmelons exhibited weight loss, rind darkening, and decreased mesocarp firmness regardless of disinfectant treatments (Tables 1 and 2). Zhang et al. (2015) reported that muskmelons treated with just an alginate coating were less firm than when treated with an alginate coating that contained 2% cinnamon bark oil (containing 62% to 68% of cinnamaldehyde) (Baratta et al. 1998; Simić et al., 2004). The lack of firmness retention in our EO-treated melons may be due to the different coating or the different chemical composition of CN used (54% eugenol).

Storage time did not have a significant effect on any compositional attribute (Table 2); the averages for flesh pH and SSC were 6.62 ± 0.26 and 8.1 ± 1.5 *Brix*, respectively, with 23.5 ± 2.9 µg·g⁻¹ of β-carotene and 20.1 ± 5.8 mg·100 g⁻¹ of total AsA.

‘Galia’ muskmelon harvested at an advanced ripening stage and held for 21 d at 15 °C had no change in mesocarp pH (Ergun et al., 2005). Zhao et al. (2011) reported that AsA initially remained stable in ‘Galia’ melons harvested at half-slip but then decreased significantly after 13 d storage at 13 °C. No change of β-carotene was observed in a range of orange-fleshed muskmelon cultivars that were harvested at full-slip and stored at 7 °C (14 d) and 21 °C (3 d) (Hodges and Lester, 2006). In agreement with previous studies, our results suggested that there were no significant changes in the composition of muskmelon mesocarp over 3 weeks in storage under low temperature (4 °C).

Changes in SSC of stored muskmelon have been reported as neutral (Ergun et al., 2005; Lester, 1989), decreased (10% to 17%) (Ferrante et al., 2008; Muniz et al., 2008), or increased (19%) followed by decreased (35%) (Zhao et al., 2011), depending on cultivar, ripeness, and storage temperature, and duration. In our study, SSC was affected by disinfectant treatment (P = 0.01). Melons sprayed with the TO had a lower SSC ( < 0.05) than those treated with CN or DI but were not different from the CBO treatment or CL control (Table 3). We hypothesize that TO induced oxidative stress in the peel or outer mesocarp of muskmelons, changing localized carbohydrate metabolism and transport in the fruit and subsequently decreasing flesh SSC.

No ethylene was detected in the cavity of melons stored at 4 °C for 7–14 d. Internal CO₂ content was ± 0.1% higher in stored muskmelon with whey protein–based treatments, with or without EOs (Table 3), whereas the water-based control treatments DI and CL had less internal CO₂. Whey protein is a well-known and excellent gas barrier in the food coating industry (Hong and Krochta, 2006; Miller and Krochta, 1997), and inclusion of whey protein in the emulsions used on muskmelon may have decreased gas permeability, resulting in slightly higher internal CO₂ content (still below 0.5%). However, this small increase in CO₂ is unlikely to have an off-flavor effect as a 5% to 7% internal CO₂ level was needed for consumers to detect off-flavor in coated ‘Galia’ melons (Fallik et al., 2005).

### Table 1. P values for variance components of all shelf life and composition variables of muskmelon collected through Experiment 1 and Experiment 2.

| Variance component | Wt loss | Surface color | pH | Soluble solids content | β-carotene | Total ascorbic acid | Flesh firmness | Internal carbon dioxide |
|--------------------|---------|---------------|----|------------------------|------------|---------------------|---------------|------------------------|
| Disinfectant (D)   | 0.58    | 0.50          | 0.22 | 0.01                  | 0.84       | 0.56                | 0.03          | <0.01                  |
| Storage time (S)   | 0.01    | 0.01          | 0.21 | 0.15                  | 0.12       | 0.51                | 0.02          | —                      |
| S*D                | <0.01   | 0.22          | 0.69 | 0.28                  | 0.22       | 0.20                | 0.20          | 0.85                   |
| Harvest (H)        | 0.37    | 0.28          | 0.10 | 0.29                  | 0.57       | 0.45                | 0.76          | —                      |
| H*D                | 0.16    | 0.29          | 0.28 | 0.95                  | 0.16       | 0.08                | 0.94          | 0.60                   |
| H*S*D              | 0.18    | 0.18          | 0.03 | 0.28                  | 0.48       | <0.01               | 0.39          | 0.44                   |
| Melon ID           | <0.01   | <0.01         | —   | —                     | —          | —                   | —             | —                      |

*Analysis was performed with mixed model (SAS Enterprise Guide 6.1; SAS Institute Inc., Cary, NC). Variance components shown in italics were random effects. Depending on individual data structure, the best model was identified for each variable, and variance components that were not included for a particular variable were marked as “—”.

### Table 2. Influence of storage time on ‘Athena’ muskmelon quality.

| Days of storage | Wt loss (%) | Rind color | Flesh firmness (N) | pH | Soluble solid content (°Brix) | β-carotene (µg·g⁻¹ FW⁻¹) | Total ascorbic acid (mg/100 g FW⁻¹) |
|-----------------|------------|------------|--------------------|----|-----------------------------|--------------------------|-------------------------------|
| 0               | 0          | 4.1 b      | 14.16 a            | 6.85 | 8.4                         | 25.03                     | 19.78                         |
| 7               | 1.0 c      | 4.2 b      | 11.60 b            | 6.69 | 8.5                         | 24.09                     | 20.10                         |
| 14              | 1.9 b      | 4.4 a      | 9.40 c             | 6.60 | 8.0                         | 23.41                     | 22.94                         |
| 21              | 2.6 a      | 4.6 a      | 7.86 d             | 6.45 | 7.7                         | 22.13                     | 17.64                         |
| P value         | 0.01       | 0.01       | 0.02               | 0.21 | 0.15                        | 0.12                      | 0.51                          |

*Data combined all disinfectant treatments for each storage time because of a lack of effect for disinfectant treatment, except for soluble solids content.

*Rind color was determined by averaging subjective ratings for top (away from the ground spot) and bottom (ground spot) of each melon on a 1–6 scale, with 1 being greenest and 6 being most orange/brown.

*FW⁻¹ = fresh weight.

*Multiple comparisons were made for each parameter (by column) when P value for storage time was significant (P ≤ 0.05), and values with the same letter were not significantly different at P ≤ 0.05 using Tukey’s honestly significant difference.
Table 3. Disinfectant effect on postharvest quality attributes and internal carbon dioxide content of ‘Athena’ muskmelon after storage at 4 °C.

| Disinfectant treatment | Wt loss (%) | Rind color | Flesh firmness (N) | pH | Soluble solid content (° Brix) | β-carotene (µg/g FW) | Total ascorbic acid (mg/100 g FW) | Internal carbon dioxide concn (%) |
|------------------------|-------------|------------|--------------------|----|-------------------------------|--------------------|-------------------------------|-------------------------------|
| CN/WP                  | 1.9         | 4.4        | 35.94 a            | 6.57| 8.3 a                          | 23.55              | 21.43                         | NA                            |
| CBO/WP                 | 1.9         | 4.5        | 35.96 a            | 6.64| 8.1 ab                         | 23.17              | 19.28                         | 0.40 ab                       |
| TO/WP                  | 1.8         | 4.3        | 32.80 a            | 6.48| 7.5 b                          | 23.01              | 20.14                         | 0.40 ab                       |
| DI                     | 1.5         | 4.2        | 34.06 a            | 6.64| 8.4 a                          | 23.73              | 21.77                         | 0.29 c                        |
| CL                     | 2.2         | 4.3        | 32.96 a            | 6.59| 8.0 ab                         | 22.83              | 18.27                         | 0.33 bc                       |
| WP                     | NA          | NA         | NA                 | NA | NA                            | NA                 | NA                            | NA                            |
| P value                 | 0.58        | 0.50       | 0.03               | 0.22| 0.01                          | 0.84               | 0.56                          | <0.01                         |

Storage time for each parameter was combined because of a lack of effect on storage time, except for weight loss, rind color and flesh firmness.

This project was designed as a proof of concept to determine if EOs can cause any negative effects on melons. Although it was funded by an Organic Research & Extension Initiative grant, only conventional produce was available for these experiments. Because the nature of the application is the same despite the cultural practices, results of this study should apply to conventional as well as organic produce. Conventional produce, which also poses risks for microbial contamination, could also benefit if EO application is proved to be effective as an antimicrobial treatment without negative quality impact.

This study focused on the physiological effects and appearance of muskmelon after EO treatments, whereas future studies on potential off-flavor are necessary to determine if EOs have the potential for practical application (Ponce et al., 2011). In addition, the EO products proposed in this study included whey protein as an emulsifier. Although a low cost by-product, whey protein poses allergen concerns and may not conform with certain religious or philosophical dietary restrictions, suggesting its application as a commercial product requires further assessment.

**Conclusion**

Muskmelon weight loss and rind color increased and mesocarp firmness decreased after storage at 4 °C and 80% to 95% RH in response to EO disinfectants emulsified with whey protein. Emulsions incorporating whey protein slightly increased internal CO2 in stored melons. No changes in total AsA, β-carotene, pH, and soluble solids were found through 21 d storage, except for TO, which decreased melon SSC. Our results indicate that use of TO, CBO, and CN as disinfectants had minimal effect on appearance and overall quality of muskmelons.

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