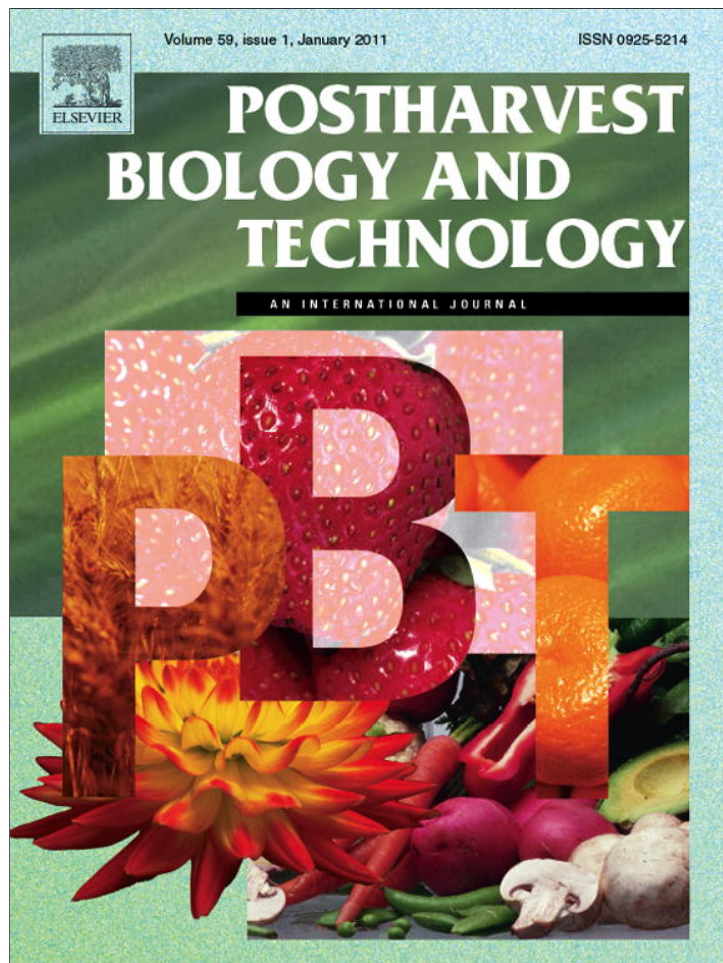


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Effect of edible coatings on the quality of fresh blueberries (Duke and Elliott) under commercial storage conditions

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ABSTRACT

The effects of edible coatings, Semperfresh™ (SF), acid-soluble chitosan (ACH), water-soluble chitosan (WCH), calcium caseinate (CC), and sodium alginate (SA) on the fruit quality of fresh blueberries during storage was studied in 2006 and 2008. Fruit were washed in 200 $\mu\text{L L}^{-1}$ chlorinated water before applying coatings, packaged in vented or non-vented clam-shell containers, and then stored at 2 °C for 1 week, followed by storage at room temperature (20 °C) for up to 15 d for quality evaluation. The ACH, WCH, and WCH+SA coatings helped reduce the decay rate of 'Duke' or 'Elliott' fruit during room temperature storage. Results from 2006 showed that SF coating decreased weight loss of 'Duke' after 6 d of room temperature storage, CC-coated 'Elliott' fruit had delayed fruit ripening as evidenced by higher TA, lower pH, and greater firmness than control during storage, and washing and coating did not significantly affect antioxidant capacity and total phenolics content of 'Duke' and 'Elliott'. Fruit in non-vented containers had reduced weight loss and increased firmness than those in vented containers as demonstrated in 2008 study. Our results suggest that edible coatings have potential for retaining quality of pre-washed, ready-to-eat fresh blueberries under commercial storage conditions, when appropriate coating material, container, and method of applying the coatings are used.

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1. Introduction

Fresh highbush blueberries have a shelf life of 1–8 weeks depending on stage of fruit ripeness, method of harvest, presence of fruit disease, and storage conditions (temperature, relative humidity, and atmosphere; Hancock et al., 2008). Post-harvest respiration and transpiration cause quality deterioration of fresh fruit, limiting shelf life. In addition, bioactive compounds may degrade rapidly during post-harvest storage, partly due to the oxidation of polyphenolics with exposure to light and oxygen (Connor et al., 2002).

Several preservation technologies, including cold storage, UV irradiation, modified atmosphere packaging and ozonation, have been used to reduce deterioration, prolong shelf life, and retain the nutritional value of fresh blueberries (Connor et al., 2002; Zheng et al., 2003; Chiabrando et al., 2006; Trigo et al., 2006). In addition, edible coatings have been studied for extending shelf life of some fresh berry fruits (Park, 1999; Han et al., 2004; Vargas et al., 2006; Ribeiro et al., 2007). Edible coatings may control the internal gas atmosphere of the fruit, minimizing fruit respiration rate (Park, 1999) and may serve as a barrier to water vapor, reducing

moisture loss and delaying fruit dehydration (Baldwin et al., 1995). In addition, some edible coating materials, such as chitosan, have shown delayed decay of the fruit, possibly due to a direct or indirect defense response of the fruit to chitosan (Park et al., 2005). Along with increased interest in ready-to-eat fruit with high quality and safety, edible coatings may provide a means to provide pre-washed, ready-to-eat blueberries – a product not presently available in stores.

Polysaccharides, proteins, lipids and their combinations may be used as coating materials for fresh produce (Baldwin et al., 1995). Chitosan (1,4-linked 2-amino-2-deoxy- β -D-glucan), a derivative of chitin, has excellent film-forming and antimicrobial functions and has been successfully used to control quality loss of fresh strawberries (*Fragaria x ananassa*) and raspberries (*Rubus idaeus*; Han et al., 2004; Park et al., 2005; Vargas et al., 2006; Ribeiro et al., 2007), sliced mango fruits (*Mangifera indica*; Chien et al., 2007), citrus (*Citrus* sp.; Fornes et al., 2005), fresh-cut water chestnut (*Trapa natans*; Pen and Jiang, 2003), and many other fruits and vegetables (Lin and Zhao, 2007). Caseinate, a milk protein-based material, has excellent oxygen barrier properties and has been studied in carrots (*Daucus carota*; Mei and Zhao, 2003), apples (*Malus sylvestris*) and potatoes (*Solanum tuberosum*; Letien et al., 2001), celery (*Apium graveolens* var. *dulce*; Avena-Bustillos et al., 1997), and strawberries (Vachon et al., 2003) for controlling post-harvest respiration. Semperfresh™, a commercial coating product

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of sucrose-fatty acid ester, was reported to effectively decrease weight loss of hardy kiwifruit (*Actinidia arguta*; Fisk et al., 2008), cherry (*Prunus avium*; Yaman and Bayoindirli, 2002), and summer squash (*Cucurbita pepo*; Kaynas and Ozelkok, 1999), and extend shelf life of pineapple (*Ananas comosus*) for up to 5 weeks by preventing moisture loss (Nimitkeatkai et al., 2006). Sodium alginate is a natural linear polysaccharide and has many attractive physical and biological properties, such as moisture retention, gel-forming capability, and good biocompatibility (Pei et al., 2008).

The objectives of this study were to investigate the effectiveness of chitosan, calcium caseinate, Semperfresh™ and sodium alginate based coatings for enhancing the shelf life and retaining the antioxidant properties of pre-washed, ready-to-eat highbush blueberry cultivars under commercial storage conditions.

2. Materials and methods

2.1. Materials

2.1.1. Fruit

This study was conducted in the 2006 and 2008 growing seasons. In 2006, two common fresh market highbush blueberry cultivars, Duke and Elliott, were hand harvested by a commercial picking crew from a farm in Sheridan, Oregon in mid-July and mid-Aug., respectively. In 2008, 'Elliott' fruit were harvest from the same farm in mid-Aug. Harvested fruit were immediately packed in a 177 mL plastic "clam-shell" containers (industry standard) and transported to the Food Science laboratory at Oregon State University, Corvallis, OR, USA (see "Packaging Container and Storage").

2.1.2. Coatings

Food-grade coating materials were used including: acid-soluble chitosan (ACH: Vanson Inc., Redmond, WA, USA; 89.8% deacetylation) extracted from shrimp shells; water-soluble chitosan (WCH: Nantong Xingcheng Biochemical Industrial Limited Co., Nantong, China; 90.5% deacetylation, 63.5% carboxylation); calcium caseinate (CC: Alanate 385, NZMP, Santa Rosa, CA, USA; 92.9% protein and 1.4% calcium); sodium alginate (SA: TICA-algin®400 powder, TIC Gums, Belcamp, MD, USA); and Semperfresh™ (SF: AgriCoat Industries Ltd., England; distributed by Pace International, Seattle, WA, USA) which is a mixture of sucrose esters of fatty acids, sodium carboxymethylcellulose, and mono-diglycerides of fatty acids. Other materials used in the coating formulation were glycerol (Fisher Scientific Inc., Fairawn, NJ, USA), glacial acetic acid (Baker Adamson, Morristown, NJ, USA), and Tween 20 (Sigma-Aldrich, Inc., St. Louis, MO, USA).

2.2. Preparation of coating solutions

A 2% (w/v) ACH coating solution was prepared by dissolving ACH in 1% aqueous acetic acid with 50% glycerol (w/ACH dry weight), adding 0.15% Tween 20 (w/v), homogenizing (Polytron PT 10-35, Kinematica AG, Littau, Switzerland) for 90 s at 50 s⁻¹, and then storing overnight at room temperature. A 2% CC coating solution was prepared by dissolving 2% CC in deionized water and adding 50% glycerol (w/CC dry weight) and 0.15% Tween 20 (w/v). The mixture was homogenized for 1 min at 50 s⁻¹ and shaken in a 60 °C water bath for 30 min, followed by cooling to room temperature. The SF coating solution was prepared by diluting 50% SF concentrate with deionized water to 1%, and mixing with 50% glycerol (w/SF weight) and 0.15% Tween 20 (w/v). A 3% (w/v) WCH coating solution was prepared by dissolving WCH in distilled water with 25% glycerol (w/WCH dry weight) and 0.15% Tween 20 (w/v), homogenizing for 90 s at 50 s⁻¹, and then storing overnight at room temperature. A 1.5% (w/v) WCH and 1% (w/v) SA coating solution (WCH + SA) was prepared by mixing 3% WCH solution and 2% SA (w/v) solution at

a 1:1 ratio with 25% glycerol (w/WCH + SA dry weight) and 0.15% Tween 20 (w/v) and homogenizing for 90 s at 50 s⁻¹.

2.3. Fruit sample preparation, packaging and storage

The major goal of applying coatings on fresh blueberries was to develop ready-to-eat fresh fruit with a similar or longer shelf life than unwashed, control fruit. Hence, fruit were first sanitized by soaking for 30 s in NaOCl solution (containing 200 µL L⁻¹ total chlorine) prepared by diluting commercial bleach solution (Clorox Regular Bleach, ~6% [w/w] NaOCl, Clorox Co., Oakland, CA, USA) with distilled water and then twice rinsed with distilled water for 1 min each to remove the residual chlorine. Washed fruit were drained and air-dried on stainless steel screen for 30 min prior to coating application. In 2006, 'Duke' fruit were randomly assigned to one of four treatments: unwashed and coated with SF, washed and coated with ACH, CC, or SF. 'Elliott' fruit were randomly assigned to one of two treatments: unwashed and coated with CC, or washed and coated with CC, based on observations that CC-coated 'Duke' fruit from the earlier harvest season had a more natural looking "bloom" (natural waxy coating on unwashed fruit). In 2008, washed 'Elliott' fruit were coated with WCH or WCH + SA to evaluate the potential of using chemically modified and blended polymers as coating materials. In both seasons, an unwashed (natural waxy bloom present) and washed (in water, removing much of the bloom) control (without coating) were used for comparison. All treatment coatings were applied twice, by dipping fruit in the coating solution for 30 s, draining on a stainless steel screen for 30 min, and then repeating the same procedure to achieve a uniform surface coating.

In 2006, 170 g fruit were packaged in vented, plastic "clam-shell" containers (industry standard, 9756Z, Pactiv Corp., Mexico). In 2008, 120–130 g fruit were packaged in vented or non-vented (VP756RP, Inline Plastic Corp., Salt Lake City, UT, USA) "clam-shell" containers for comparing the influences of container structures on the quality of fruit during storage. The experimental unit was one "clam-shell" container. At each sampling time, three individual containers/replications were selected per treatment for measuring the quality attributes.

To simulate commercial storage conditions, treated fruit were stored in a cooler at 2 ± 1 °C and 88% relative humidity (RH) in the dark for 1 week, and were then removed and placed at room temperature (to simulate retail display conditions) at 20 ± 3 °C and 30% RH under normal room light for up to 15 d.

2.4. Fruit physicochemical quality analyses

Fruit physicochemical quality attributes were measured before cold storage and then (after 1 week of cold storage), at 0, 3, 6, 9 and 12 d at room temperature in 2006 and at 0, 5, 10 and 15 d at room temperature in 2008. In 2006, total antioxidant and phenolic content of 'Duke' and 'Elliott' fruit were also evaluated at the same sampling times during storage. Only unblemished fruits were used for quality analysis.

Percent total soluble solids (TSS; % soluble solids), pH, and titratable acidity (TA) were measured following the procedures as described by Fisk et al. (2008), where TA was reported as percent malic acid (mass/mass) on the basis of fresh weight of fruit. About 10 blueberries from each container were pooled and measured for TSS, pH and TA. Firmness (FN) of fresh blueberries was measured by compression using a pre-calibrated BioWorks FirmTech2 Instrument (BioWorks, Inc., Wamego, KS, USA), and the firmness is defined as the slope of the line between the minimum and maximum deflection thresholds. A sub-sample of fruit (25 berries/replication) were set on their side (calyx end to the side) in indentations on the turntable and tested individually through

the compression of the load (with reference size as 18.87 mm and deflection thresholds as 0.51–1.47 mm), and the mean for the replicate was recorded as $N\text{ mm}^{-1}$. Percentage weight loss (WL) was calculated as the fresh weight change of fruit at each sampling time divided by the initial weight of the fruit. Decay rate (DR) was defined as percentage of fruit with a visible lesion. Decayed fruit were discarded after sampling.

The extraction of polyphenolics was carried out using a modified method of Rodriguez-Saona and Wrolstad (2001). Briefly, fruit were cryogenically powdered with liquid nitrogen; a 5 g powdered sample was mixed with 100% (v/v) aqueous acetone (EMD Chemicals Inc., Gibbstown, NJ, USA), ultrasonicated for 1 min and centrifuged for 5 min, and the remaining filtrate was re-extracted twice using 70% (v/v) aqueous acetone. The aqueous phase at the top was combined and transferred into a glass centrifuge bottle with 50 mL of chloroform (Mallinckrodt Baker Inc., Phillipsburg, NJ, USA) added and mixed. After centrifugation at 50 s^{-1} for 30 min, the aqueous phase was collected to a rotary evaporator (Brinkmann Instruments, Westbury, NY, USA) to remove the residual acetone. The extract was diluted in deionized water to desired concentration and stored at -70°C until analysis.

Antioxidant content (AC) was determined using DPPH assay (Brand-Williams et al., 1995). The sample extract was mixed with 1.5 mL DPPH (1, 1-diphenyl-2-picrylhydrazyl) (Kasel Kogyo Co. Ltd, Tokyo, Japan) in a small screw-cap test tube, vortexed, and set at room temperature for 5 min. The water-soluble antioxidant content was determined spectrophotometrically at 517 nm with the absorbance of ascorbic acid (Mallinckrodt Baker Inc.) used as a standard. Results with two replications were expressed as mass of ascorbic acid equivalents (AAE) per fresh weight (FW) mass of sample (g kg^{-1}).

Total phenolic content (TPC) was measured by a modified method from Singleton and Rossi (1965). A series of test tubes containing 7.5 mL deionized water and 0.5 mL Folin-Ciocalteu reagent (Sigma-Aldrich, Inc.) were prepared. A 0.5 mL diluted extract was mixed with 0.5 mL of 50, 100, 150, 200 mg kg^{-1} gallic acid (Sigma-Aldrich, Inc.) solutions, respectively, and 0.5 mL deionized water was used as control. After the solutions were mixed by a vortexer and set at room temperature for 10 min, they were mixed with 3 mL 20% (w/v) Na_2CO_3 , and then placed in water bath at 40°C for 20 min. The samples were immediately cooled to room temperature in an ice bath for 3 min. The absorbance of samples and a standard were analyzed by spectrophotometer at 765 nm. TPC was calculated as mass of gallic acid equivalents (GAE) per fresh weight (FW) mass of sample (g kg^{-1}). AC and TPC measurements were performed at storage times of 0, 6, and 12 d for both cultivars with two replicates.

2.5. Statistical analyses

All the measurements were conducted in triplicate. Data analyses were performed by ANOVA (analysis of variance) and GLM (general linear model) using SAS statistical software 9.01 (SAS institute, Cary, NC). Multiple comparisons among the treatments with significant differences tested in ANOVA were conducted by using LSD (least significant difference) at $p < 0.05$.

3. Results and discussion

3.1. 2006 season

3.1.1. pH, total acidity, and total soluble solids

The effect of cold storage and coating treatment during cold storage are shown in Table 1. In 'Duke', pH, TA and TSS were not significantly affected by cold storage or coating treatments during

Table 1

The effect of 1-week cold storage (2°C and 88% relative humidity) and coating treatment during cold storage on the fruit quality of 'Duke' and 'Elliott' in 2006.^a

| Coating and cold storage treatments ^b | pH | TA ^c (%) | TSS ^c (%) | FN ^c (N mm^{-1}) |
|--|---------------------|---------------------|----------------------|--|
| Duke | | | | |
| Unwashed | 3.81 | 0.63 | 13.7 | 1.80 |
| Washed | 3.94 | 0.61 | 13.7 | 1.74 |
| Unwashed SF | 3.95 | 0.65 | 12.8 | 1.69 |
| Washed SF | 3.94 | 0.63 | 12.8 | 1.68 |
| Washed ACH | 3.96 | 0.64 | 12.5 | 1.67 |
| Washed CC | 3.92 | 0.66 | 12.8 | 1.68 |
| Sig. (<i>p</i> value) | 0.91 | 0.99 | 0.44 | 0.16 |
| Pre-cold storage | 3.85 | 0.65 | 13.2 | 1.75 a |
| Post-cold storage | 3.98 | 0.62 | 12.7 | 1.67 b |
| Sig. (<i>p</i> value) | 0.22 | 0.44 | 0.14 | 0.03* |
| Elliott | | | | |
| Unwashed | 3.25 a ^d | 1.10 a | 14.2 | 1.94 |
| Washed | 3.25 a | 1.09 a | 13.8 | 1.88 |
| Unwashed CC | 3.23 a | 1.19 ab | 14.5 | 1.80 |
| Washed CC | 3.07 b | 1.29 b | 13.8 | 1.85 |
| Sig. (<i>p</i> value) | 0.03* | 0.04* | 0.16 | 0.34 |
| Pre-storage | 3.18 | 1.12 a | 14.0 | 1.84 |
| Post-storage | 3.22 | 1.21 b | 14.2 | 1.89 |
| Sig. (<i>p</i> value) | 0.13 | 0.04* | 0.39 | 0.40 |

^a Means are averaged overall coating or storage treatments ($n = 18$ and 12 for storage of 'Duke' and 'Elliott', respectively; $n = 6$ for each coating treatment).

^b "Unwashed" and "washed" (not coated) served as controls; "unwashed SF": unwashed and coated with Semperfresh™; ACH, acid-soluble chitosan; CC, calcium caseinate.

^c TA, titratable acidity; TSS, percent total soluble solids; FN, firmness.

^d Means followed by the same letters in the same column are not significantly different ($p > 0.05$) (only those with significant difference among treatments or storages are marked, as shown by *p* values followed by an asterisk).

cold storage ($p > 0.05$) (Table 1). During room temperature storage, the coating treatments did not cause significant difference in pH, TA and TSS of differently treated samples (Fig. 1A–C). The TA declined significantly over storage time (Fig. 1A). The pH tended to increase during the first 3 d of storage, but then declined, reaching 3.84 at day 9, significantly lower than the pH of 4.20 at day 3 (Fig. 1B). In contrast, TSS tended to decrease during the first 3 d of storage, but increased with longer storage, reaching 13.2% at day 9, which was significantly higher than 12.0% at day 3 ($p < 0.05$) (Fig. 1C).

In 'Elliott', both pH and TSS was not significantly affected by cold storage ($p > 0.05$), but TA of fruit increased after cold storage ($p < 0.05$) (Table 1). Coating treatments did not affect TSS of fruit ($p > 0.05$), but CC coating significantly increased TA and lowered pH of washed fruits during cold storages ($p < 0.05$). During room temperature storage, fruit pH stayed relatively stable during the first 6 d of storage, but increased from 6 to 12 d. Washed CC-coated and unwashed CC-coated fruit had higher TA and lower pH, particularly at the end of the storage period, than those washed and unwashed control fruit (Fig. 2A and B).

During post-harvest storage, acid metabolism as a result of fruit ripening which continues by converting starch and acid to sugar, has been shown to decrease TA and increase pH and TSS in other crops (Thompson, 1996; Verma and Joshi, 2000). Our results with CC-coated 'Elliott' fruit confirmed previous studies that coating with CC helped control changes in post-harvest physicochemical properties, such as pH and TA. The protein-based CC coating delayed post-harvest respiration of fruit by providing a strong gas barrier on the surface of fruit (Letien et al., 2001; Khwaldia et al., 2004; Lin and Zhao, 2007). However, the similar change was not observed in CC-coated 'Duke' fruit. As an early harvested variety of blueberry, 'Duke' had a higher pH and lower TA than 'Elliott' (Table 1), which might cause the difference in coating effects.

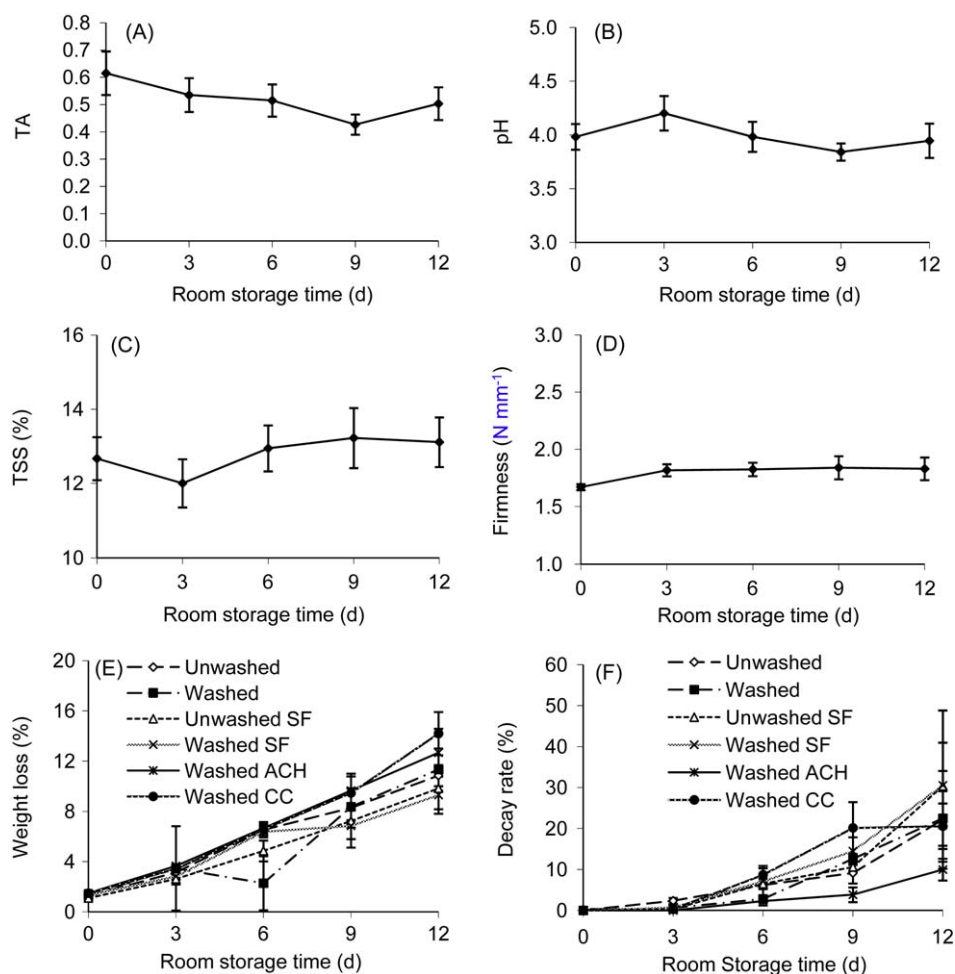


Fig. 1. Physicochemical properties of 'Duke' fruit during room temperature storage in 2006 season: (A) total acidity (TA); (B) pH; (C) total soluble solids (TSS); (D) firmness; (E) weight loss; and (F) decay rate. In (E) and (F), \diamond unwashed, Δ unwashed SF, * washed ACH, \blacksquare washed, \times washed SF, and \bullet washed CC. Since no significant differences ($p > 0.05$) in TA, pH, TSS, and firmness among different treatments were identified, mean values of all treatments are reported for these parameters. Mean \pm SE; $n = 18$ (for all except (E) and (F) where $n = 3$).

3.1.2. Firmness

The FN of 'Duke' fruit declined from 1.75 to 1.67 N mm^{-1} after 1 week of cold storage, but was not affected by coating treatments during cold storages ($p > 0.05$) (Table 1). During room temperature storage, FN was not affected by coating treatments either (Fig. 1D). The mean FN increased initially during the first 3 d of storage (from 1.67 to 1.82 N mm^{-1}), then remained stable through the following 12 d of storage.

The FN of 'Elliott' fruit was neither affected by 1 week of cold storage nor by coating treatments during cold storage ($p > 0.05$) (Table 1). Unlike 'Duke', coating affected FN in 'Elliott' during room temperature storage, as CC-coated fruit had significantly higher FN than uncoated fruit ($p < 0.05$) (Fig. 2D). The FN of coated samples slightly increased during the first 9 d of room temperature storage, and then declined, while the FN of uncoated 'Elliott' fruit continuously decreased throughout the storage period.

Fruit softening, one of the important quality deteriorations during post-harvest storage, is generally caused by the hydrolysis of starch to sugar and the degradation of pectin in the fruit cell wall associated with fruit ripening (Thompson, 1996). In contrast, water loss of fruit may lead to hardening. Both fruit softening and hardening affect the measured fruit firmness. In this study, CC coating helped maintain the firmness of 'Elliott' during room temperature storage. Calcium caseinate (CC) has been shown to provide calcium ions which may be chelated by adjacent acidic pectin polymers in the cell wall through non-covalent linkage forming an "egg

box" model at the biochemical level during storage (Seymour et al., 1993).

3.1.3. Weight loss

The WL of 'Duke' increased throughout the room temperature storage period (Fig. 1E). No significant differences were observed after 3 d of room temperature storage ($p > 0.05$), but WL of unwashed SF and washed SF-coated 'Duke' (9.8%, 9.3% WL, respectively) was significantly lower than other treatments at 12 d of room temperature storage. Similar to 'Duke', WL of 'Elliott' increased during room temperature storage, up to a mean of 13% at day 12 ($p < 0.0001$; Fig. 2E). There was no significant effect of coating on WL of 'Elliott' during room temperature storage ($p = 0.06$).

Migration of water from the fruit to the environment is the major cause of weight loss of fruit during storage. Our results were consistent with previous studies that a hydrophobic coating material, such as SF, provided a barrier to water loss (Morillon et al., 2002), while ACH and CC were hydrophilic coating materials with relatively high moisture permeability. Therefore, it may be necessary to incorporate lipids into hydrophilic coating formulae for better control of water loss of coated blueberries if this is a major goal when applying coatings.

3.1.4. Decay rate

Fruit decay rate in both blueberry cultivars increased during room temperature storage (Figs. 1F and 2F). Although there was no

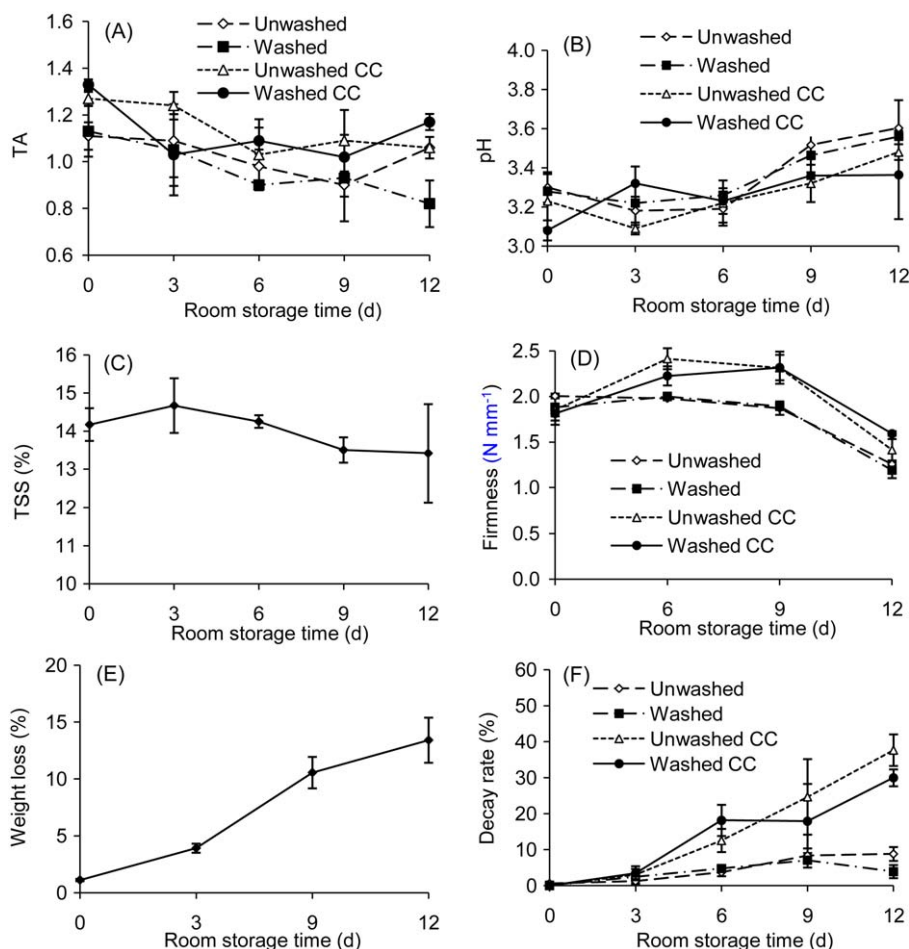


Fig. 2. Physicochemical properties of 'Elliott' fruit during room temperature storage in 2006 season: (A) total acidity (TA); (B) pH; (C) total soluble solids (TSS); (D) firmness; (E) weight loss; and (F) decay rate. In (A), (B), (D) and (F), \diamond unwashed, Δ unwashed CC, \blacksquare washed, and \bullet washed CC. Since no significant differences ($p > 0.05$) in TSS and weight loss among different treatments were identified, mean values of all treatments are reported for these parameters. $n = 3$ (for all except (C) and (D) where $n = 12$).

overall treatment effect on DR of 'Duke' ($p = 0.069$), washed ACH-coated fruit had a lower DR at days 9 and 12 than other treatments ($p < 0.05$) (Fig. 1F). In contrast, both washed and unwashed CC-coated 'Elliott' fruit had a significantly higher DR than uncoated fruit from day 6 of room temperature storage (Fig. 2F).

Fruit decay in blueberry is usually caused by fungi, with Anthracnose (*Colletotrichum acutatum*) being identified as the most common causal fungus, followed by Alternaria (*Alternaria* spp.) and Botrytis (*Botrytis cinerea*) (Wang et al., 2010). The anti-fungal function of ACH coating to prevent fruit decay has been well reported in several studies (Zhang and Quantick, 1998; Han et al., 2004; Park et al., 2005; Chien et al., 2007). The antimicrobial activity of chitosan is probably caused by the interaction between chitosan and the microbial cell membranes, which leads to the leakage of proteinaceous and other intracellular constituents. Chitosan can also penetrate to the nuclei of fungi and interferes with RNA and protein synthesis (Rabea et al., 2003). However, this anti-fungal property may be limited by several factors. Dipping and washing in chlorinated water may remove the natural waxy bloom on the surface of blueberries, thus weakening adhesion and durability of coatings. Moreover, residual water on the surface of blueberries, after washing, possibly diluted or dissolved applied coating materials, making it difficult to form a uniform and durable layer of edible films on the fruit surface (Lin and Zhao, 2007). However, CC, as a protein-based material, may provide additional nutrients for fungi to grow when the fruit is contaminated, potentially leading to an increased decay rate. Washing with a chlorine solution has been shown to decrease fruit decay (Butota et al., 2008).

3.1.5. Antioxidant content

The antioxidant content (AC) of unwashed 'Duke' fruit was significantly higher than that of other samples right after coating treatment, before cold storage (Table 2). After 1 week of cold storage, the AC degraded 22% in unwashed 'Duke' fruit while AC remained stable in washed, unwashed SF-coated, and washed ACH-coated fruit, but increased 32% and 25% in washed SF- and washed CC-coated fruit, respectively.

There was an increase in AC of 'Duke' during the room temperature storage period, from an initial 2.59 to 3.29 g kg⁻¹ after 12 d (Fig. 3A). Weight loss of 'Duke' (up to 14%) during room temperature storage might have caused the increase in AC because it is calculated on the basis of fresh fruit weight.

In 'Elliott', the AC in unwashed and washed CC-coated fruit declined 7% and 4%, respectively, after cold storage, but there was no effect of cold storage on AC of washed and unwashed CC-coated fruit (Table 2). Connor et al. (2002) reported that fresh 'Elliott' harvested before turning fully blue showed an increase in antioxidant activity after harvest and during cold storage. Therefore, changes in antioxidant activities are likely affected by storage conditions and stage of fruit ripeness at harvest. In our study, the AC of all 'Elliott' fruit declined after 6 d of room temperature storage, but was relatively stable from 6 to 12 d (Fig. 4A).

3.1.6. Total phenolic content

The TPC of all 'Duke' fruit decreased significantly after 1 week of cold storage, resulting in a 27–49% loss depending on treatment ($p < 0.0001$) (Table 2). According to Verma and Joshi (2000) and

Table 2
The effect of 1-week cold storage (2 °C and 88% relative humidity) and coating treatment during cold storage on the antioxidant and total phenolic content of 'Duke' and 'Elliott' in 2006.^a

| Coating and cold storage treatments ^b | | AC ^c g kg ⁻¹ | TPC ^c g kg ⁻¹ |
|--|-------------------|------------------------------------|-------------------------------------|
| Duke | | | |
| Unwashed | Pre-cold storage | 3.38 ± 0.13 | 4.16 ± 0.10 |
| | Post-cold storage | 2.64 ± 0.02 | 2.12 ± 0.03 |
| Washed | Pre-cold storage | 2.42 ± 0.04 | 3.49 ± 0.08 |
| | Post-cold storage | 2.55 ± 0.08 | 2.11 ± 0.07 |
| Unwashed SF | Pre-cold storage | 2.57 ± 0.22 | 3.59 ± 0.29 |
| | Post-cold storage | 2.49 ± 0.11 | 2.45 ± 0.22 |
| Washed SF | Pre-cold storage | 1.83 ± 0.03 | 3.10 ± 0.07 |
| | Post-cold storage | 2.41 ± 0.12 | 2.08 ± 0.10 |
| Washed ACH | Pre-cold storage | 2.81 ± 0.08 | 4.02 ± 0.15 |
| | Post-cold storage | 2.69 ± 0.49 | 2.60 ± 0.30 |
| Washed CC | Pre-cold storage | 2.24 ± 0.11 | 3.32 ± 0.43 |
| | Post-cold storage | 2.79 ± 0.16 | 2.42 ± 0.19 |
| Elliott | | | |
| Unwashed | Pre-cold storage | 7.13 ± 0.14 | 4.99 ± 0.96 |
| | Post-cold storage | 6.65 ± 0.10 | 5.53 ± 0.15 |
| Washed | Pre-cold storage | 7.23 ± 0.03 | 5.56 ± 0.19 |
| | Post-cold storage | 7.20 ± 0.35 | 6.26 ± 0.63 |
| Unwashed CC | Pre-cold storage | 6.93 ± 0.45 | 5.31 ± 0.61 |
| | Post-cold storage | 7.11 ± 0.02 | 6.65 ± 0.27 |
| Washed CC | Pre-cold storage | 7.10 ± 0.22 | 5.54 ± 0.19 |
| | Post-cold storage | 6.79 ± 0.07 | 6.31 ± 0.10 |

^a Data are reported as mean ± SE (n = 3).
^b "Unwashed" and "washed" (not coated) served as controls; "unwashed SF", unwashed and coated with Semperfresh™; ACH, acid-soluble chitosan; CC, calcium caseinate.
^c AC, Antioxidant content; TPC, Total phenolic content; fresh weight basis.

Seymour et al. (1993), the oxidation of phenolics in 'Duke' catalyzed by polyphenoloxidase and peroxidase during post-harvest storage is a major reason for the fast decline in TPC. Unlike 'Duke', the TPC of 'Elliott' did not change after 1 week of cold storage in unwashed (mean = 5.26 g kg⁻¹) and washed control fruit

(5.91 g kg⁻¹) (p > 0.05), but increased 25% in unwashed CC-coated fruit and 14% in washed CC-coated fruit, agreeing with the results reported by Connor et al. (2002) in fresh 'Elliott' fruit.

During room temperature storage, the coating treatments did not significantly affect TPC of 'Duke' and 'Elliott'. The mean TPC of 'Duke' increased initially, but remained relatively stable after 6 d of storage (Fig. 3B), while the mean TPC of 'Elliott' decreased from days 0 to 6, but increased from days 6 to 12 (Fig. 4B).

3.1.7. Comparison of two blueberry cultivars

Although we could not statistically compare the two cultivars in this study due to differences in ripening season, we did observe differences between 'Duke' and 'Elliott' in post-harvest quality and response to coating treatment. 'Duke' is an early-season cultivar with harvest starting in late June and 'Elliott' is a late-season cultivar with harvest starting in August in the Willamette Valley, OR. 'Elliott' fruit had a lower pH and higher TA than those of 'Duke', while 'Duke' had a larger fruit size (data not shown). In respect to antioxidant capacity of the two cultivars, the AC of 'Elliott' was higher than that of 'Duke'. Our results agree with a previous report that the later harvested 'Elliott' had a higher antioxidant activity than earlier harvested ones such as 'Bluecrop' and 'Jersey' (Connor et al., 2002).

3.2. 2008 season

3.2.1. pH, total acidity, and total soluble solids

One week of cold storage, coating and packaging container treatments did not affect the pH, TA, and TSS of 'Elliott' fruit during cold storage (p > 0.05) (Table 3).

During room temperature storage, the average TA of the fruit decreased from 0.96% on day 0 to 0.83% on day 15 (p < 0.05) (Fig. 5A). The pH of fruit tended to increase during the first 10 d of storage, and then declined (Fig. 5B). Unwashed fruit had the lowest

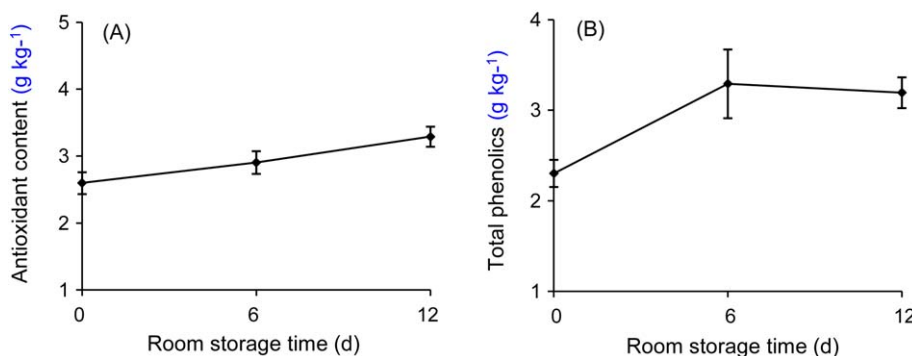


Fig. 3. Antioxidant content (A) and total phenolic content (B) of 'Duke' fruit during room temperature storage in 2006 season. Since no significant differences (p > 0.05) among different treatments were identified, mean values of all treatments are reported (mean ± SE, n = 18).

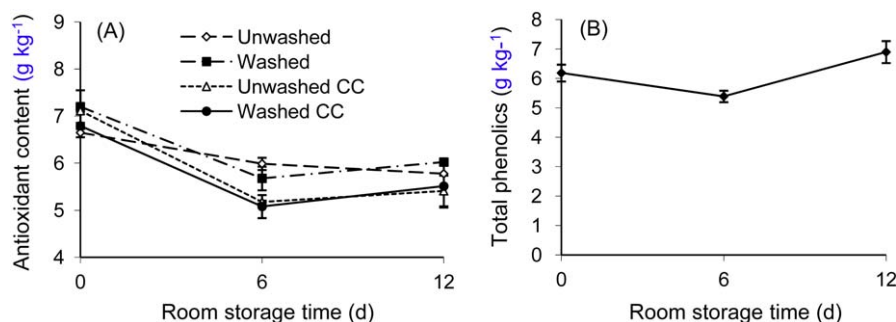


Fig. 4. Antioxidant content (A) and total phenolic content (B) of 'Elliott' fruit during room temperature storage in 2006 season. Since no significant differences (p > 0.05) in total phenolic content among different treatments were identified, mean values of total phenolic content are reported.

pH (3.12–3.14) over the storage period. WCH and WCH + SA coated fruit had higher pH than those of controls, which might be caused by the slight alkalinity of WCH (pH = 7.45) and WCH + SA (pH = 7.76) coating solutions. The fruit in vented containers had higher TSS than those in non-vented containers ($p < 0.05$) (Fig. 5C), likely because of increased WL and thus concentration of sugars.

3.2.2. Firmness

The FN of 'Elliott' fruit decreased from 2.63 to 2.49 N mm⁻¹ after 1 week of cold storage. During cold storage, washed control had lower FN than unwashed control. However, FN was greater in fruit coated with WCH or WCH + SA. Different packaging containers did not cause significant difference in FN during cold storage (Table 3).

During the 10 d of room temperature storage, the FN of unwashed control fruit decreased from 2.71–2.87 to 2.15–2.64 N mm⁻¹ (Fig. 5D). Washed control fruit had a lower FN (2.36–2.39 N mm⁻¹) than that unwashed control fruit on day 0, indicating that washing fruit removed the natural bloom, thus reduced firmness. Coating with WCH or WCH + SA and packing in non-vented containers significantly increased the FN of fruit during room temperature storage ($p < 0.05$), in which the

FN of coated fruit packed in non-vented containers increased to 5.45–5.56 N mm⁻¹ on day 15, while those packed in vented containers reached 4.07–4.19 N mm⁻¹ on day 10. Fruit softening usually occurs during post-harvest storage. The observed increase in fruit firmness probably resulted from the additional resistance provided by the coatings during the measurement.

3.2.3. Weight loss

WL of the fruit in vented containers was significantly higher than those in non-vented containers during room temperature storage ($p < 0.05$) (Fig. 5E). Non-vented containers effectively prevented the migration of water from the fruit to the environment. No significant difference in WL was observed between uncoated and coated fruit ($p > 0.05$), which may be due to the hydrophilic nature of WCH and SA coating materials with limited water barrier property.

3.2.4. Decay rate

Coating and container treatments significantly affected DR during room temperature storage (Fig. 5F). Washed control fruit had a higher DR than unwashed control fruit in both types of containers, indicating that the waxy bloom on the surface of blueberry might

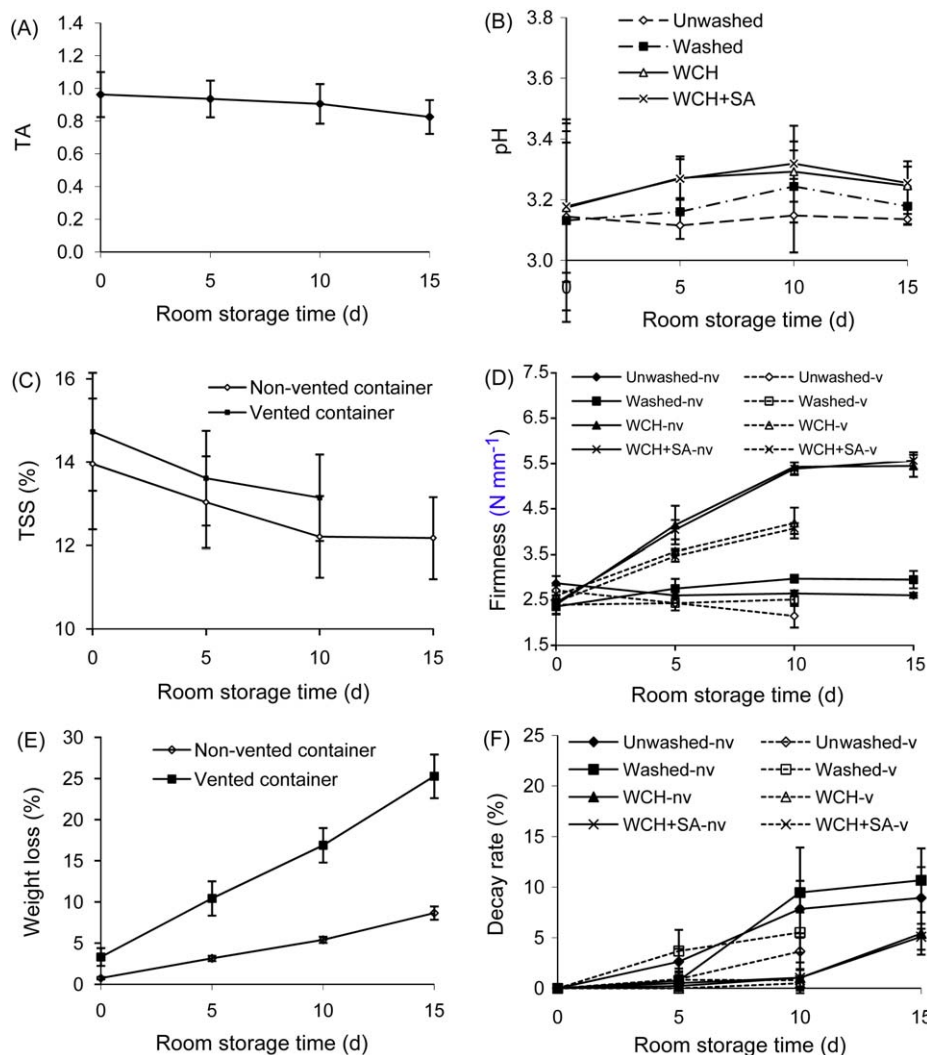


Fig. 5. Physicochemical properties of 'Elliott' fruit during room temperature storage in 2008 season: (A) total acidity (TA); (B) pH; (C) total soluble solids (TSS); (D) firmness; (E) weight loss and (F) decay rate. For legend in (D): -nv indicates non-vented container; -v indicates vented container. Since no significant differences ($p > 0.05$) in TA among different treatments and containers were identified, mean values of all treatments and containers are reported for this parameter. Since no significant differences ($p > 0.05$) in pH between different containers were identified, mean values of both containers for each treatment are reported for this parameter. Since no significant differences ($p > 0.05$) in TSS and weight loss among different treatments were identified, mean values of all treatments for each container are reported for these parameters. All fruit quality attributes (except weight loss) were not measured for the samples in vented containers on day 15 due to the severe shrinkage of the fruits.

Table 3

The effect of 1-week cold storage (2 °C and 88% relative humidity), coating and container treatments during cold storage on the fruit quality of 'Elliot' in 2008.^a

| Coating, cold storage and container treatments ^b | pH | TA ^c (%) | TSS ^c (%) | FN ^c (N mm ⁻¹) |
|---|--------|---------------------|----------------------|---------------------------------------|
| Unwashed | 3.08 | 1.00 | 13.97 | 2.74 a ^d |
| Washed | 3.16 | 0.99 | 14.38 | 2.38 b |
| WCH | 3.27 | 0.89 | 14.89 | 3.52 c |
| WCH+SA | 3.27 | 0.87 | 14.11 | 3.52 c |
| Sig. (<i>p</i> value) | 0.2013 | 0.1110 | 0.4714 | <0.0001* |
| Pre-cold storage | 3.27 | 0.88 | 14.33 | 2.63 a |
| Post-cold storage | 3.16 | 0.96 | 14.34 | 2.49 b |
| Sig. (<i>p</i> value) | 0.1617 | 0.1013 | 0.9816 | 0.0268* |
| Non-vented container | 3.15 | 0.96 | 13.96 | 2.45 |
| Vented container | 3.16 | 0.96 | 14.73 | 2.54 |
| Sig. (<i>p</i> value) | 0.9023 | 0.9294 | 0.1543 | 0.1112 |

^a Means are averaged overall coating, storage or container treatments (*n* = 16 for pre-storage; *n* = 32 for post-storage; *n* = 12 for each coating treatment; *n* = 16 for each container).

^b "Unwashed" and "washed" (not coated) served as controls; "WCH", washed and coated with water-soluble chitosan; "WCH+SA", washed and coated with the mixture of water-soluble chitosan and sodium alginate.

^c TA, titratable acidity; TSS, percent total soluble solids; FN, firmness; DR, decay rate; WL, weight loss.

^d Means followed by the same letters in the same column are not significantly different (*p* > 0.05) (only those with significant difference among treatments or storages are marked, as shown by *p* values followed by an asterisk).

be a natural physical protection against mold. WCH or WCH+SA coating reduced DR of fruit packed in non-vented containers from 8.9–10.7% to 5.1–5.4% at 15 d of storage, and from 3.6–5.5% to 0.5–0.8% on fruit packed in vented containers at 10 d of storage. The WCH used in this study is the product of chitosan carboxylation synthesized by the reaction of chitosan and chloroacetic acid in 2-propanol in the presence of KOH (Guo et al., 2006). The fungistatic activities exerted by carboxymethyl chitosan have been reported by other researchers (Muzzarelli et al., 2001; Guo et al., 2006; Zhong et al., 2007). The fruit coated with WCH+SA had a higher DR than fruit coated with WCH alone in vented containers, indicating that mixing SA with WCH diluted the antifungal effect of WCH. Fruit packed in non-vented containers without coating had more decay than those in vented containers (*p* < 0.05), likely a result of an accumulation of moisture inside the container favorable for the growth of mold. However, this difference was not observed on WCH or WCH+SA coated fruit in different containers.

3.2.5. Comparison of two storage containers

Non-vented container provided a good barrier to water evaporation and gas exchange, and therefore effectively delayed fruit ripening and dehydration, leading to the reduced weight loss and increased firmness of fruit. However, the moisture accumulated in non-vented container might promote the growth of mold, thus induced a higher DR of enclosed fruit. Therefore, coating with anti-fungal material is in need to effectively control the growth of mold, especially for fruit packaged in non-vented container.

3.3. Comparison of different coatings

The coatings evaluated in this study showed different effects on preserving the post-harvest quality of fresh blueberries. The hydrophobic coating material, SF, provided a better water barrier property than other hydrophilic coating materials, thus reduced WL of coated 'Duke' fruit during the room temperature storage. The protein-based CC coating delayed post-harvest respiration of fruit by providing a strong gas barrier on the surface of fruit, as evidenced by increased TA and lowered pH in CC-coated 'Elliot' during both cold and room temperature storages. CC coating also improved FN of 'Elliot' fruit during room temperature storage. How-

ever, increased DR observed in CC-coated 'Elliot' fruit indicates that additional anti-fungal ingredient is needed in CC coating when applied on fruit. Both ACH and WCH coatings significantly reduced DR in coated 'Duke' and 'Elliot' fruit as a result of the anti-fungal activity of chitosan. Blending SA in WCH coating did not provide additional advantages but slightly diluted WCH's anti-fungal activity on 'Elliot' fruit stored in vented container.

4. Conclusions

Results from this study indicate the possibility of using edible coatings to develop ready-to-eat fresh blueberries with no reduction in shelf life. The key for success is using an appropriate coating material, container, and method of applying the coatings. In this study, different coatings showed various effects on the post-harvest quality of pre-washed fresh blueberries. Both acid-soluble and water-soluble chitosan coatings showed potential for reducing rate of decay of 'Duke' and 'Elliot' during room temperature storage. Semperfresh™ coating helped reduce weight loss of 'Duke', while calcium caseinate coatings tended to delay the fruit ripening and to improve the firmness of 'Elliot' during room temperature storage. Reduction of weight loss and retention of fruit firmness in 'Elliot' was most desirable in non-vented containers. However, the moisture accumulation in non-vented containers can promote the mold decay of enclosed fruit. By using chitosan coating, the mold growth can be effectively controlled for fruit packed in non-vented containers. The current dipping method used to apply coatings might have reduced the efficacy of the coatings; new methods of applying coatings should be investigated. The use of electrostatic spraying for coating application is currently being evaluated by the authors for achieving a more uniform coating and to avoid removal of the natural waxy layer on the surface of blueberries and thus maximize the potential benefits of coatings. In addition, a separate sensory study should be conducted in the future to evaluate if these edible coatings have any impact on the most relevant quality related attributes such as taste, flavour, texture and visual appearance.

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