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Effect of chitosan-based edible coating on antioxidants, antioxidant enzyme system, and postharvest fruit quality of strawberries (*Fragaria* x *aranassa* Duch.)

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ABSTRACT

The severity of decay in strawberries stored at either 5 °C or 10 °C was significantly reduced and the shelf-life was extended by immersing fruits in chitosan solutions of 0.5, 1.0 and 1.5 g/100 mL for 5 min at 20 °C as compared to the control. Strawberries treated with chitosan also maintained better fruit quality with higher levels of phenolics, anthocyanins, flavonoids (ellagic acid, ellagic acid glucoside, *p*-coumaroyl glucose, quercetin 3-glucoside, quercetin 3-glucuronide, kaempferol 3-glucoside, kaempferol 3-glucuronide, cyanidin 3-glucoside, pelargonidin 3-glucoside, cyanidin 3-glucoside-succinate, and pelargonidin 3-glucoside-succinate), antioxidant enzyme activity [catalase (CAT), glutathione-peroxidase (GSH-POD), guaiacol peroxidase (G-POD), dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDAR)], and oxygen radical absorbance capacity for peroxyl radicals (ROO•), hydroxyl radical radicals (•OH; HOSC) and 2,2-Di (4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) than the untreated fruits. Chitosan treatments retarded the decrease of ascorbic acid (ASA) and reduced glutathione (GSH) content and β-1,3-glucanase activities compared to control. The high contents of antioxidants, antioxidant activity, ASA and GSH and high activity of β-1,3-glucanase in the treated strawberries reinforced the microbial defense mechanism of the fruit and accentuated the resistance against fungal invasion. Therefore, the application of chitosan coating could be favorable in extending shelf-life, maintaining quality and controlling decay of strawberries.

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1. Introduction

Strawberries are a good source of natural antioxidants (Heinonen, Meyer, & Frankel, 1998; Wang, Cao, & Prior, 1996). In addition to the usual nutrients such as vitamins and minerals, strawberries are also rich in anthocyanins, flavonoids, and phenolic acids (Heinonen et al., 1998). Our previous study showed that strawberries have high oxygen radical absorbance activity and enzymatic system for active oxygen species detoxification (Wang & Lin, 2000; Wang & Zheng, 2001). However, strawberries are highly perishable, susceptible to rapid spoilage, and have short market shelf-life (Hardenburg, Watada, & Wang, 1986). In addition to rapid deterioration in quality, they are also very susceptible to microbial invasion. The shelf life of strawberries is usually terminated by decay. Therefore it is necessary to develop strategies to reduce decay and increase the storage life of the fruit. Proper postharvest handling of strawberries is critical to marketing success. Refrigeration is widely used to reduce spoilage and extend the shelf-life of strawberries. Heat and controlled atmosphere are effective in retarding decay and softening of strawberries (Gil, Holcroft, & Kader, 1997). However, exposure to heat treatment or high concentrations of CO_2 could adversely affect the color change in strawberry fruit. Heat treatment (Civello, Martinez, Chaves, & Añón, 1997) and high CO_2 cause a reduction in red color intensity and a decrease in anthocyanin content of strawberry fruit (Gil et al., 1997). As CO_2 level increases, the concentrations of pelargonidin glycosides and ascorbic acid decrease (Gil et al., 1997). This suggests that high CO_2 concentrations may have a stimulating effect on the oxidation of ascorbic acid or an inhibition of mono- or dehydroascorbic acid reduction to ascorbic acid. Anthocyanins, flavonoids, and total antioxidant activity of both cultivars are higher in air-stored fruit than in CO_2-stored fruit. It appears that high CO_2 storage generally decreases total phenolics, total anthocyanins, and oxygen radical absorbance capacity (ORAC) values (Gil et al., 1997).

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Natural antimicrobial compounds such as methyl jasmonate (MJ), essential oils such as thymol, menthol, eugenol and allyl isothiocyanate (AITC) and other natural volatile compounds have been investigated as alternative methods to reduce postharvest deterioration and prolong storage life of strawberries (Wang, Chen, & Yin, 2010; Wang, Wang, Yin, Parry, & Yu, 2007). Some of these natural compounds have been found to affect the levels of antioxidants and antioxidant enzyme activities.

Chitosan is a linear polysaccharide composed of randomly distributed β-(1–4)-linked D-glucosamine and *N*-acetyl-D-glucosamine units. The physico-chemical properties of chitosan have been well studied (Arvanitoyannis, 1999; Arvanitoyannis, Kolokuris, Nakayama, Yamamoto, & Aiba, 1997; Arvanitoyannis, Nakayama, & Aiba, 1998). Chitosan-based edible coating is derived from natural sources by deacetylation of chitin; is harmless to humans, pets, wildlife, and the environment; and has been studied for efficacy in inhibiting decay and extending shelf life of fruits (Aider, 2010; No, Meyers, Prinyawiwatkul, & Xu, 2007). Chitosan and its derivatives have been shown to inhibit the growth of a wide range of fungi (Aider, 2010; No et al., 2007). They can also trigger defensive mechanisms in plants and fruits against infections caused by several pathogens. Postharvest studies have shown that chitosan treatments might have potential to inhibit fungal growth in strawberries (Han, Zhao, Leonard, & Traber, 2004; Zhang & Quantick, 1998). Recent studies on the sensory evaluation of chitosan-coated strawberries have reported that chitosan solution prepared at a low acid concentration does not change astringency of the fruit and chitosan coatings do not change consumer acceptance of strawberries in storage (Bautista-Banos et al., 2006). However, no information is available on the effect of chitosan on antioxidant capacity in strawberries. This study was carried out to study the effect of chitosan treatment on reducing decay and also to examine its effect on activities of antioxidant enzymes and β-1,3-glucanase in strawberries after storage at 5 °C and 10 °C for 0–12 days.

2. Materials and methods

2.1. Plant materials and treatments

Strawberries (*Fragaria* x *ananassas* Duchesne ex Rozier 'Earliglow') used in this study were grown at a farm near Beltsville, Maryland, USA and were hand-harvested at a commercially mature stage, sorted to eliminate damaged, shriveled, unripe fruit, and selected for uniform size and color. Selected berries were randomized and used for the experiments. The pH values for control and all chitosan solutions were adjusted to 5.6 with 1.0 mol/L NaOH, and Tween-80 (0.05 g/100 mL) was added to the solution as a surfactant. Chitosan solutions were made by dissolving 0.5, 1.0 or 1.5 g chitosan in acetic acid (0.5 mL acetic acid/100 mL de-ionized H_2O).

Strawberries were randomly distributed into four groups. Three groups were assigned to the three treatments while the fourth group provided as the untreated control. The treatments consisted of immersing fruits for 5 min at room temperature (20 °C) in: (a) 0.5 g chitosan (b) 1.0 g chitosan (c) 1.5 g chitosan. The control berries also were immersed in 0.5 mL acetic acid/100 mL de-ionized H_2O with Tween-80 (0.05 g/100 mL). Fruits were allowed to dry for 2 h at 20 °C. Fifteen strawberries were placed into each of the 1-L polystyrene containers with snap-on lids. Three containers were used for each sampling date (3-day interval) at each storage temperature (5 °C or 10 °C). The strawberries were then subsequently stored at 5 °C or 10 °C.

2.2. Decay evaluation

Each berry was visually examined at 3-day intervals during storage period. Strawberries showing surface mycelia development or bacterial lesions were considered decayed. Results were expressed as the percentage of fruits infected.

2.3. HPLC analysis of berry anthocyanins and phenolic compounds

Triplicate composite samples of 10 g fresh weight (fw) each cut from 15 fruits were extracted 3 times with 10 mL acetone (50 mL acetone/100 mL H_2O) using a Polytron (Brinkmann Instruments, Inc., Westbury, NY). The homogenized samples from the acetone extracts were then centrifuged at 14,000× *g* for 20 min at 4 °C. The supernatants from the above extracts were concentrated to dryness using a Buchler Evapomix (Fort Lee, NJ) in a water bath at 35 °C and were dissolved in 10 mL of acidified water (3 mL formic acid/100 mL H_2O) and then passed through a C_{18} Sep-Pak cartridge (Waters Corporation, MA), which was previously activated with methanol followed by water and then aqueous formic acid (3 mL formic acid/100 mL H_2O). Anthocyanins and other phenolics were adsorbed onto the column while sugars, acids, and other water-soluble compounds were eluted with 10 mL of formic acid (3 mL formic acid/100 mL H_2O). Anthocyanins and other phenolics were then recovered with 4.0 mL of acidified methanol containing formic acid (3 mL formic acid/100 mL H_2O) and used for total anthocyanin, total phenolic, HPLC, and antioxidant activity analysis. For HPLC analysis, the methanol extracts were passed through 0.45-μm membrane filters (Millipore, MSI, Westboro, MA) and 20 μL were analyzed by HPLC. The samples were determined using a Waters (Waters Corporation, MA) HPLC system coupled with a photodiode array detector (Waters 996 Series) and equipped with two pumps (600E system controller). Samples were injected at ambient temperature (20 °C) into a reverse phase NOVA-PAK C_{18} column (150 × 3.9 mm, particle size 4 μm) with a guard column (NOVA-PAK C_{18}, 20 × 3.9 mm, particle size 4 μm) (Waters Corporation, MA). The mobile phase consisted of aqueous formic acid (2.5 mL formic acid/100 mL H_2O) (A) and HPLC grade acetonitrile (B). The flow rate was 1 mL/min, with a gradient profile consisting of A with the following proportions of B: 0–1 min, 3%, 1–10 min, 3–6% B; 10–15 min, 6% B; 15–35 min, 6–18% B; 35–40 min, 18–20% B; 40–45 min, 20–100% B; 45–50 min, 100% B. The phenolic compounds in fruit extracts were identified by their UV spectra, recorded with a diode array detector, and by chromatographic comparison with authentic markers. Retention times and spectra were compared to those of the pure standards and the results were confirmed by co-injection with authentic standards. Individual phenolic acid, flavonols, and anthocyanins were quantified by comparison with an external standard of ellagic acid, ellagic acid glucoside, *p*-coumaroyl glucose, quercetin 3-glucoside, quercetin 3-glucuronide, kempferol 3-glucoside, kempferol 3-glucuronide, cyanidin 3-glucoside, pelargonidin 3-glucoside, cyanidin 3-glucoside-succinate, and pelargonidin 3-glucoside-succinate. Each standard was dissolved in methanol at a concentration of 1 mg/mL and five dilute solutions from these stock solutions were used to prepare calibration curves of each standard. Recoveries were measured by extracting the recovered amounts of pure substances added to strawberries before the experiment. Three replicates from each sample were used for HPLC analyses. Scanning between 250 and 550 nm was performed and data were collected by the Waters 996 3-D chromatography data system.

2.4. Total anthocyanin and total phenolic content

One mL from the above extracts was assayed for total soluble phenolics using Folin–Ciocalteu reagent (Slinkard & Singleton, 1977). The extracts were purified to remove interfering substances, such as sugars, aromatic amine, sulfur dioxide, ascorbic acid, organic acids, Fe (II), as well as nonphenolic organic