PHILIPP AGRIC SCIENTIST
Vol. 93 No. 1, 66-75
March 2010

ISSN 0031-7454

Quality of Fresh-Cut Pears (*Pyrus bretschneideri* Rehd cv. Huangguan) Coated with Chitosan Combined with Ascorbic Acid and Rosemary Extracts

Chenlong Xiao[1,2], **Wen Luo**[2], **Mingyang Liu**[2], **Liwei Zhu**[2], **Min Li**[3], **Hongshun Yang**[4] and **Yun Deng**[1,2,*]

[1]SJTU-Bor Luh Food Safety Center, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai, 200240, P.R. China
[2]Department of Food Science and Engineering, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai, 200240, P.R. China
[3]Engineering College, Guangdong Ocean University, Zhanjiang 524025, P.R. China
[4]College of Food Science and Technology, Henan University of Technology, 140 S Songshan Rd, Zhengzhou, Henan 450052, China
[*]Author for correspondence; e-mail: y_deng@sjtu.edu.cn; Tel: + 86-21-34206613, Fax: + 86-21-34206918

The combined effects of chitosan coating plus antioxidants on the quality of fresh-cut 'Huangguan' pears (*Pyrus bretschneideri* Rehd) were investigated. Pear wedges were immersed in 2% (w/v) chitosan solution with 0.03% rosemary or in 0.5% (w/v) ascorbic acid, followed by dipping in 2% (w/v) chitosan solution. Physico-chemical and sensory qualities of the fruits were evaluated during 3 d of storage at 20 °C. Results indicated that chitosan + rosemary and ascorbic acid + chitosan improved the preservation of fresh-cut pear compared with the control. Compared with ascorbic acid + chitosan, chitosan + rosemary suppressed polyphenol oxidase activity and reduced the loss of phenolic content, retained higher *L* and *h* values, and scored higher for color and visual appearance. No significant differences in electrolyte leakage, soluble solids content, pH, firmness and weight loss were observed between the two treatments. Ascorbic acid sharply increased in the coated samples after pretreatment with 0.5% ascorbic acid. Data obtained in this study suggest that chitosan incorporated with rosemary improved the antioxidant protection and sensory qualities of fresh-cut pears, providing a great advantage in the reduction of browning, which is the main problem of quality deterioration in fresh-cut produce.

Key Words: ascorbic acid, browning, chitosan, firmness, fresh-cut pears, rosemary

INTRODUCTION

Pear *(Pyrus bretschneideri* Rehd cv. Huangguan) has attracted great interest in China because of its nutritional and organoleptic properties. It has yellow skin, white flesh and a moderate sweet-sour ratio; it is juicy, crispy and lacking in stone cells (Feng et al. 2008).

Fresh-cut fruits are one of the fastest growing products in the food industry. However, the greatest hurdle to the commercial marketing of pears is their limited shelf-life, due to excessive browning of the tissue when cut, and softening, decay and off-flavor development (Ding et al. 2007; Gonzalez-Aguilar et al. 2008). Correlations between browning, phenolic content and/or polyphenol oxidase (PPO) have been determined for pears (Gil et al. 1998; Chung et al. 2009). Inhibition of browning reactions by adding antioxidants, excluding oxygen, or inhibiting the activity of the responsible enzymes are some of the ways to decrease the loss of quality in pears (Gil et al. 1998; Robles-Sánchez et al. 2009).

The use of browning inhibitors is restricted in food processing due to toxicity and adverse effects on texture,

taste and flavor (González-Aguilar et al. 2005). Ascorbic acid is generally recognized as a safe, inexpensive and consumer-friendly compound that is commonly used to prevent enzymatic browning of fresh-cut pears and fruits by reducing the o-quinones to diphenols. Ascorbic acid treatment at 15 mg L^{-1} had a significant effect in decreasing the browning of cut surface and effectively maintained the sensory quality of minimally processed carambola stored at 7 °C for 5 d (Ding et al. 2007). The use of ascorbic acic + $CaCl_2$ + citric acid also significantly reduced color deterioration and loss of firmness but did not affect the sensory characteristics of fresh-cut mango (Gonzalez-Aguilar et al. 2008).

A post-cutting dip of 2% (w/v) ascorbic acid, 1% (w/v) calcium lactate and 0.5% (w/v) cysteine adjusted to pH 7.0 significantly inhibited cut surface browning and flesh softening and prolonged the shelf-life of 'Bartlett' pear slices (Gorny et al. 2002). However, ascorbic acid alone or combined with calcium salt dipping treatments did not seem to completely prevent the browning of pear wedges throughout the storage period (Gorny et al. 1998; Oms-Oliu et al. 2006). Different fruits respond differently to different physical and chemical treatments, hence, the need to determine the reasonable combination of various techniques for lengthening their storage life (Bico et al. 2009).

Edible coating can extend the shelf life of fresh-cut produce. Chitosan is the second most abundant natural polymer in nature after cellulose, and is composed of N-acetyl-D-glucosamine units with β (1–4) glycosidic bounds (Deng et al. 2009). Chitosan, as an ideal non-toxic preservative coating, provides a barrier against external elements and therefore increases the shelf life of fresh-cut produce by excellent antimicrobial action and decreases gas exchange; loss of water, flavors and aroma; and solute migration toward the cuticle (Deng et al. 2009). Numerous studies have been conducted to develop and apply chitosan coating containing natural plant extracts for improving fresh fruits and processed fruits and vegetables (Rojas-Graü et al. 2009).

Rosemary extracts are natural and non-toxic and contain carnosic acid, rosemarinic acid and carnosol which have strong anti-senescence, anti-septic, antibacterial and antioxidant properties (Stefanovits-Bányai et al. 2003). Rosemary is used commercially as a good source of antioxidants and is widely used in the food industry to prevent oxidative degradation of foods (Stefanovits-Bányai et al. 2003; Ponce et al. 2008). The antioxidant activity of rosemary extracts is associated with the presence of several phenolic diterpenes, which break free radical chain reactions by hydrogen donation. However, little information is available concerning the effect of rosemary on the browning characteristics of fresh-cut produce. Our study was therefore conducted to evaluate the combined effect of chitosan + rosemary extracts and chitosan + ascorbic acid on the browning characteristics and the physico-chemical and sensory properties of fresh-cut pears stored at 20 °C.

MATERIALS AND METHODS

Raw Materials

Pears (*Pyrus bretschneideri* Rehd cv. Huangguan) harvested in Hebei, China were supplied by a local distributor and brought to the laboratory. The pears were selected on the basis of uniform color, size (255–265 g), hardness and the absence of visible physical and fungal infection; the fruits were stored at 2 ± 1 °C at approximately 95% relative humidity prior to processing.

Shrimp chitosan with a molecular weight of 1.5×10^5–1.7×10^5 (90% degree of deacetylation, viscosity of 200 cps, food-grade, water-soluble, odorless and tasteless powder with moisture content ≤10%) was purchased from Nantong Xincheng Biological lndustrial Co. Ltd., Nantong, China. The chemicals used in this study were Folin-Ciocalteu reagent, L-ascorbic acid, 2,4-dinitrophenylhydrazine, glycerol, Tween 80 and glacial acetic acid (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China). Other chemicals used for analyses were of analytical reagent grade (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China).

Preparation of Edible Coating

Chitosan solution was prepared according to the method of Deng et al. (2009) with slight modification. Briefly, chitosan (2%, w/v) was dispersed in an aqueous solution of glacial acetic acid (1%, v/v) at 4 ºC. After stirring overnight at room temperature, 1.5% glycerol (w/v) and 0.2% Tween 80 (v/v) were added to the mixture. The mixture was homogenized (SENCO S312, Shanghai SENCO Technology Co. Ltd, China) at 1000 rpm for 10 min. In order to guarantee the stability of the emulsions, the pH was adjusted to 5.6 with 1 mol L^{-1} NaOH. Finally, the solution was strained through eight layers of cheesecloth and degassed under vacuum of 100 mm Hg at room temperature. Chitosan film solutions were stored at 4 ºC for 1–2 d. Rosemary extract containing 22 ± 4% phenolic diterpenes (carnosic acid, carnosol and rosmarinic acid as stated by the manufacturer) was purchased from Guizhou Red Star Development Duyun Luyou CO., Ltd. (China); 0.03% rosemary extract was added for a final chitosan solution.

Fresh-cut Process

Pear fruits were dipped in a 7500 ppm HClO solution for 5 min (Bico et al. 2009), washed with sterilized distilled water and blotted with tissue paper to remove excess water. The fruits were peeled, the core tissue completely removed and the remaining tissue cut manually into 6–8 wedges (ca. 20 mm thickness) per pear. The wedge samples were placed in an ice bath immediately after cutting. The fruit slices were randomly assigned to one of three treatments:

(1) Control – wedges dipped in distilled water were used. Previous research has reported that chitosan coating alone delayed increase in weight loss and retained greater firmness, total soluble solids and titratable acidity content compared with the control, and decreased respiration rate and membrane permeability of pear fruits compared with the control (Lin et al. 2008). Therefore, we did not use chitosan coating alone as control, and directly used distilled water dipping as control.

(2) Ascorbic acid + chitosan (AA+CH) – the wedges were dipped in 0.5% (w/v) ascorbic acid solution (Gorny et al. 2002) for 3 min, dried using tissue paper for 3 min, then dipped into the chitosan coating solution without rosemary extract for 2 min.

(3) Chitosan + rosemary extract (CH+R) – the samples were immersed in the chitosan coating solution with 0.03% rosemary extract for 2 min. After drainage of excess coating solution or water, about 130 g of pear wedges were immediately placed in foam trays, covered with unsealed individual polyethylene film bags and stored at 20 ºC and 60–70% RH for up to 3 d. Three replicates from each treatment for analyses were taken at day 0, 1 and 3.

Color Measurement

The color of the cut surface was determined using a Color Difference Meter (WSC-S, Shanghai Precise Scientific Apparatus, Shanghai, China). CIE-*Lab* color parameters were recorded as *L*, *a*, and *b*. Color values of *L* (lightness or darkness) and hue angle [h = arctan (b/a), color itself, 0^o = red–purple, 90^o = yellow, 180^o = bluish-green, and 270^o = blue] were measured. Measurements were performed by placing the sample slices over the standard plate. Eight slices per treatment were used. The *L* and *h* values were used as indicators of the browning intensity of the cut surface (Oms-Oliu et al. 2008). The lower *L* and *h* values represented the increase in the degree of browning discoloration.

Electrolyte Leakage Test

Electrolyte leakage was expressed by the relative leakage rate according to the method of Deng et al. (2005a) with slight modification. A sample (~ 7 g) was rinsed with de-ionized water, gently blotted with tissue paper to remove excess water, and then incubated in 100 mL de-ionized water at 21 °C for 2 h. Initial electrolyte leakage was determined using a digital conductometer (FE30, Mettler Toledo). The sample was placed directly in boiling water for 30 min and cooled to 21 °C to assess total electrolytes. Relative leakage was expressed as percentage of total electrolytes.

Firmness Test

Firmness was measured using a TA-XT2i Texture Analyzer (Stable Micro Systems Ltd., UK) with a 4-mm diameter cylinder penetrometer probe. The probe was penetrated through the wedges (ca. 20 mm thickness) at a constant speed of 5 mm s^{-1}. The maximum force encountered was registered. Ten wedges from each treatment were used for the analysis.

Weight Loss Determination

The moisture content of the samples was gravimetrically measured by drying samples in a forced-air oven at 105 °C to a constant final weight. Weight was registered before and after drying by using an analytical balance (PL203, Mettler Toledo). Each experiment was performed in triplicate. Weight loss was calculated according to the formula (Bico et al. 2009):

$$weight\ loss(\%) = 100 - 100 \times DM(\%)_{day0} / DM(\%)_{dayN}$$

where $DM(\%)_{day\ 0}$ is dry matter at day 0 and $DM(\%)_{day\ N}$ is dry matter at day N.

Soluble Solids Content, pH and Ascorbic Acid Content Determination

Samples (10 g) from each treatment were crushed and homogenized in 100 mL boiled distilled water (pH 8.3) using a juice extractor (ACDEO3-029751, Shunde OUKE Electric Appliance Co. Ltd., China). The mixture was centrifuged at 5,000 ×*g* for 15 min in an Anke LXJ-B centrifuge (Shanghai Anting Scientific Instrument Factory, China), and the supernatant was used to determine soluble solids content, pH and ascorbic acid content. Soluble solids content (SSC) was determined by a hand-held refractometer (%) (WYT-J, Chendu Optical Apparatus Co., China). The pH was measured by means of a pH meter (FE20, Mettler Toledo). Ascorbic acid was determined using the 2, 6-dichloroindophenol titration method (AOAC 1990).

Total Phenolic Content Determination

Phenols were measured spectrophotometrically using

the Folin-Ciocalteu procedure (Singleton and Rossi 1965), with some modification. Briefly, samples (5 g) were extracted three times with 50 mL 100% methanol. The extracts were pooled and then centrifuged at 10,000 × g for 10 min in an Anke LXJ-B centrifuge (Shanghai Anting Scientific Instrument Factory, China). The supernatant (1 mL) was mixed with 9 mL of distilled H_2O in a 25 mL volumetric flask before adding 1.5 mL Folin-Ciocalteu reagent. After a reaction time of 6 min, 6 mL of 10% Na_2CO_3 solution was added. The mixture was made up to 25 mL with distilled H_2O. The phenol content was measured at 765 nm using a spectrophotometer (UNIC UV-2100, UNIC (Shanghai) Equipment Co. Ltd, China) after a reaction time of 90 min. The concentration of total phenolic compounds was determined by comparison with the absorbance of chlorogenic acid used at different concentrations as standard.

PPO Activity Analysis

Polyphenol oxidase (PPO) (EC 1.10.3.1) activity was measured using the method described by Deng et al. (2009). Five grams of samples were ground in 50 mL of 0.05 mol L^{-1} potassium dihydrogen phosphate buffer (pH 6.8) with a mortar and pestle. After rapid homogenization, the mixture was centrifuged at 8,000 ×g for 15 min at 4 °C in a Hermle Z233MK-2 refrigerated microcentrifuge (Germany). The clear supernatant was used to determine PPO activity. The enzyme solution (0.2 mL) was added to a mixture of 3 mL of 0.05 M phosphate buffer (pH 6.8), and 1.0 mL of 0.02 M catechol (pH 7.0) as substrate. PPO activity was measured in a spectrophotometer (UNIC UV-2100, UNIC (Shanghai) Equipment Co. Ltd, China) at 398 nm. One unit of PPO activity was defined as the amount of enzyme which results in 0.01 increase in absorbance per minute under assay conditions. Each determination was run in triplicate.

Sensory Analyses

Sensory evaluation of fresh-cut pears was carried out using a simple triangle test at days 1 and 3 of storage. Eight panelists, aged between 20 and 50 years old, were recruited among students and laboratory workers. The panelists were asked to score each sample for firmness, color, juiciness, flavor, taste and visual appearance based on a nine-point scale [1: extremely poor, 3: poor, 5: acceptable (limit of marketability), 7: good, 9: excellent (fresh sample)] (Deng et al. 2005a). The panelists were trained before each evaluation. Consistent references of sample exhibiting extreme browning and off-flavor were presented along with the samples; all evaluations were made by the same panelist to minimize variability. The panelists received three samples of three treatments per evaluation in random order. Samples were presented to the panelists in individual rooms provided with white lights. Each panelist was instructed to cleanse his or her mouth with distilled water, chew the sample and give scores for the indexes, then do oral cleansing again before proceeding to evaluate the next sample.

Statistical Analysis

Experimental design was completely randomized. The data were analyzed using ANOVA (P<0.05). Mean differences were established by Duncan's multiple range tests. The data were analyzed using SAS 8.0 statistical data analytical software.

RESULTS AND DISCUSSION

Change in Color

Browning reactions are detrimental to quality and limit the consumer acceptability of minimally processed products. Changes in L color value or hue angle (h) were used as indicators of browning intensity of fresh-cut pear surface (Oms-Oliu et al. 2008). Ascorbic acid + chitosan and chitosan + rosemary extracts resulted in higher L value and h angle (P<0.05), indicating less browning in the cut surface of pear wedges compared with the control (Table 1). At each test point, the L and h values in chitosan + rosemary-treated fruits were higher than in the samples treated with ascorbic acid + chitosan

Table 1. L and h values of fresh-cut pears during 3 d of storage at 20 °C.

Treatment	L		h	
	Day 1	Day 3	Day 1	Day 3
Control	62.86±7.77c	58.91±9.16c	83.59±4.29c	74.18±2.99b
AA + CH	64.52±4.70b	60.76±7.19b	85.27±3.07b	77.10±7.22ab
CH + R	67.15±8.63a	63.60±4.70a	88.14±5.17a	79.50±1.38a

Control: uncoated, AA + CH: ascorbic acid + chitosan, CH + R: chitosan + rosemary
Day 0 values: L = 78.46 ± 0.84; h = 97.21± 7.16
Values are expressed as mean ± SD. Values within a column followed by a common letter are not significantly different (P< 0.05) according to Duncan's multiple range tests.

Table 2. Electrolyte leakage of fresh-cut pears during 3 d of storage at 20 °C.

Treatment	Relative Leakage (%)	
	Day 1	Day 3
Control	37.0±0.70a	38.2±0.10a
AA + CH	34.46±1.04bc	36.8±1.10b
CH + R	33.5±0.42c	37.0±0.63b

Control: uncoated, AA + CH: ascorbic acid + chitosan, CH + R: chitosan + rosemary
Day 0 value: 27.8 ± 3.67
Values are expressed as mean ± SD. Values within a column followed by a common letter are not significantly different ($P < 0.05$) according to Duncan's multiple range tests.

($P<0.05$), except for *h* value at day 3. This suggested that rosemary is an effective anti-browning agent that may be incorporated in edible coatings. Ponce et al. (2008) also found that chitosan enriched with 1% rosemary oleoresins enhanced antioxidant protection of minimally processed squash, offering a great advantage in the prevention of browning reactions.

Changes in Electrolyte Leakage

The amount of electrolyte leakage is a function of membrane permeability. An increase in electrolyte leakage indicates a rise in membrane permeability and reflects deterioration in the quality of fruits and vegetables (Deng et al. 2005a; Chung et al. 2009). As shown in Table 2, electrolyte leakage increased gradually in the pear wedges at all treatment conditions during storage. Significant differences were found between the control and the other treatments ($P<0.05$). However, there were no statistical variations between ascorbic acid + chitosan and chitosan + rosemary. By day 3, the leakage rate of the control samples was about one-fold higher than that of the others. This result indicated that ascorbic acid + chitosan and chitosan + rosemary maintained membrane integrity and reduced electrolyte leakage. Similar findings were reported by Lin et al. (2008) that electrolyte leakage in chitosan-coated fruit exhibited ca.3% lower than the control fruit. Chung et al. (2009) found that browning in fresh-cut apples was correlated with levels of electrolyte leakage. In addition, Cantos et al. (2002) suggested that, except for either the enzymes associated with browning or polyphenol substrate concentration, membrane integrity is potentially a major factor in controlling the rate of browning.

Changes in Firmness and Weight Loss

Changes in firmness in the different treatments are shown in Table 3. Flesh firmness decreased with storage time in all treatments due to the release of water and other compounds as a consequence of the cutting process. Similar results were reported in minimally processed carambola (Ding et al. 2007) and in fresh-cut mango (Gonzalez-Aguilar et al. 2008). At longer storage, the control fruits had the fastest softening rate, losing about 15.9% of their firmness in about 3 d. In fruits coated with chitosan + rosemary and ascorbic acid + chitosan, firmness also decreased in 3 d, but to a less extent (9.6% and 10.4% lower, respectively, compared with the control). There were no significant variations between samples coated with chitosan + rosemary and ascorbic acid + chitosan ($P<0.05$). Ding et al. (2007) stated that treatment of the minimally processed fruit with ascorbic acid did not affect flesh firmness because ascorbic acid mainly functions as a reducing agent, as an antioxidant and as a metal sequestering agent but does not produce stress resistance in plants (Borenstein 1987).

Chitosan coating could decrease the reduction in firmness, the total soluble solids and the titratable acidity of 'Yali' pears stored at 25 ºC (Lin et al. 2008). The use of alginate or gellan edible coatings on fresh-cut apples effectively controlled moisture loss, preventing loss of turgor and reducing fruit softening (Rojas-Graü et al. 2007). However, no significant protection against

Table 3. Firmness and weight loss of fresh-cut pears during 3 d of storage at 20 °C.

Treatment	Firmness (N)		Weight Loss (%)	
	Day 1	Day 3	Day 1	Day 3
Control	14.44±2.20b	13.15±1.37b	5.81±0.13a	3.54±0.39a
AA + CH	14.77±1.76a	14.12±1.37a	3.55±0.31b	3.30±0.17b
CH + R	14.62±0.83ab	14.01±4.76a	3.67±0.24b	3.23±0.07b

Control: uncoated, AA + CH: ascorbic acid + chitosan, CH + R: chitosan + rosemary
Day 0 value: firmness 15.63 ±1.67
Values are expressed as mean ± SD. Values within a column followed by a common letter are not significantly different ($P< 0.05$) according to Duncan's multiple range tests.

loss of firmness was observed in cubes treated with SemperFresh coating applied on 'Kent' and 'Keitt' mango cubes (Gonzalez-Aguilar et al. 2008). Loss of firmness strongly depends on the enzymatic hydrolysis of cell wall substances (Deng et al. 2005b). Additionally, softening is often related to water loss, which is responsible for loss of turgor and crispness of fresh-cut fruits (Deng et al. 2008). In the present study, the highest weight loss and depletion in firmness of fresh cut fruit was observed in the control compared with the chitosan + rosemary and the ascorbic acid + chitosan treatments (Table 3).

The highest weight loss was observed in the control samples throughout storage (Table 3). Particularly, at day 1, weight loss was approximately 5.81%. Kays (1991) observed that more than 4–6% weight loss (from the total fresh weight) was accompanied by visible wilting or wrinkling of the surface of fresh-cut produce. In our study, weight loss was less than 4% for all coated fruits, which means the coated fruits maintained their freshness during storage. There were also no significant changes between the chitosan + rosemary and the ascorbic acid + chitosan treatments (P<0.05). Lin et al. (2008) found both chitosan coating and chitosan + ascorbic acid coating delayed increase of weight loss in 'Yali' pears at room temperature compared with the control, hence, resulting in delayed fruit shriveling and slow deterioration in fruit quality. Similar superior effects of the other edible coating treatments on weight loss have been observed in other fruits (Rojas-Graü et al. 2007; Bico et al. 2009; Deng et al. 2009). This was ascribed to the fact that edible coating on the fruit surface can retain the fluid and delay migration of moisture from the fruit to the environment and decrease the respiration rate.

Changes in pH, Soluble Solids Content and Ascorbic Acid

Compared with fruits at day 0, an increase in soluble solids content occurred throughout 3 d at 20 °C (Table 4), due to the hydrolysis of starch into sugar by phosphorylase enzymes in the fruit (Spayd et al. 1990). Our findings are in agreement with those on minimally processed carambola (Ding et al. 2007). Other authors indicated that the levels of glucose, fructose and sucrose in slices treated with different anti-browning agents increased during the first 6 d of storage and then decreased toward the end of storage (González-Aguilar et al. 2005). This difference may be associated with variation in the characteristics of the evaluated cultivars, particularly in sugars, upon ripening. Lin et al. (2008) also found that pear coated with chitosan retained higher total soluble solid content compared with the control. No significant difference was observed between ascorbic acid + chitosan and chitosan + rosemary treatments (P<0.05). This result indicated that the soluble solid content was not significantly affected by either ascorbic acid or rosemary treatment, similar to the findings on minimally processed carambola treated with ascorbic acid (Ding et al. 2007). For fresh-cut pineapple slices, however, the ascorbic acid treatments generally maintained higher sugar levels without a significant change after 6 d of storage at 10 °C (Robles-Sánchez et al. 2009).

pH of the wedge pears decreased at day 1, then increased at day 3 in all treatments. Chitosan + rosemary treatment effectively maintained lower pH value compared with the control and the ascorbic acid + chitosan treatment at day 3. No significant differences for pH were observed between ascorbic acid + chitosan and chitosan + rosemary except for day 3 (P< 0.05). This means that the ascorbic acid or the rosemary treatment had no significant effect on the pH of fresh cut pears, similar to the findings of Ding et al. (2007) for minimally processed carambola treated with ascorbic acid.

Changes in ascorbic acid content of sliced pear in relation to the different treatments are shown in Table 4. Significant changes in the levels of ascorbic acid were detected between the control and all coated wedges during the storage period at 20 °C (P<0.05). For example, at day 3, there was 8.88

Table 4. Soluble solids content (SSC), ascorbic acid (AA) and pH of fresh-cut pears during 3 d of storage at 20 °C.

Treatment	SSC (%)		pH		AA (mg/100 g FW)	
	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3
Control	8.97±0.06a	9.07±0.06b	4.46±0.03a	4.85±0.02a	3.67±0.13c	2.30±0.23[c]
AA + CH	9.10±0.10a	9.54±0.05a	4.41±0.01a	4.83±0.02a	9.69±0.07a	8.88±0.53a
CH + R	9.03±0.06a	9.67±0.06a	4.40±0.02a	4.78±0.01b	4.59±0.13b	2.76±0.23b

Control: uncoated, AA + CH: ascorbic acid + chitosan, CH + R: chitosan + rosemary, FW: fresh weight
Day 0 values: SSC = 8.73 ± 0.15; pH = 4.56 ± 0.03; AA = 4.24 ± 0.12
Values are expressed as mean ± SD. Values within a column followed by a common letter are not significantly different ($P < 0.05$) according to Duncan's multiple range tests.

mg per 100 g fresh weight of ascorbic acid in the ascorbic acid + chitosan-treated fruits, about 3.2-fold and 3.9-fold higher than in the chitosan + rosemary-treated fruits and the control, respectively. This finding is similar to those of González-Aguilar et al. 2005) on fresh-cut pineapple slices applied with ascorbic acid and of Robles-Sánchez et al. (2009) on fresh-cut 'Kent' mango treated with 1% ascorbic acid and 1% citric acid. Cocci et al. (2006) reported a 20-fold increase in ascorbic acid content in minimally processed apples after treatment of the tissues with a mixture of 1% ascorbic acid and 1% citric acid.

The use of coating on the surface of fresh-cut pears can also help reduce O_2 diffusion and, consequently, better preserve the ascorbic acid content of fresh-cut pears (Oms-Oliu et al. 2008). Ascorbic acid content sharply increased after treatment with 2% ascorbic acid. However, ascorbic acid content in the ascorbic acid + chitosan-treated fruits increased from day 0 to day 1 but decreased again toward the end of storage. This finding concurs with that of Robles-Sánchez et al. (2009) on fresh-cut pineapple, and is attributed to the fact that ascorbic acid is most likely converted to dehydroascorbic acid and further degraded to 2, 3 diketo-gluconic acid. The ascorbic acid level in pear wedges treated with chitosan + rosemary followed a similar pattern, although to a less extent.

Changes in Total Phenolic Content and Polyphenol Oxidase (PPO) Activity

Browning is not only related to the presence of PPO but also to the presence of phenolic substrates and the availability of oxygen. Changes in total phenolic content and PPO activity are given in Table 5. Significant differences were noted among the three treatments (P<0.05). After 3 d storage, reduction in the total phenolic content in the control was 24%, 21% in the ascorbic acid + chitosan-treated fruits and 13% in the chitosan + rosemary-treated samples. This trend suggests that the chitosan coating plus ascorbic acid or rosemary could prevent the decrease in phenolic content to a varying degree. Campaniello et al. (2008) reported that the use of chitosan coating could reduce losses in phenolic compounds and minimize the occurrence of browning in fruits. As an efficient reducing agent, 2% (m/v) ascorbic acid treatment delayed the loss of phenolic compounds in fresh-cut apple (Gil et al. 1998).

Oxidation of phenolic compounds into quinines via PPO inherent in tissue is believed to be a major cause of browning in many fruits and vegetables (Deng et al. 2005a). Compared with the control, both ascorbic acid + chitosan and chitosan + rosemary significantly suppressed increase in the PPO activity level of fruits (Table 5) (P<0.05). The lower L and h values (Table 1), as well as worsening visual appearance of the fruits (Fig. 1) could be related to the higher PPO activity of the fresh cut wedges. Talcott et al. (2003) also observed that the addition of both rosemary extract at 0–0.4% (v/v) and ascorbic acid (800 mg L^{-1}) were influential in altering the total phenolic content and the antioxidant capacity of muscadine juice. Application of ascorbic acid (0.05–0.1 M) to fresh-cut pineapple slices diminished the loss of phenol content and the PPO activity (González-Aguilar et al. 2005). In our study, there was a large increase in PPO activity in the control but the reverse was observed in the other treatments. This increase is related to the reaction of the uncoated control sample toward enzymatic reactions. In addition, the level of PPO in ascorbic acid + chitosan-treated fruit was 2.9-fold and 2.5-fold higher than that of the chitosan + rosemary-treated samples at 1 d and at 3 d, respectively.

The combination of chitosan coating and rosemary offered the best protection against reduction in the phenolic content and the increase in PPO of the pear wedges. The antioxidant ability of rosemary is far higher than that of other natural antioxidants such as ascorbic acid, α-tocopherol and tea polyphenols (http://www.allproducts.com/). On the other hand, Ponce et al. (2008)

Table 5. Total phenolic content and polyphenol oxidase (PPO) activity of fresh-cut pears during 3 d of storage at 20 °C.

Treatment	Total Phenolic Content (mg/g FW)		PPO Activity (U/g FW/min)	
	Day 1	Day 3	Day 1	Day 3
Control	0.27±0.01c	0.26±0.01c	18.62±0.20a	18.33±1.21a
AA +CH	0.31±0.04b	0.27±0.04b	5.69±0.83b	3.48±0.80b
CH + R	0.32±0.04a	0.28±0.05a	1.99±0.31c	1.39±0.68c

Control: uncoated, AA + CH: ascorbic acid + chitosan, CH + R: chitosan + rosemary, FW: fresh weight
Day 0 values: total phenolic content = 0.35 ± 0.01, PPO = 6.72 ± 0.12
Values are expressed as mean ± SD. Values within a column followed by a common letter are not significantly different ($P < 0.05$) according to Duncan's multiple range tests.

found that chitosan coating plus rosemary oleoresin was less effective in reducing PPO activity in butter lettuce than in Romaine lettuce or butternut squash. The different results may be due to the fact that changes in phenolic composition and PPO activity may be strongly influenced in fruits not only by processing methods and conditions but also by variety and stage of maturity, among others.

Sensory Attributes

Sensory attributes, namely, firmness, color, juiciness, flavor, taste and visual appearance of fresh-cut pear wedges are shown in Figure 1. Sensory testing was not performed beyond 3 d of storage for microbiological reasons. At day 1, the chitosan-coated pear wedges scored higher than the control in terms of overall sensory attributes ($P < 0.05$). In particular, the control showed the most significant decrease in visual appearance (4.8) to the extent of being unacceptable to consumers, largely due to significant shriveling or wrinkling (Table 3) and browning. Chitosan + rosemary-treated fruits had higher scores for color and visual appearance parameters than the ascorbic acid + chitosan-treated samples, while the score for taste was low for the former. No significant differences in terms of flavor, juiciness and firmness were observed between the two treatments ($P<0.05$) were observed. After 3 d, all treatments resulted in a reduction in sensory quality compared with day 1. There were significant differences in sensory scores between the control and the other treated samples ($P<0.05$), with the control scoring the lowest (≤5), indicating loss of commercial value. Ascorbic acid + chitosan-treated samples were given higher scores for flavor and taste than the chitosan + rosemary-treated samples, but were given lower scores for visual appearance and color. No statistical variations were detected for firmness ($P<0.05$). As a whole, the combination of chitosan and rosemary was more effective in the reduction of browning and deterioration of the quality of fresh-cut pears stored at 20 °C.

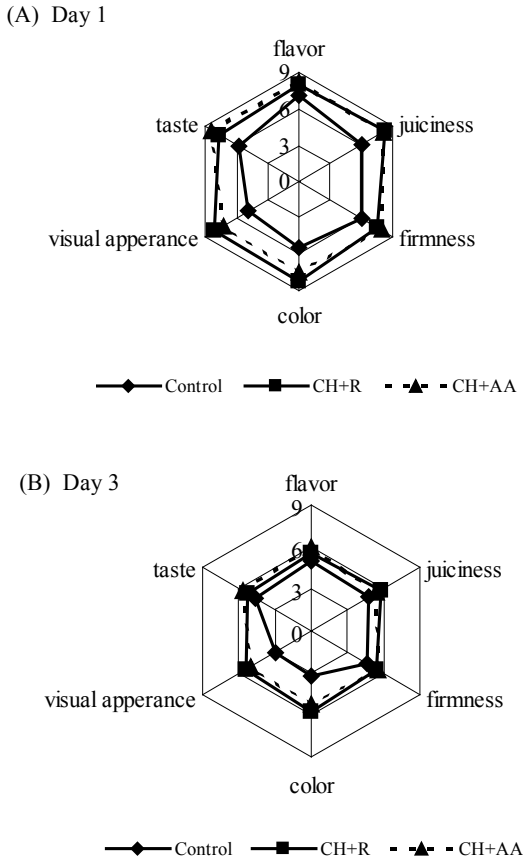

Fig. 1. Sensory profiles of fresh-cut pear wedges stored at 20 °C. Control: uncoated, AA + CH: ascorbic acid + chitosan, CH + R: chitosan + rosemary.

CONCLUSION

Chitosan coating combined with rosemary/ascorbic acid maintained the quality of fresh-cut pear and diminished browning, softening and decay, which are major problems of this fruit during storage. Compared with ascorbic acid + chitosan, chitosan + rosemary treatment inhibited polyphenol oxidase activity, reduced loss of phenolic content, retained higher L and h values, and resulted in a lower rate of softening and sensory degradation in the fresh-cut fruit after 3 d of storage at 20 °C. The use of a combination of chitosan coating with rosemary extract may be a potential method to improve the antioxidant protection and the sensorial attributes, and to delay the deterioration of quality in fresh-cut pears. More research in the future is required to investigate the growth of microorganisms, as well as the physiological mechanisms contributing to quality preservation in relation to this treatment combination.

ACKNOWLEDGMENT

This research was supported by the National Undergraduate Innovative Test Program (Project 081024832) and the National Natural Science Foundation of China (Project 30600420).

REFERENCES CITED

[AOAC] Association of Official Analytical Chemists. 1990. Vitamin C (Ascorbic acid), 2,6-dichloroindophenol titrimetric method. In: Helrich K, editors. Official

Methods of Analysis. 15th ed. Virginia: Arlington. p. 1058-1059.

BORENSTEIN B. 1987. The role of ascorbic acid in foods. Food Tech 41: 98-99.

BICO SLS, RAPOSO MFJ, MORAIS RMSC, MORAIS AMMB. 2009. Combined effects of chemical dip and/or carrageenan coating and/or controlled atmosphere on quality of fresh-cut banana. Food Control 20: 508-514.

CAMPANIELLO D, BEVILACQUA A, SINIGAGLIA M, CORBO MR. 2008. Chitosan: Antimicrobial activity and potential applications for preserving minimally processed strawberries. Food Microbiol 25: 992-1000.

CANTOS E, TUDELA JA, GIL MI, ESPIN JC. 2002. Phenolic compounds and related enzymes are not rate-limiting in browning development of fresh-cut potatoes. J Agric Food Chem 50: 3015-3023.

COCCI E, ROCCULI P, ROMANI S, ROSA M DALLA. 2006. Changes in nutritional properties of minimally processed apples during storage. Postharvest Biol Technol 39: 265-271.

DENG Y, LUO W, ZHU LW, XIAO CL. 2009. Effect of chitosan-beewax composite coating on physiology and quality of frozen Yellow-flesh peach (*Prunus persicu* L. *Batsch*.). Trans CSAE (accepted).

DENG Y, WU Y, LI YF. 2005a. Effects of high O_2 levels on post-harvest quality and shelf life of table grapes during long-term storage. Eur Food Res Technol 221: 392-397.

DENG Y, WU Y, LI YF. 2005b. Changes in firmness, cell wall composition and cell wall hydrolases of grapes stored in high oxygen atmospheres. Food Res Int 38: 769-776.

DENG Y, ZHU LW, LUO W, XIAO CL, SONG XY, CHEN JS. 2009. Changes in physical properties of chitosan films at subzero temperatures. Ital J Food Sci 4 (21): 1-11.

DING P, AHMAD SH, GHAZALI HM. 2007. Changes in selected quality characteristics of minimally processed carambola (*Averrhoa carambola* L.) when treated with ascorbic acid. J Sci Food Agric 87:702-709.

FENG YX, LI LM, GUAN JF, JI H, SUN YI. 2008. Effect of functional modified atmosphere packaging on storage quality and browning of 'Huangguan' pear. J Hebei Agric Sci 12 (11):16 -17, 32.

GIL MI, GORNY JR, KADER AA. 1998. Responses of 'Fuji' apple slices to ascorbic acid treatments and low-oxygen atmospheres. HortScience 33(2): 305-309.

GONZALEZ-AGUILAR GA, CELIS J, SOTELO-MUNDO RR, DE LA ROSA LA, RODRIGO-GARCIA J, ALVAREZ-PARRILLA E. 2008. Physiological and biochemical changes of different fresh-cut mango cultivars stored at 5 °C. Int J Food Sci Technol 43: 91-101.

GONZÁLEZ-AGUILAR GA, RUIZ-CRUZ S, SOTO-VALDEZ H, VÁZQUEZ-ORTIZ F, PACHECO-AGUILAR R, WANG CY. 2005. Biochemical changes of fresh-cut pineapple slices treated with anti-browning agents. Int J Food Sci Technol 40: 377-383.

GORNY JR, GIL MI, KADER AA. 1998. Postharvest physiology and quality maintenance of fresh-cut pears. Acta Horticult 464:231-236.

GORNY JR, HESS-PIERCE B, CIFUENTE RA, KADER AA. 2002. Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. Postharvest Biol Technol 24: 271-278.

KAYS SJ. 1991. Postharvest Physiology of Perishable Plant Products. New York: Van Nostrand Reinhold. p. 356-357.

LIN L, WANG BG, WANG M, CAO JK, ZHANG JJ, WU Y, JIANG WB. 2008. Effects of a chitosan-based coating with ascorbic acid on post-harvest quality and core browning of 'Yali' pears (*Pyrus bretschneideri* Rehd.). J Sci Food Agric 88: 877-884.

OIL-SOLUBLE ROSEMARY EXTRACT RM-A61. 2009. Hainan Super Biotech Co. LTD. Allproducts.com. Retrieved August 13, 2009 from the World Wide Web: http://www.allproducts.com/manufacture100/rosemary008/product2.html.

OMS-OLIU G, AGUILÓ-AGUAYO I, MARTÍN-BELLOSO AO. 2006. Inhibition of browning on fresh-cut pear wedges by natural compounds. J Food Sci 71(3): S216-S224.

OMS-OLIU G, ODRIOZOLA-SERRANO I, SOLIVA-FORTUNY R, MARTÍN-BELLOSO O. 2008. Antioxidant content of fresh-cut pears stored in high-O_2 active packages compared with conventional low-O_2 active and passive modified atmosphere packaging. J Agric Food Chem 56: 932-940.

PONCE AG, ROURA SI, DEL VALLE CE, MOREIRA MR. 2008. Antimicrobial and antioxidant activities of edible coatings enriched with natural plant extracts:

In vitro and *in vivo* studies. Postharvest Biol Technol 49: 294-300.

ROBLES-SÁNCHEZ RM, ROJAS-GRAÜ MA, ODRIOZOLA-SERRANO I, GONZÁLEZ-AGUILAR GA, MARTÍN-BELLOSO O. 2009. Effect of minimal processing on bioactive compounds and antioxidant activity of fresh-cut 'Kent' mango (*Mangifera indica* L.). Postharvest Biol Technol 51: 384-390.

ROJAS-GRAÜ MA, SOLIVA-FORTUNY R, MARTÍN-BELLOSO O. 2009. Edible coatings to incorporate active ingredients to fresh-cut fruits: a review. Trends Food Sci Technol, doi:10.1016/j.tifs.2009.05.002.

ROJAS-GRAÜ MA, TAPIA MS, RODRÍGUEZ FJ, CARMONA AJ, MARTÍN-BELLOSO O. 2007. Alginate and gellan based edible coatings as support of antibrowning agents applied on fresh-cut Fuji apple. Food Hydrocolloid 21(1): 118-127.

SINGLETON VL, ROSSI JA. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Viticul 16(3): 144-158.

SPAYD SE, MORRIS JR, BALINGEN WE, HIMELRICK DJ. 1990. Maturity standards, harvesting, postharvest handling and storage. In: Galleta GJ, Himelrick DJ, editors. Small Fruit Management. New Jersey: Prentice Hall. p. 504-528.

STEFANOVITS-BÁNYAI É, TULOK MH, HEGEDÜS A, RENNER C, VARGA IS. 2003. Antioxidant effect of various rosemary (*Rosmarinus officinalis* L.) clones. Acta Biologica Szegediensis 47(1-4):111-113.

TALCOTT ST, BRENES CH, PIRES DM, DEL POZO-INSFRAN D. 2003. Phytochemical stability and color retention of copigmented and processed muscadine grape juice. J Agric Food Chem 51: 957-963.