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MOHAMMAD ALI SHIRI

DAVOOD BAKHSHI

MAHMOOD GHASEMNEZHAD

MONAD DADI

ALEXANDROS PAPACHATZIS

See next page for additional authors

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Chitosan coating improves the shelf life and postharvest quality of table grape (*Vitis vinifera*) cultivar Shahroudi

Mohammad Ali SHIRI[1], Davood BAKHSHI[1], Mahmood GHASEMNEZHAD[1], Monad DADI[1], Alexandros PAPACHATZIS[2,*], Helen KALORIZOU[2]

[1]Department of Horticultural Sciences, Faculty of Agriculture, University of Guilan, Rasht, Iran
[2]Technological Education Institute of Larissa, Department of Plant Production, Larissa, Greece

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Abstract: Table grapes (*Vitis vinifera*) of the cultivar Shahroudi were coated with 0.5% or 1% chitosan and then stored at 0 °C for 60 days. Shelf-life qualitative traits including rachis and berry appearance and berry cracking, shattering, decay, browning, acceptability, and flavor were evaluated. Treated berries showed less weight loss, decay, browning, shattering, and cracking. Flavor-related factors such as total soluble solids (TSS), titratable acidity (TA), and TSS/TA ratio levels were higher in treated fruits; however, there was no significant difference between the fruits treated with 0.5% and 1% chitosan. Changes of the total phenolics, catechin, and antioxidant capacity of the berries coated with chitosan were delayed, while quercetin 3-galactoside and total quercetin were higher in the control treatment. Overall, fruits coated with chitosan had greater external adequacy than untreated ones.

Key words: Chitosan, grape, phenolics, quality, storage

1. Introduction

Postharvest deterioration of grapes can be due to physical, physiological, or pathological factors that may occur in the vineyard (preharvest) or after harvest. For example, rachis dehydration is a physical deterioration associated with high vapor pressure deficit between the rachis and the environment during the pre- and postharvest periods (Crisosto et al. 1994). Skin browning is the main physiological problem associated with an excessively mature Princess table grape cultivar (Vial et al. 2005).

Botrytis cinerea can also infect grapes during berry development, remaining latent until harvest or appearing during storage (Holz et al. 2003). Micropore density on the surface of the berry has been highly correlated with *B. cinerea* infection in table grapes (Gabler et al. 2003). Therefore, the integration of canopy management and fungicide treatments before harvest with the use of SO_2 and cold storage (–0.5 °C) after harvest are the commercial strategies implemented to control this disease; however, hairline cracking and bleaching develop under high SO_2 postharvest management (Zoffoli et al. 2008). Edible coatings have long been known to protect perishable food products from deterioration by retarding dehydration, suppressing respiration, improving textural quality, helping retain volatile flavor compounds, and reducing microbial growth (Debeaufort et al. 1998; Özdemir et al. 2010).

Over the last decade, much attention has been paid to the potential of natural polymers such as polysaccharides and proteins in food packaging applications. Most of these macromolecules can be processed into films or applied as fruit surface coatings to reduce respiration and transpiration rates due to their high permselectivity (CO_2/O_2) coefficient and partial moisture barrier. They also improve the mechanical handling properties of the produce and help maintain its structural integrity (Baldwin 1994). Chitosan, a mostly deacetylated β-(1-4)-linked D-glucosamine polymer, is a structural component of fungal cell walls. Chitosan has been reported to enhance resistance against many fungal diseases when applied as either a pre- or postharvest treatment (Reglinski et al. 2005). In addition, chitosan can be directly antimicrobial and has been shown to interfere with the germination and growth of several phytopathogenic fungi, including *B. cinerea* (Ben-Shalom et al. 2003). Furthermore, various investigators have demonstrated that a chitosan coating has the potential to inhibit decay and hence prolong the storage life of a variety of produce such as citrus fruit (Chien et al. 2007).

* Correspondence: papachad@teilar.gr

In recent years, consumers' interest in the health enhancement role of specific foods or physically active food components, so-called nutraceuticals or functional foods, has exploded (Hasler 1998). Phenolic compounds are widely distributed in plant foods and therefore are important constituents of the human diet. Phenolic compounds may act as an antioxidant and protect foods from oxidative deterioration. In recent years, the numbers of studies conducted to determine the antioxidant activity of phenolic compounds have increased due to the possible role of reactive oxygen species in the pathogenesis of degenerative diseases such as atherosclerosis, cancer, and chronic inflammation. The phenolic compounds from grapes contribute to the desired color, corpulence, astringency, flavor, and vitamins; participate in oxidoreduction and condensation reactions; and have bactericidal properties.

The objectives of this study were to investigate the effectiveness of chitosan postharvest treatments for the postharvest traits of table grapes. In addition, the influence of chitosan on fruit nutritional quality, especially total phenolic content and antioxidant capacity, was evaluated.

2. Materials and methods

2.1. Plant materials and treatments

Table grapes (*Vitis vinifera*) of the cultivar Shahroudi were harvested at the ripe stage from a commercial vineyard in North Khorasan Province in northeastern Iran. The harvest time was determined by the total soluble solids concentration (TSS), which was 19.5% (TSS for the Shahroudi cultivar ranges between 18% and 20% at the ripe stage).

Fruits were selected for size and color uniformity. Blemished, damaged, or diseased berries were discarded carefully. After preparation, fruits were weighed to about 1 kg and then randomly distributed into 3 groups before treatment. Low molecular weight chitosan (Sigma Chemicals, USA) solution (1%, w/v) was prepared by dissolving chitosan in 0.5% (v/v) glacial acetic acid (Ghasemnezhad et al. 2010) under continuous stirring. The pH of the solution was adjusted to 5.2 using 1 N NaOH, and the solution was then autoclaved at 121 °C for 20 min. Grape bunches were dipped in the solutions for 1 min and then left for 2 h at room temperature to be dried. Samples of the control were dipped in the solution at pH 5.2 containing no chitosan. Treated grapes were packed in polypropylene bags of 20 × 20 cm^2 with a 29.2 pmol s^{-1} m^{-2} Pa^{-1} oxygen transmission rate film and stored in an experimental storage room with a temperature of 0 °C and 90 ± 5% relative humidity for 60 days. At the end of the storage stage, packages were brought out and kept at room conditions (24 °C and 70 ± 5% relative humidity) for 5 days. Evaluation of qualitative traits was performed on days 0, 30, 60, and 65 with 3 replications.

2.2. Qualitative traits

Treated grapes with chitosan intended for quality evaluation were randomized, treated, packaged, and stored as described above. Bunches of each treatment were examined by evaluation of berry and rachis appearance, incidence of cracked and shattered berries, decay, browning, acceptability, and flavor by a panel of 5 trained judges (Xu et al. 2007). The visual characteristics, including berry and rachis appearance, were scored in daylight. Fruit flavor was evaluated under red light in a taste room with individual booths in order to avoid the interference of visual judgment. Detailed evaluation information and the units employed are described in Table 1.

Table 1. Postharvest qualitative traits and methods used for evaluating Shahroudi table grapes after storage for 65 days at 0 °C (relative humidity > 90).

Quality parameters	Methods of evaluation and units
Rachis appearance	Visual index of clusters: 1 = fresh and green, 2 = green, 3 = semidry, 4 = 50% dry, 5 = completely dry.
Berry appearance	Visual index of clusters: 1 = excellent, 2 = good, 3 = slightly dull, 4 = <50% brownish and soft berries, 5 = >50% brownish and soft berries.
Cracking	Number of cracked berries kg^{-1}
Shattering	Number of shattered berries kg^{-1}
Flavor	Flavor acceptability using a 5-point scale: 1 = excellent, 2 = good, 3 = acceptable, 4 = poor, 5 = unacceptable.
Browning	Number of brown berries kg^{-1}
Decay	Number of decayed berries kg^{-1}
Acceptability	Acceptability was evaluated on a scale of 9 to 1 where 9 = excellent, no defects; 7 = very good, minor defects; 5 = fair, moderate defects; 3 = poor, major defects; 1 = unusable.
Berry abscission	Number of abscissed berries kg^{-1}

2.3. Weight loss

Grapes were weighed at the beginning of the experiment just after treatment, at days 30 and 60 of low temperature storage, and finally 5 days after exposure to room conditions. Weight loss was expressed as percentage of initial weight.

2.4. Total soluble solid, titratable acidity, and TSS/TA ratio

Total soluble solid (TSS) contents were determined with a desktop digital refractometer (CETI, Belgium) for the juice of 10–20 berries for each treatment. Titratable acidity (TA) was determined by titration of 25 mL of filtered juice with the addition of 0.1 N NaOH solution to the juice to reach a pH of 8.2.

2.5. Total phenolic content

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method as described by Singleton et al. (1999), with minor modifications. Polyphenol extraction was carried out with 10 mL acidic methanol added to 1 g of fine powder of specimen, kept at 4 °C, with the mixture then filtered through ordinary filter paper. Next, 150 µL of this extract was diluted with 350 µL of distilled water, and then 2.5 mL of Folin–Ciocalteu reagent and 2 mL of 7.5% sodium carbonate were added to the mixture. This reaction solution was shaken in a shaker and kept in the dark for 2 h. The absorbance of the samples was measured at 765 nm with a UV/Vis spectrophotometer. Gallic acid was used as a standard for obtaining the calibration curve. Data were expressed as milligram of gallic acid equivalent (mg GAE) per gram of fruit fresh weight.

2.6. Phenolic composition

Quercetin and catechin were determined at the beginning and at the end of the storage time using high-performance liquid chromatography (HPLC) as described by Bakhshi and Arakawa (2006). At least 20 berries were peeled carefully and ground to fine powder using liquid nitrogen, and 2 mL of extraction solvent (methanol and acetic acid, 85:15, v/v) was added to 1 g of skin powder and then kept in a refrigerator overnight. The samples were centrifuged for 10 min at 10,000 rpm. The supernatant of centrifuged samples was filtered through a 0.45-µm syringe filter, and then 50 µL of the filtered sample was injected into a HPLC instrument (Waters, USA) equipped with a UV-Vis detector (Waters Dual λ Absorbance 2487) and a C18 column (Waters Symmetry C18, 5 µm, 4.6 × 150 mm; Waters, Ireland) set at 280 nm (for catechins) and 350 nm (for quercetins). The catechin standard was purchased from Sigma-Aldrich Chemicals, and quercetin 3-galactoside was obtained from Extrasynthese, France.

2.7. Antioxidant capacity

The antioxidant capacity was measured by the scavenging of 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) radicals according to the method of Brand-Williams et al. (1995) with minor modifications. Two milliliters of 0.15 mM DPPH solved in methanol was added to 1 mL of grape berry extracts and mixed well. Absorbance of the mixture was measured at 517 nm after 30 min. The inhibition percentage for each sample was calculated as follows and expressed as antioxidant capacity:

$$\% \text{ inhibition} = 100 (A_0 - A_x) / A_0,$$

where A_0 is the absorbance of blank solution and A_x is the absorbance of juice-containing media.

2.8. Statistical analysis

The experiment was conducted using a completely randomized design with 3 replications and 10 clusters per replication. A preliminary test was run prior to the main experiment reported here. Data were analyzed as a 2-factor linear model via the PROC MIXED procedure of SAS software (ver. 9.1, 2002–2003), where treatments and storage time were the factors.

3. Results

Sensory quality decreased during storage (Table 2). Fruit treated with chitosan was commercially acceptable after 60 days of storage followed by 5 days kept under room temperature conditions. All fruit showed a progressive loss of weight during storage (Table 3). Fruit weight loss is mainly related to the respiration rate and moisture evaporation of fruits. In this study, chitosan coating significantly ($P < 0.01$) reduced weight loss during storage as it enables epidermal tissues to control water loss and reduce respiratory exchange.

The concentrations of total soluble solids and titratable acidity decreased over time during shelf life evaluation (Table 3), which was consistent with fruit quality decline. Chitosan coating treatment delayed the decrease in concentrations of total soluble solids and titratable acidity. There was no significant difference between the 2 levels of chitosan concentrations applied in this study. The TSS/TA ratio of table grape is reported in Table 3. The results show that TSS/TA significantly ($P < 0.01$) increases during storage time. For all fruits and treatments the increase in TSS/TA (ripening index) was attributable to a decrease in TA rather than to a decrease in TSS.

The total phenolic content changes of table grapes during storage are shown in Figure 1. It was observed that total phenolic content decreased significantly during early storage (124.1 to 63.3 mg GAE 100 g FW^{-1}) and then increased in the last stage (79.5 mg GAE 100 g FW^{-1}). The highest phenolic content was found with 1% chitosan (99.8 mg GAE 100 g FW^{-1}) and the lowest was found in the control (86.9 mg GAE 100 g FW^{-1}) after 65 days of storage.

The changes of flavonoids evaluated in this study, including catechin, quercetin 3-galactoside, and total quercetin, are shown in Figures 2–4. Catechin, quercetin 3-galactoside, and total quercetin content decreased

Table 2. Influence of chitosan coating and storage time on Shahroudi table grapes during storage at 0 °C.

Treatments	Storage times	Fruit quality								
		Rachis appearance	Berry appearance	Cracking	Shatter	Flavor	Browning	%Decay	Acceptability	Berry abscission (%)
Control	**0**	1.0 e	1.0 e	0.0 f	0.0 g	1.0 d	0.0 f	0.0 f	9.0 a	0.0 f
	30	3.7 bc	2.3 c	5.7 b	6.5 de	2.0 bcd	2.3 cd	17.7 c	7.0 b	11.6 cd
	60	4.0 b	3.3 b	6.6 b	13.2 b	2.7 b	3.3 b	29.4 b	5.0 c	17.2 b
	60 + 5	5.0 a	4.0 a	10.4 a	23.4 a	4.7 a	4.7 a	42.7 a	3.0 d	24.6 a
Chitosan 0.5%	**0**	1.0 e	1.0 e	0.0 f	0.0 g	1.0 d	0.0 f	0.0 f	9.0 a	0.0 f
	30	2.7 d	1.0 e	0.9 ef	2.6 f	1.3 cd	1.3 e	3.5 f	8.3 a	1.7 f
	60	4.0 b	1.7 d	1.9 cde	7.5 cd	1.7 bcd	2.3 cd	11.1 de	6.3 b	7.6 e
	60 + 5	4.3 ab	2 cd	2.6 c	8.3 cd	2.7 b	2.7 bc	14.9 cd	5.0 c	13.9 bc
Chitosan 1%	**0**	1.0 e	1.0 e	0.0 f	0.0 g	1.0 d	0.0 f	0.0 f	9.0 a	0.0 f
	30	2.7 d	1.0 e	0.9 ef	2.1 fg	1.0 d	1.0 e	3.1 f	9.0 a	1.0 f
	60	3.0 cd	1.7 d	1.1 def	5.1 e	1.3 cd	1.7 de	10.2 e	7.0 b	7.3 e
	60 + 5	4.3 ab	1.7 d	2.2 cd	9.2 c	2.3 bc	2.3 cd	13.4 de	6.3 b	8.3 de

Means of 3 replicates with the same letters were not statistically significantly different ($P \leq 0.01$).

Table 3. Influence of chitosan coating and storage time on Shahroudi table grapes during storage at 0 °C.

Treatments	Storage times	Weight loss (%)	TSS (%)	TA(%)	TSS/TA
Control	**0**	0.0 h	19.5 a	0.53 a	36.5 de
	30	0.25 f	16.0 bc	0.45 bc	35.6 e
	60	0.5 c	15.1 cd	0.35 d	42.8 cde
	60 + 5	1.24 a	13.7 d	0.2 e	67.9 a
Chitosan 0.5%	**0**	0.0 h	19.5 a	0.53 a	36.5 de
	30	0.06 g	19.4 a	0.48 b	40.4 cde
	60	0.31 e	18.3 a	0.42 c	43.2 cd
	60 + 5	0.82 b	17.8 ab	0.33 d	53.5 b
Chitosan 1%	**0**	0.0 h	19.5 a	0.53 a	36.5 de
	30	0.09 g	18.9 a	0.47 b	39.8 cde
	60	0.4 d	18.4 a	0.42 c	44.4 c
	60 + 5	0.9 b	18.0 ab	0.32 d	57.0 b

Means of 3 replicates with the same letters were not statistically significantly different (P ≤ 0.01).

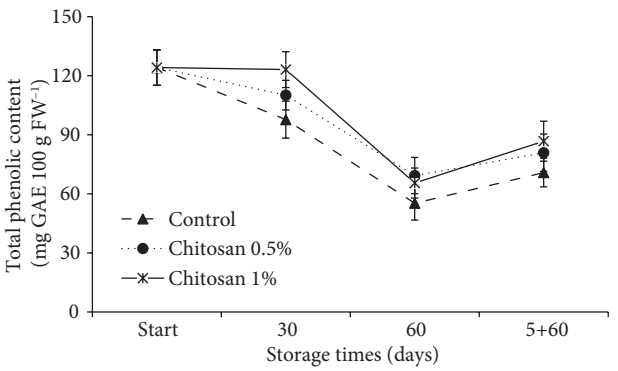

Figure 1. Changes in total phenolic content of table grapes treated with chitosan during storage at 0 °C. Vertical bars indicate standard error (n = 3).

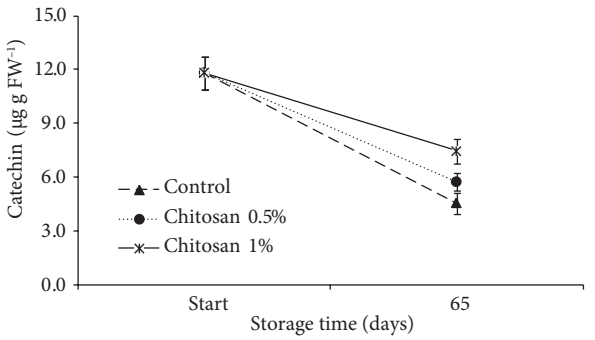

Figure 2. Changes in catechin of table grapes treated with chitosan during storage at 0 °C. Vertical bars indicate standard error (n = 3).

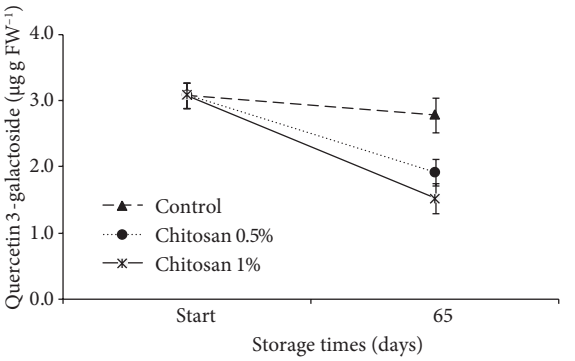

Figure 3. Changes in quercetin 3-galactoside of table grapes treated with chitosan during storage at 0 °C. Vertical bars indicate standard error (n = 3).

significantly (P < 0.01) at the end-point of storage. Chitosan-treated grapes had higher catechin content than the control, with an inverse of quercetin 3-galactoside and total quercetin content.

It was found that antioxidant capacity decreased significantly (P < 0.01) during 60 days of storage (55.8% to 36.3% DPPH scavenging activity [DPPHsc]) and thereafter increased (40.3% DPPHsc) in the last stage (Figure 5). Fruits treated with chitosan had significantly higher antioxidant capacity than the control (48.8%, 47.3%, and 38.9% DPPHsc in chitosan at 1% and 0.5% and the control, respectively). Park et al. (2004) suggested that chitosan may eliminate various free radicals by the action of nitrogen on the C-2 position of the chitosan. The results of the present study (Figure 5; Table 4) show that there is

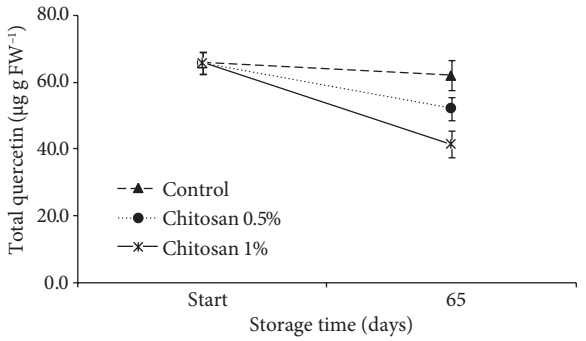

Figure 4. Changes in total quercetin of table grapes treated with chitosan during storage at 0 °C. Vertical bars indicate standard error (n = 3).

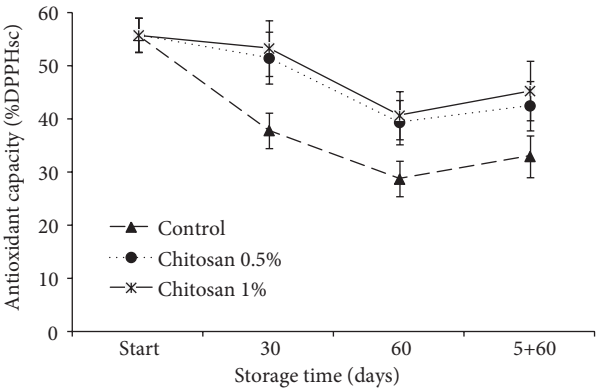

Figure 5. Changes in antioxidant capacity of table grapes treated with chitosan during storage at 0 °C. Vertical bars indicate standard error (n = 3).

Table 4. Correlation coefficients between total phenolic content, antioxidant capacity, and phenolic composition in Shahroudi table grapes treated with chitosan, compared to control, during storage at 0 °C.

	Catechin (µg g FW^{-1})	Quercetin 3-galactoside (µg g FW^{-1})	Total quercetin (µg g FW^{-1})	Total phenolic content (mg GAE 100 g FW^{-1})	Antioxidant capacity (% DPPHsc)
Catechin (µg g FW^{-1})	1	0.59 **	0.53 *	0.91 **	0.77 **
Quercetin 3-galactoside (µg g FW^{-1})		1	0.96 **	0.61 **	0.32 ^{ns}
Total quercetin (µg g FW^{-1})			1	0.59 **	0.33 ^{ns}

*, **, and ns: Significantly different values at $P \leq 0.05$ and $P \leq 0.01$, and nonsignificant.

a positive relationship between total phenolic content and antioxidant capacity (r = 0.79). Interestingly, there was no significant correlation between antioxidant capacity and quercetins, which is in agreement with the findings of Pinelo et al. (2004), who reported that the antioxidant capacity of flavonoids is related to variations in antiradical activities such as quercetin ≤ resveratrol < catechin.

4. Discussion

Loss of grape quality due to weight loss, color changes, accelerated softening, and high incidence of berry decay and rachis browning (Crisosto et al. 2002) leads to shelf-life reduction. The storage-life extension because of chitosan coating might be due to the formation of a semipermeable layer that regulates gas exchanges, reducing transpiration loss. Forming a semipermeable layer on live crops reduces CO_2 from cellular respiration in tomatoes, tangerines, and pears (Bautista-Baños et al. 2006). Low storage temperature was thought to be directly correlated with the reduction of CO_2 production, which was clearly demonstrated in the present study.

In general, the positive effect of fruit coatings with polysaccharides like chitosan is based on their hygroscopic properties that enable the formation of a water barrier and consequently reduce external water transfer (Morillon et al. 2002). In addition, chitosan coating has been reported to be a useful part of a strategy to reduce the weight loss caused by pathogen isolates that are resistant to currently used postharvest fungicides (Chien et al. 2007), which is evident in this study (Table 3). The antifungal property of chitosan might be related to its forming a physical barrier against infection, reducing the conidial germination and mycelial growth of *B. cinerea* and resulting in the long-lasting protection of grape berries against gray mold (Romanazzi et al. 2002).

Moreover, the polycationic properties of chitosan provide this polymer with the possibility of forming films by the breakage of polymer segments and subsequently reforming the polymer chain into a film matrix or gel; this can be achieved by evaporating a solvent, thus creating hydrophilic and hydrogen bonding and/or electrolytic and ionic cross linking (Butler et al. 1996). These films are an

excellent oxygen barrier, and their mechanical properties are comparable to many medium-strength commercial polymer films (Park et al. 2002). Romanazzi (2010) reported that application of chitosan coating inhibited respiration rates of table grapes, strawberries, and sweet cherries. Thus, the higher levels of total soluble solids and titratable acidity in the fruits coated with chitosan found in this study may be due to the reduction of the oxygen supply on the fruit surface, which inhibited respiration (Yonemoto et al. 2002); however, chitosan's impact on O_2 and CO_2 concentration, exchange, and action in relation to fruit respiration requires further investigation.

Chitosan also has the potential to induce defense-related enzymes (Bautista-Baños et al. 2006) and phenolic contents in plants. These results are compatible with the findings of Liu et al. (2007), who reported that the production of phenolic compounds was induced in tomato plants and fruit treated with chitosan. Chitosan may inhibit the activity of polyphenol oxidase, an enzyme that is involved in the process of phenolic compound degradation (Jiang and Li 2001).

In this study, increase in total phenolic content in the last stage may be due to stress from the fruits being exposed to market conditions. Ali et al. (2007) reported that higher phenolic compound levels could increase antioxidant activity and also showed a linear correlation between phenolic content and antioxidant activity. When plant cells were exposed to abiotic or biotic stress such as elicitor treatment, glucose-6-phosphate dehydrogenase was the first enzyme of the pentose phosphate pathway acting to provide precursors for phenolic synthesis. The phenylpropanoid pathway leads to biosynthesis of flavonoids and pigments as well as lignin and phenolic compounds (Xu et al. 2007). Khan et al. (2003) found that chitosan oligomers could elevate phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase activities in soybean leaves. This may lead to the induction of other phenolic compounds, including phenolic acids like chlorogenic acid and flavonoids such as catechins and quercetins, produced via the phenylpropanoid and flavonoid pathway where PAL is a leading enzyme. Flavonoids are phenolic derivatives, and they are found in substantial amounts in grapes. This explains the decrease in flavonoids associated with total phenolic content during storage, as shown in Table 4.

Common fruit polyphenol oxidase (PPO) substrates are flavonoids and related compounds such as quercetin and catechin (Nagai and Suzuki 2001). It has also been reported that quercetin and catechin are oxidized directly by PPO (Jiménez and García-Carmona 1999). The decrease in phenolic composition in this study was probably due to oxidation by polyphenol oxidase (Yamaguchi et al. 2003). The inhibition of PPO activity by chitosan coating has also been observed with longan fruit, fresh-cut Chinese water chestnut, and litchi fruit stored at low temperature (Jiang et al. 2005). Quercetin, in contrast, was always influenced by oxygenation, indicating its higher reactivity with oxygen as compared to the other compounds (Castellari et al. 2000). The major natural substrates found for the oxidative enzymes are hydroxycinnamic acid esters and monomeric and dimeric flavans and quercetin glycosides. In intact cells, phenolic compounds are located in the vacuoles and protected from the PPO or peroxidase enzymes in the chloroplasts and mitochondria by physical barriers (Awad and De Jager 2000).

It is well known that the bioactivity of chitosan, including antioxidant ability, is mainly attributed to the activity of hydroxyl and amino groups. There are 3 kinds of hydrogen sources: NH_2 of C2, and OH of C3 and C6. It is difficult for 3-OH to take part in the reaction because of steric hindrance. The potential mechanism of these results needs to be further researched (Xie et al. 2001).

The mechanism by which phenolic compounds are able to scavenge free radicals is yet to be exactly established. In any case, it seems to be clear that the basic structure of compounds and other structural factors are very important in the scavenging mechanism (Sadeghipour et al. 2005). As reported by Heijnen et al. (2001), the aromatic OH groups are the reactive centers, primarily the 3,4-dihydroxy catechol group, and their activity can be enhanced by electron donating effects of other substituents. This is clearly verified in our study, where antioxidant capacity (Figure 5) decreased with declining total phenolics (Figure 1) and catechins (Figure 2). Even though chitosan demonstrates antioxidant activity, the above could be due to the high content of phenolic acids in comparison with the control samples, which is partly similar to findings of other reports (Guo et al. 2006).

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