CHITOSAN COATING OF RED TABLE GRAPES AND FRESH-CUT HONEY MELONS TO INHIBIT *FUSARIUM OXYSPORUM* GROWTH

REYHAN IRKIN[1,3] and METIN GULDAS[2]

[1]Susurluk College, Balikesir University, Susurluk TR10600, Balikesir, Turkey
[2]Karacabey Vocational School, Uludag University, Bursa, Turkey

[3]Corresponding author.
TEL: +90266-865-71-53;
FAX: +90266-865-71-55;
EMAIL: rirkin@hotmail.com;
reyhan@balikesir.edu.tr

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ABSTRACT

Chitosan is a modified, natural biopolymer derived by deacetylation of chitin. Mycelial growth of *Penicillium chrysogenum*, *Fusarium oxysporum*, *Aspergillus parasiticus*, *As. fumigatus* and *As. niger* was determined by measuring colony diameters on Petri plates. The red grape and honey melon samples inoculated with *F. oxysporum* were immersed into chitosan solution, and then kept at 4C for 7 days. The fungal isolates were tested using with seven concentrations of chitosan (0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0%). The effective concentrations that require reducing the radial growth of the fungus on the media were determined as 57.6, 62.5 and 73.1% for *Pe. chrysogenum*, *F. oxysporum* and *As. parasiticus*, respectively. The chitosan coatings caused to decrease *F. oxysporum* growth in table grapes and honey melons and delayed changes in their external color. Chitosan is suitable to use as an antifungal edible film in the food industry in the near future.

PRACTICAL APPLICATIONS

Chitosan has inhibitory activity for the growth of *Penicillium chrysogenum*, *Fusarium oxysporum* and *Aspergillus parasiticus in vitro*. Chitosan coatings can be used as an edible film for table grapes and honey cut melons. Hunter color values are better than the control samples for chitosan-coated fruits. It might be said that 1.5% w/v chitosan concentration can show optimum edible coating application for grapes and melons.

INTRODUCTION

Molds and their mycotoxigenic products are important because of their carcinogenic effects on humans, and post-harvest diseases result in major losses of fruits and vegetables. *Aspergillus parasiticus* produces four of the major aflatoxins as B1, B2, G1 and G2. *As. niger* is the most common species of the genus *Aspergillus*, a fungus that produces a black mold on plants (very common in lettuce, tomatoes or kales). *As. fumigatus* could cause spoilage of food products after pasteurization and it produces gliotoxin. *Fusarium* species, especially *F. oxysporum*, has harmful effects on humans and plants. Sambutoxin is produced by *F. oxysporum* and causes esophageal cancer in humans and economical losses in agriculture. These molds can be prevented by the applications of synthetic chemical fungicides. But, synthetic chemical fungicides are potentially harmful on human health. However, many pathogens can be resistant to these chemicals. Moreover, public concern over the indiscriminate use of synthetic fungicides has been growing. Thus, it is significant to develop new alternatives for fungal control nowadays as organic sources (Farooq *et al.* 2005; Gumus *et al.* 2005; Jay *et al.* 2005; No *et al.* 2007; Badawy and Rabea 2009; Guler *et al.* 2009; Ziani *et al.* 2009; Meng *et al.* 2010; Meca *et al.* 2012).

Chitosan is one of the most widely present amino polysaccharides in nature. It has been generally used in food production for clarification and deacidification of fruit juices (fining agent), for purification of water, for antioxidative maintenance in muscle foods and in pharmaceutical areas, for example, and for drug delivery systems because it has good biocompatibility, biodegradability, film-forming property and antimicrobial activity (Cooksey 2005; Ham-Pihavant *et al.* 2005; Srinivasa and Tharanathan 2007; Wang *et al.* 2007). In Fig. 1, the production of chitosan and chitin from crustacean shells is given. Chitosan, together

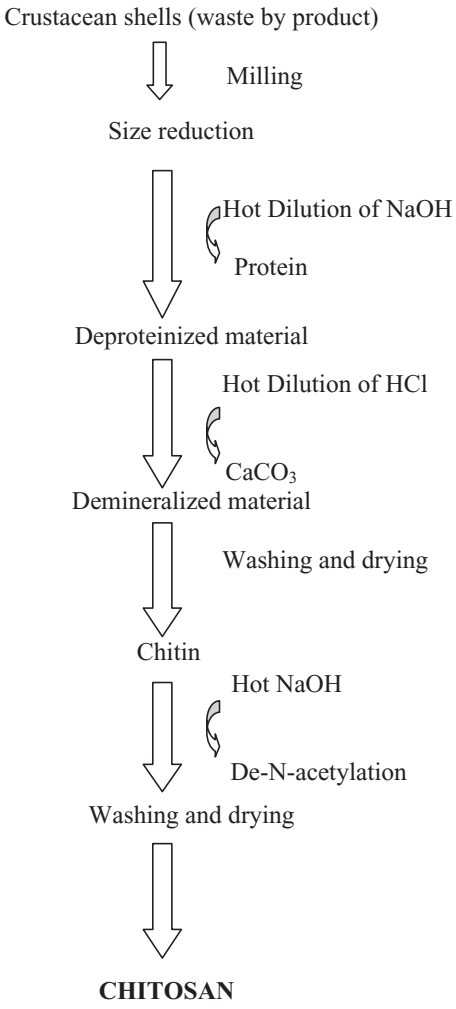

FIG. 1. PRODUCTION STEPS OF CHITIN /CHITOSAN (SRINIVASA AND THARANATHAN 2007)

with its derivatives, has been reported as a promising alternative to control postharvest diseases. In addition, chitosan and oligochitosan as fungicides are effective in inhibiting spore germination, germ tube elongation and mycelial growth of fungal phytopathogens, such as *Alternaria solani*, *Botrytis cinerea*, *Fusarium*, *Rhizopus stolonifer*, *Penicillium* and *Phytophthora capsici* (Khalaf 2004; Meng *et al*. 2010; Qin *et al*. 2012; Ren *et al*. 2012). Many researchers have studied the antimicrobial action of chitosan on fungal or bacterial strains (Sebastien *et al*. 2006; Rabea *et al*. 2009). It was stated that *As. fumigatus*, *As. parasiticus*, *F. oxysporum* in seafoods, *Cladosporium*, *B. cinerea*, *Pe. digitatum*, *Pe. italicum* and *Rhizopus* spp. in fruits and vegetables, *As. niger*, *Pe. chrysogenum*, *Pe. expansum*, *Pe. notatum* and *R. nigricans* in breads were inhibited by chitosan applications in the previous research. The antimicrobial activity of chitosan against a range of foodborne filamentous fungi, yeasts and bacteria has attracted attention as a potential food preservative of natural origin (No *et al*. 2007; Chittenden and Singh 2009; Cota-Arriola *et al*. 2011).

Molds cause postharvest decay of table grapes (*Vitis vinifera* L.) and are mainly controlled by extensive use of fungicides; some consumers develop allergic reactions to them (Xu *et al*. 2007; Fernandez-Caballero *et al*. 2009). Bio-based edible materials are an emerging low-cost technique suitable for short-term preservation of intact or freshly cut fruits and vegetables. Therefore, from a practical viewpoint, the antifungal activity of chitosan that has a great potential of bio-based materials was tested for some fresh fruits previously. Positive results have been determined to control fungal growth on freshly cut fruits such as mangoes, sweet cherries, strawberries, apples and pears with chitosan coatings in previous research (Valencia-Chamorro *et al*. 2009; Aider 2010; Assis and Britto 2011; Vu *et al*. 2011; Yang *et al*. 2012; Yu *et al*. 2012).

In addition to antifungal effect, chitosan coating prevents color loss, browning and undesired appearance of fruits. It was previously shown that chitosan coating on fruits lead to increase in phenolic compounds and suppressed the decline in anthocyanin content of pomegranates, strawberries, fresh-cut pears, table grapes and raspberries (Dong *et al*. 2004; Han *et al*. 2005; Chien *et al*. 2007a; Ansorena *et al*. 2011; Shiri *et al*. 2012; Ghasemnezhad *et al*. 2013; Xu *et al*. 2013).

In this study, inhibition effects of chitosan against *Pe. chrysogenum*, *F. oxysporum*, *As. parasiticus*, *As. fumigatus* and *As. niger* were determined *in vitro* conditions at first. Second, effective inhibition concentration of chitosan was determined on table grapes and freshly cut honey melons, which were artificially contaminated with *F. oxysporum* during 7 days of cold storage at 4C. The color changes occurred on experimental fruit samples were also measured during the storage.

MATERIALS AND METHODS

Fungal Isolation

Stock cultures of *Pe. chrysogenum*, *F. oxysporum*, *As. parasiticus*, *As. fumigatus* and *As. niger* supplied by Erciyes University, Kayseri, Turkey, were kept frozen (−25C) in potato dextrose broth (PDB; 254920, Difco, Detroit, MI) supplemented with 30% glycerol. All fungal cultures were grown on potato dextrose agar (PDA; Merck, Darmstadt, Germany) slants. Ten milliliters of 1% v/v Tween 20 (Sigma-Aldrich, St. Louis, MO) was added for conidia collection. Conidia were harvested by centrifugation at 1,000 rpm for 10 min and washed with sterile distilled water. This step was repeated three times. One milliliter of these conidia suspensions was added into the sterile 50 mL PDB (Difco) and

were incubated for 24 h at 30C (Yin and Tsao 1999; Rabea *et al.* 2009). Potato dextrose suspension concentrations of filamentous fungi contained approximately 10^4 conidia/mL.

Preparation of Chitosan Solutions and Determination of Mycelial Growth

The chitosan (Sigma-Aldrich) solutions in seven concentrations (0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% w/v) were investigated to determine the mycelial growth of fungi using PDA *in vitro* conditions (Martinez-Camacho *et al.* 2010; Ziani *et al.* 2010).

The chitosan solution was prepared by dissolving chitosan 85% deacetylated in 0.25 N HCl with continuous stirring at 50C. Insoluble material was removed by centrifugation, and chitosan was precipitated by neutralization with 1 N NaOH, washed three times with deionized water and air dried. For incorporation into the PDA, purified chitosan was dissolved by stirring in 0.25 N HCl and adjusting the pH to 5.6 using 1 N NaOH. Chitosan solution was added to the medium after autoclaving and poured into 90-mm Petri dishes to yield a total volume of 15 mL per dish. Thirty microliters from an actively growing PDB culture of fungi was placed fungus side down in the center of each Petri dish. Sterile water with the same volume of 0.25 N HCl solution added to all concentrations of chitosan was used as a negative control to check whether it had any effect on the fungi (Munoz *et al.* 2009).

F. oxysporum inoculation culture was obtained by sterile 50-mL PDB (Difco) conidia suspension according to Yin and Tsao (1999). The fungi were inoculated on PDA and incubated at 24C for 7 days in the dark. Mycelial growth was determined by measuring colony diameters. The inhibitory rate of fungal growth was calculated. Three replicates were used for each chitosan concentration and each experiment was repeated twice (Chittenden and Singh 2009).

Preparation of Red Table Grapes and Melons

Red table grapes (*V. vinifera* L.) and honey melons (*Cucumis melo*) were obtained from a market. The grape grains were separated from clusters and melons were cut into $2 \times 2 \times 1$ cm dimensions. Before treatment, grape grains and melon pieces were washed with tap water, then disinfected with 2% sodium hypochlorite (Merck) in food grade for 4 min and rinsed with sterilized water. After draining for 5 min on a clean paper towel, they were grouped for the treatments. First, all grape grains and melon pieces were dipped into *F. oxysporum* inoculation solution for attachment of the microorganism to the experimental fruit samples. Then, they were put into sterile glass containers and incubated for 3.5 h at 25–30C (Badawy and Rabea 2009; Meng *et al.* 2010).

Chitosan (Sigma-Aldrich; 0.5, 1.0, 1.5, 3.0% w/v) was dissolved in 150 mL of lactic acid solution (1% in distilled water, v/v) and added 0.75 g glycerin. This solution was heated up to 100C with continuous stirring (Wu *et al.* 2000; Guldas *et al.* 2010). Melons and grapes were randomly assigned to five groups. One group was inoculated with *F. oxysporum* and dipped for 2 min into a sterile water (control), and the other fruit groups were dipped into various concentrations of chitosan solutions (0.5, 1.0, 1.5, 3.0%, w/v) for 2 min inoculation separately. The dipped samples were dried for 1 h at room temperature conditions, then put into polystyrene plastic cups, closed with the covers (fruit salad cups) and stored at 4C during 7 days (Badawy and Rabea 2009).

Fungal Analysis

For the determination of *F. oxysporum* counts during the storage period, samples of 10 g from each group were taken and homogenized in 90 mL of sterile peptone water using a stomacher (BagMixer, Interscience, Saint-Nom-la-Bretèche, France). Serial dilutions were carried out, and 0.1 mL from each solution was spread to the PDA for 5 days at 25C. Samples were prepared in triplicate, and the counts between 30 and 300 colony-forming units (cfu/g) were only considered.

Color Analysis

All samples were chopped with a blender (Arzum Misto Grande, Istanbul, Turkey), and then the color of the samples were measured using a HunterLab DP-9000 colorimeter (Reston, VA). L^* (lightness), a^* (redness–greenness) and b^* (yellowness–blueness) values were recorded. Color coordinates range from $L = 0$ (black) to $L = 100$ (white), –a (greenness) to +a (redness), and –b (blueness) to +b (yellowness). Color measurements were carried out at 1, 3, 5 and 7 days (Chien *et al.* 2007a,b; Djioua *et al.* 2010).

Proximate Analysis

All samples were chopped with a blender (Arzum Misto Grande) and the pH values were recorded using Hanna Instruments model HI221 microprocessor (Woonsocket, RI) pH meter.

Statistical Analyses

All analyses were repeated three times and the data were recorded. Statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS) 15.0 software for Windows (SPSS Inc., Chicago, IL). One-way analysis of

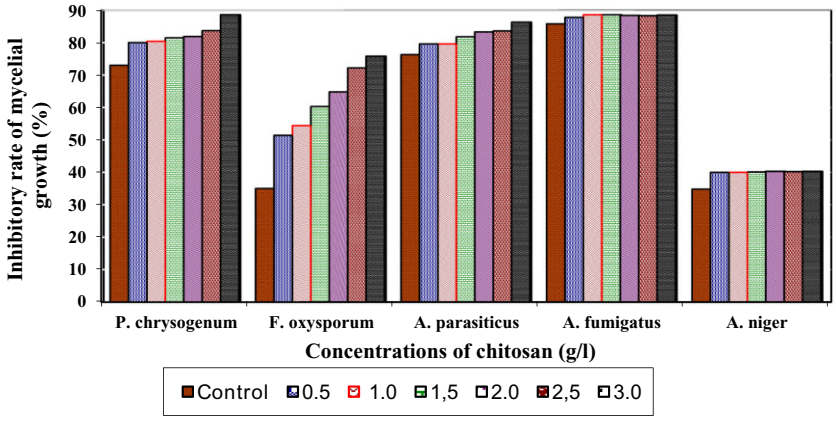

FIG. 2. EFFECTS OF CHITOSAN ON INHIBITORY RATE OF MYCELIAL GROWTH (5) OF TESTED FUNGI AFTER 7 DAYS OF INOCULATION

variance test was applied for determining mean differences. The level of significance among means was determined by the Tukey's honestly significant difference test. All data were presented as mean value with their standard error (SE) indicated (mean ± SE). Differences were accepted as significant when $P < 0.05$ (Ozdamar 2004).

RESULTS AND DISCUSSION

The tested fungi strains versus chitosan were *Pe. chrysogenum*, *F. oxysporum* and *As. parasiticus* in the study. Fungal growth of *Pe. chrysogenum*, *F. oxysporum* and *As. parasiticus* was significantly affected by all chitosan concentrations ($P < 0.05$) after 7 days of incubation. However, *As. fumigatus* and *As. niger* were not inhibited with chitosan significantly ($P > 0.05$). The difference in inhibition zones between 2.5 and 3.0% of chitosan against *Pe. chrysogenum*, *F. oxysporum* and *As. parasiticus* was found statistically different ($P < 0.05$), whereas the difference between 0.5 and 1.0% of chitosan concentrations was not significant ($P > 0.05$).

Compared with the control, 3% of chitosan inhibited the mycelial growth by 57.6, 62.5 and 73.1% for *Pe. chrysogenum*, *F. oxysporum* and *As. parasiticus*, respectively. Inhibitory rate of chitosan against tested fungi is seen in Fig. 2. In Aider's (2010) review, it was concerned that *F. solani* growth can be inhibited with 4 mg/L of chitosan in a liquid nutrient medium. In Al-Hetar *et al.*'s (2011) research, 8 mg/mL concentration of chitosan was used to obtain maximum 76.36% of *F. oxysporum* inhibition on PDA media.

There were several studies about antimicrobial inhibition of chitosan in the last years, but there were very limited data about chitosan's antifungal activity in food products. Therefore, it was not possible to compare the results directly in terms of antifungal effect of chitosan on food commodities. The antifungal activity of chitosan and its ability to reduce the *in vitro* growth of many fungi has been demonstrated by Munoz *et al.* (2009). *Colletotrichum* spp. was inhibited with 2.5 and 2.0% of chitosan by 63.16 and 39.42%, respectively. Palma-Guerrero *et al.* (2010) reported that the antifungal action of chitosan has been studied for the last years, but is still little understood. They found that the plasma membrane forms a barrier to chitosan in chitosan-resistant, but not chitosan-sensitive fungi. The plasma membranes of chitosan-sensitive fungi were shown to have more polyunsaturated fatty acids than chitosan-resistant fungi. It was concluded that their permeabilization by chitosan may be changed depending on the membrane fluidity.

Chitosan has been used in numerous industrial and food applications because of its biological and functional properties. Chitosan is nontoxic for humans and has a low environmental impact. The International Commission on Natural Health Products recognized chitin as a natural product for the 21st century in 2005. Chitosan was generally recognized as safe by the United States Food and Drug Administration based on the scientific procedures for use in foods (Mahae *et al.* 2011).

The results of Qin *et al.* (2006) suggest that water-soluble chitosan is not appropriate for antimicrobial agent, and water-insoluble chitosans with molecular weight (M_w) around 5×10^4 have potential for using as a preservative in acidic foods.

Changes in pH

The pH values of grapes and honey melons did not show significant changes during 7 days of storage. Coated grapes (0.5, 1.0, 1.5 and 3.0%) showed slightly greater mean values as 3.75, 3.81, 3.82 and 3.88 than the control (3.7), respectively. Acidity slightly changed depending on the organic acid content of the fruit. The mean pH values of honey melons (0.5, 1.0, 1.5 and 3.0%) were 6.23, 6.24, 6.32 and 6.33 for coated ones and 6.2 for uncoated ones, respectively. It can be said that chitosan coating causes a slight increase in pH values.

TABLE 1. *FUSARIUM OXYSPORUM* COUNTS (CFU/G) IN RED GRAPES AND MELONS TREATED WITH CHITOSAN DURING STORAGE (DAYS)

	1	3	5	7
Red grape				
Control (%)	$1.05.10^6 \pm 0.8^a$	$1.20.10^5 \pm 0.6^b$	$7.38.10^5 \pm 0.6^c$	$8.73.10^5 \pm 0.7^d$
0.5	$6.33.10^5 \pm 0.9^a$	$5.23.10^5 \pm 0.6^b$	$1.75.10^5 \pm 0.5^c$	$1.74.10^5 \pm 0.7^c$
1.0	$6.13.10^5 \pm 0.5^a$	$4.76.10^5 \pm 0.4^b$	$3.15.10^5 \pm 0.7^c$	$1.61.10^5 \pm 0.5^d$
1.5	$5.90.10^5 \pm 0.9^a$	$4.30.10^5 \pm 0.3^b$	$2.64.10^5 \pm 0.8^c$	$1.61.10^5 \pm 0.3^d$
3.0	$5.86.10^5 \pm 0.4^a$	$2.26.10^5 \pm 0.2^b$	$1.91.10^5 \pm 0.7^c$	$1.42.10^5 \pm 0.5^d$
Melon				
Control (%)	$1.63.10^5 \pm 0.2^a$	$2.73.10^5 \pm 0.5^b$	$4.30.10^5 \pm 0.7^c$	$9.10.10^5 \pm 0.3^d$
0.5	$6.35.10^5 \pm 0.7^a$	$4.50.10^5 \pm 0.3^b$	$2.85.10^5 \pm 0.4^c$	$1.89.10^5 \pm 0.9^d$
1.0	$6.25.10^5 \pm 0.8^a$	$4.28.10^5 \pm 0.8^b$	$2.47.10^5 \pm 0.6^c$	$1.41.10^5 \pm 0.7^d$
1.5	$5.71.10^5 \pm 0.3^a$	$4.23.10^5 \pm 0.2^b$	$2.21.10^5 \pm 0.8^c$	$1.26.10^5 \pm 0.5^d$
3.0	$5.80.10^5 \pm 0.5^a$	$4.73.10^5 \pm 0.3^b$	$2.13.10^5 \pm 0.8^c$	$1.06.10^5 \pm 0.5^d$

Values (±standard deviation) in a line with different superscripts are significantly different ($P < 0.01$), $n = 5$.

Changes in *F. oxysporum* Counts during the Storage

Chitosan coatings are known to possess a protective effect against mold growth on fresh fruits and vegetables (Vu *et al.* 2011). In this study, *F. oxysporum* counts in control samples increased to 8.73×10^5 cfu/g in red grapes and 9.10×10^5 cfu/g in honey melons at 7 days of the storage (Table 1).

Generally, *F. oxysporum* counts in inoculated samples with *F. oxysporum* decreased depending on the chitosan concentration used. In the red grape samples artificially contaminated with *F. oxysporum* with 0.5, 1.0 and 1.5% chitosan, the counts were inhibited between 72.5 and 73.7% at the end of 7-day storage. However, treatment with 3.0% of chitosan caused the highest inhibition as 75.7%. Similarly, in another study, it was reported that *F. oxysporum* growth was completely inhibited by 3% of chitosan (Bautista-Banos *et al.* 2006). Assis and Britto (2011) also found inhibitory effects of chitosan coating on mycelial fungi growth (*Penicillium* spp. and *Alternaria* spp.) for sliced apples in 2 g/L concentration. According to the results of Yu *et al.* (2012), chitosan did not show full protection effect in pear fruits versus *Pe. expansum* infection even when it was used at tested highest experimental concentration 1%. However, Al-Hetar *et al.* (2011) obtained maximum 76.36% inhibition on *F. oxysporum* in 8 mg/mL concentration of chitosan.

Similarly, there were limited data on melons just as red grapes concerning antifungal activity of chitosan. Therefore, it was also not possible the comparison of the obtained data, directly. Whereas 0.5% of chitosan treatment caused 70% decrease in *F. oxysporum* counts of honey melon samples, treatments with 1.0 and 1.5% of chitosan led to 77 and 78% decrease, respectively. Highest inhibition rate in the honey melon samples was obtained by 3.0% of chitosan application as 82%. Statistically, the decreases observed in all *F. oxysporum* counts throughout the storage period were significant ($P \leq 0.05$).

The antifungal effect of chitosan can be caused by the biological mechanisms such as inducing morphological changes, structural alterations of the fungal cells and fruit resistance induction to pathogen attacks (Yu *et al.* 2012). In addition to these effects, antifungal activity of chitosan is also dependent on various factors such as molecular weight, chemical modification, concentration, pH and species of microorganism, etc. (Cota-Arriola *et al.* 2011; Qin *et al.* 2012; Sajomsang *et al.* 2012; Yang *et al.* 2012).

Changes in Hunter Color Values during the Storage

L values in ever red grapes or honey melon control samples decreased significantly depending on the storage period, and thus, the color of samples became darker at the end. But, when the chitosan concentration was increased, the lighter color in the samples remained unchanged at the end of storage period (Table 2). As parallel, as stated by Hernandez-Munoz *et al.* (2006), chitosan is capable of delaying external color changes in strawberries similar to other edible coatings based on natural biopolymers. It can be based on reducing the respiration rate of fruits and vegetables in consequence of edible coatings used (Yu *et al.* 2007).

L values in control samples belonging to the red grapes decreased to 40% at the end of storage period. Especially, the loss observed in red grape samples treated with 1% of chitosan decreased to 18%. The similar results were obtained in Xu *et al.*'s (2007) study; they reported that red grape clusters treated with 1% chitosan have shiny appearance compared with controls. And at the end of 4 weeks of storage, control fruits had a redder and darker color than chitosan-treated fruits. It can be concluded that chitosan coating can also prevent browning in fruits. Perdones *et al.* (2012) and Vu *et al.* (2011) also observed lighter and redder colors for strawberries coated with 1% chitosan than control groups. Chien *et al.* (2007a) determined that

TABLE 2. HUNTER COLOR VALUES OF RED GRAPES AND MELONS TREATED WITH CHITOSAN DURING STORAGE (DAYS)

	L					*a*					*b*				
	1	3	5	7		1	3	5	7		1	3	5	7	
Red grape															
Control (%)	16.32 ± 0.4^{a}	11.77 ± 0.7^{b}	10.97 ± 0.1^{c}	9.83 ± 0.6^{d}		12.52 ± 0.7^{a}	11.47 ± 0.2^{b}	10.96 ± 0.6^{c}	10.17 ± 0.4^{d}		2.86 ± 0.3^{a}	1.91 ± 0.9^{b}	1.85 ± 0.7^{c}	1.76 ± 0.5^{d}	
0.5	16.38 ± 0.8^{a}	12.79 ± 0.5^{b}	11.23 ± 0.6^{c}	10.98 ± 0.3^{c}		9.77 ± 0.6^{a}	9.42 ± 0.2^{b}	9.38 ± 0.3^{b}	9.27 ± 0.3^{c}		3.25 ± 0.6^{a}	2.27 ± 0.6^{b}	2.16 ± 0.2^{c}	2.09 ± 0.6^{c}	
1.0	16.44 ± 0.9^{a}	13.65 ± 0.3^{b}	13.54 ± 0.4^{c}	13.45 ± 0.8^{c}		8.83 ± 0.7^{a}	8.77 ± 0.5^{b}	8.72 ± 0.4^{b}	8.69 ± 0.5^{b}		1.96 ± 0.6^{a}	1.62 ± 0.3^{b}	1.58 ± 0.5^{b}	1.54 ± 0.6^{c}	
1.5	17.23 ± 0.2^{a}	14.12 ± 0.8^{b}	14.10 ± 0.9^{b}	13.96 ± 0.7^{b}		8.68 ± 0.2^{a}	8.00 ± 0.7^{b}	7.65 ± 0.6^{c}	7.56 ± 0.7^{c}		2.07 ± 0.2^{a}	1.75 ± 0.7^{b}	1.68 ± 0.4^{c}	1.62 ± 0.8^{c}	
3.0	17.57 ± 0.9^{a}	14.29 ± 0.5^{b}	14.32 ± 0.9^{b}	13.75 ± 0.3^{c}		8.19 ± 0.6^{a}	7.89 ± 0.5^{b}	7.68 ± 0.5^{c}	5.57 ± 0.3^{c}		1.47 ± 0.1^{a}	1.36 ± 0.7^{b}	1.30 ± 0.9^{c}	1.27 ± 0.6^{c}	
Melon															
Control (%)	55.33 ± 0.9^{a}	40.45 ± 0.9^{b}	39.78 ± 0.3^{c}	35.56 ± 0.2^{d}		9.44 ± 0.9^{a}	8.49 ± 0.4^{b}	7.33 ± 0.7^{c}	6.56 ± 0.3^{d}		17.92 ± 0.1^{a}	13.86 ± 0.3^{b}	12.96 ± 0.6^{c}	11.24 ± 0.4^{d}	
0.5	54.36 ± 0.2^{a}	42.74 ± 0.2^{b}	41.87 ± 0.3^{c}	40.79 ± 0.9^{c}		6.57 ± 0.3^{a}	6.54 ± 0.6^{a}	6.50 ± 0.9^{b}	6.48 ± 0.7^{b}		15.64 ± 0.2^{a}	13.40 ± 0.1^{b}	12.87 ± 0.3^{c}	11.76 ± 0.7^{d}	
1.0	54.18 ± 0.1^{a}	49.75 ± 0.3^{b}	48.76 ± 0.5^{c}	46.56 ± 0.8^{d}		5.97 ± 0.3^{a}	5.78 ± 0.9^{b}	5.67 ± 0.8^{c}	5.60 ± 0.7^{c}		13.17 ± 0.8^{a}	12.92 ± 0.9^{b}	12.48 ± 0.4^{c}	12.08 ± 0.7^{d}	
1.5	53.90 ± 0.9^{a}	52.61 ± 0.2^{b}	50.62 ± 0.4^{c}	48.25 ± 0.2^{d}		5.75 ± 0.7^{a}	5.69 ± 0.4^{b}	5.54 ± 0.6^{c}	5.48 ± 0.5^{c}		13.03 ± 0.7^{a}	12.87 ± 0.5^{b}	12.36 ± 0.3^{c}	12.09 ± 0.1^{d}	
3.0	56.24 ± 0.3^{a}	54.88 ± 0.8^{b}	53.27 ± 0.9^{c}	52.19 ± 0.1^{d}		5.33 ± 0.7^{a}	5.23 ± 0.4^{b}	5.11 ± 0.5^{c}	5.04 ± 0.5^{d}		12.73 ± 0.5^{a}	12.65 ± 0.9^{a}	12.07 ± 0.1^{b}	11.65 ± 0.3^{c}	

Values (±standard deviation) in a column with different superscripts are significantly different ($P < 0.01$), $n = 5$. *L* is a correlate of lightness; *a* and *b* are termed opponent color axes. *a* represents, roughly, redness (positive) versus greenness (negative). The colorimeter provides the values of three-color components: *L** (black–white component, luminosity) and the chromaticness coordinates, a* (green to red component), and *b** (yellow to blue component).

L values of coated, sliced red pitayas (*Hylocereus cacti*) treated with chitosan are considerably higher than uncoated fruits. They also observed that coating causes delaying the decay of fruits.

Djioua *et al.* (2010) treated mango cubes with 0.25%, 0.5% w/v chitosan solution in 0.5% (w/v) citric acid and stored for 9 days at 6C. They obtained high scores about the color of the fruits compared with the control group. They concluded that chitosan coating prevents color loss on mango fruits. Similarly, Chien *et al.* (2007b) determined that chitosan coating (treated with aqueous solution of 0.5, 1.0 or 2.0% w/v) of sliced mango fruit remained its original color, and fruits showed slower decay and browning during storage at 6C.

Dong *et al.*'s (2004) study on litchi (*Litchi chinensis* Sonn.) has shown that chitosan coating (treated with 1, 2 or 3% aqueous solution w/v) prevented browning, cracking and juice leaking of peeled fruit after 6 days of storage.

Campaniello *et al.* (2008) investigated chitosan coating on fresh-cut strawberries and they determined that high-oxygen packaging combined with chitosan coating influences the color positively.

Jiang and Li (2001) tested chitosan coating (0.5, 1.0 and 2.0% aqueous solution w/v) effects on longan fruit (*Dimocarpus Longan* Lour.). Two percent of chitosan-treated group remained in bright color until 30 days of storage, but control group became rot. They also observed that fruit discoloration was prevented by chitosan coating.

In another study, it was shown that coating of broccoli did not impart significant changes in initial color; also edible chitosan coating application slowed browning reaction on broccoli (Ansorena *et al.* 2011).

The changes seen in *L* values of honey melon samples that depended on chitosan treatments were more remarkable. The loss seen in honey melon control samples at the end of storage was 35.7%. The losses obtained in *L* values of honey melon samples were 25.0, 14.0 and 10.5% depending on chitosan concentrations used as 0.5, 1.0 and 1.5%, respectively. However, the loss seen in the honey melon samples treated with 3.0% of chitosan was only 2%.

The *a* value, which means the intensity of redness in red grape control samples, decreased to 19% parallel to deterioration of fruits during storage. The minimum loss in *a* values of red grapes was obtained from 1.5% chitosan, whereas the maximum loss was 12%. Xu *et al.* (2007) also stated that anthocyanin compounds in red table grapes, which account for their red color, changed more slowly in chitosan-treated grapes in their study.

The *a* value (darkness) in honey melon control samples decreased to 30% during the storage. The *a* value of the samples treated with chitosan decreased to 1% as minimum and 6% as maximum.

The losses seen in *b* values of red grapes were higher than obtained *a* values. The loss in *b* values of red grape control sample was 38% at the end of storage. The loss seen in *b* values decreased when the concentration of chitosan was increased. It decreased to 13% in the samples treated with 3% of chitosan. The loss seen in *b* values of honey melon control samples was 37%, whereas it was 7–8% in the sample treated with 1% of chitosan at the end of storage. Similarly, Djioua *et al.* (2010) determined that the color loss decreased parallel to *L* and *b* values as compared with control for mango cubes after 6 and 9 days. In addition, total carotenoid content remained the same and chitosan coating did not affect the carotenoids in fruits. Color results can be explained by the chitosan inhibition of polyphenol oxydase and peroxidase activity in fruits. In Gao *et al.*'s (2013) study, chitosan glucose-coated and pure chitosan-coated samples of grapes have higher scores for visual appearance and color than the control groups. They demonstrated that chitosan coating prevent formation of brown color related with prevention activity of polyphenol oxidase in grapes. Fresh table grapes with 1% w/v chitosan + putrescine solution and stored at 0C for 60 days by Shiri *et al.* (2012). They observed that chitosan coating reduced the decay, browning and cracking on grapes.

From the results of the present study, chitosan was successful in inhibiting the growth of *Pe. chrysogenum*, *F. oxysporum* and *As. parasiticus in vitro* study. In the model study of chitosan, coating also gave good results for table grapes and honey cut melons against *F. oxysporum* growth. There were no significant differences between 1.5 and 3.0% concentrations of the coating. It was reported that the high viscosity of increased chitosan concentration could limit the coating applications. Therefore, reduced concentrations that have lower viscosity of coating are expected to improve antifungal activity (Romanazzi *et al.* 2007). In addition to visual evaluation, the better hunter color values were obtained from fruits with chitosan coating. It was concluded that 1.5% of chitosan coating may be considered as an optimum edible coating concentration in the preservation of cut fruits or fruit salads.

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