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Impact of preharvest and postharvest alginate treatments enriched with vanillin on postharvest decay, biochemical properties, quality and sensory attributes of table grapes

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ABSTRACT

Alginate solution enriched with vanillin as a bioactive compound was investigated for improving preharvest and postharvest quality and safety of table grapes. Alginate treatments with or without vanillin as preharvest spray and postharvest coating were implemented on table grapes of Alphonse Lavalleé and Razaki cultivars. Fungal decay, biochemical properties, quality and sensory attributes were evaluated at day of preharvest treatment, at harvesting and during 35 days of storage at 4 ± 2 °C. Alginate treatments with or without vanillin were effective in preventing weight and firmness losses. Total soluble solids, titratable acidity, and color of grapes coated with alginate coatings with or without vanillin showed minor changes compared to control grapes. Alginate coating incorporating vanillin provided significant reduction (1.73 log CFU/g) in yeast-mold growth. Moreover, the coatings maintained greater total phenolic content and antioxidant activity compared to others during postharvest storage. In terms of sensory attributes, appearance was ranked as the highest for alginate coating without vanillin due to glossiness of alginate.

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1. Introduction

Gray mold, caused by *Botrytis cinerea*, is the most important disease which causes major quality and quantity losses in preharvest period and postharvest storage of grapes. Table grapes are commonly fumigated with sulfur dioxide (SO_2) to control decay caused by *Botrytis cinerea* in both vineyard and cold storage rooms (Gabler, Smilanick, Mansour, & Karaca, 2010). However, there is a need for natural alternatives to replace SO_2 because of many considerations related with sulfite residues, emergence of *B. cinerea* resistant strains, negative effects on quality characteristics, hazards for consumer and environment, and sustainability of grapes (Gabler et al., 2010; Parafati, Vitale, Restuccia, & Cirvilleri, 2015). Furthermore, SO_2 has been excluded in the list of generally recognised as safe (GRAS) by the Food and Drug Administration (FDA) and the usage of SO_2 is prohibited for organic products that are free of pesticide residues (Serrano et al., 2008).

Recently, there is an increasing interest in the development of natural formulations, with low impact on environment and human health, to replace fungicides and be adaptable to traditional applications in grapes (Elmer & Reglinski, 2006). Different techniques have been investigated to improve postharvest quality and functional properties of grapes such as preharvest spraying with polyamines (Mirdehghan & Rahimi, 2016), preharvest spraying and postharvest coating with chitosan (Meng, Li, Liu, & Tian, 2008), and preharvest spraying with *Aloe vera* (Castillo et al., 2010). Preharvest spraying and postharvest coating of grapes with edible solutions can be functional and reliable alternatives for SO_2 treatment. Moreover, edible coatings maintain quality characteristics and extend the shelf life of fruit through providing modified atmosphere conditions that reduce moisture and aroma losses, delay color and texture changes during storage (Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2008). Alginate is a water-soluble biopolymer extracted from brown algae and a potential component for edible coatings/films, gel production, and emulsion stabilization. It is also biocompatible and biodegradable (Pereira, Tojeira, Vaz, Mendes, & Bártolo, 2011; Rhim, 2004). Alginate based edible coatings have been used effectively for extending postharvest storage of fruit and vegetables such as pineapple (Azarakhsh, Osman, Ghazali, Tan, & Mohd Adzahan, 2014), plum (Valero et al., 2013), apple (Olivas, Mattinson, & Barbosa-Cánovas, 2007), mushroom (Jiang, Feng, & Wang, 2013), melon (Raybaudi-Massilia, Mosqueda-Melgar, & Martín-Belloso, 2008), strawberry (Fan et al., 2009), and arbutus berry (Guerreiro, Gago, Faleiro, Miguel, & Antunes, 2015).

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Moreover, incorporation of bioactive compounds such as antimicrobial agents, antioxidants, aroma compounds, nutraceuticals, and probiotics into the edible coatings represents an innovative concept (Huber & Embuscado, 2009). Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the main component of vanilla and widely used in food, beverage, and pharmaceutical industries due to its functional properties (Walton, Mayer, & Narbad, 2003). Vanillin is a phenolic compound exhibiting antioxidant and antimicrobial properties against yeasts, molds, and bacteria as well as one of the most attractive aroma compounds. It was found effective on diminishing growth of yeast at a concentration of 3000 ppm in fruit purées (Cerrutti, Alzamora, & Vidales, 1997). In addition, the inhibitory effect of vanillin (3–7 mM) on the growth of *Aspergillus flavus, A. niger, A. ochraceus*, and *A. parasiticus* in five fruit-based agar systems (apple, banana, mango, papaya, and pineapple) was significantly effective on growth rate (López-Malo, Alzamora, & Argaiz, 1995).

Literature provides limited examples of the incorporation of vanillin into edible coating formulations. Apple puree-alginate coatings incorporating vanillin (0.3% w/w), lemongrass (1.0% w/w), or oregano oil (0.5% w/w) on fresh-cut apples significantly inhibited growth of psychrophilic microorganisms, yeasts and molds. However, apple puree-alginate coating incorporating vanillin was found significantly effective on sensory quality of apples compared to apple puree-alginate coatings incorporating essential oils as lemongrass and oregano oil (Rojas-Graü et al., 2007). Volatile components especially from essential oils which have intensive odor may expose an undesirable sensory characteristics for fresh produce (Liu, 2009). In contrast, vanillin is a generally regarded as safe (GRAS) flavoring compound that is usually compatible with fruit characteristics (Matamoros-Leon, Argaiz, & Lopez-Malo, 1999). Thus, the use of bioactive compounds such as vanillin could be a new strategy.

To the best of our knowledge, there is no information available in the literature regarding the effect of preharvest alginate spray and postharvest alginate coating with or without vanillin on table grapes. Thus, the aims of this study were to investigate the effects of alginate treatments with or without (w/wo) vanillin as preharvest spray and postharvest edible coating on storage decay, biochemical properties, quality, and sensory attributes of Alphonse Lavalleé and Razaki grapes.

2. Material and methods

2.1. Fruit

Table grapes (*Vitis vinifera* L.) from two cultivars which were "Alphonse Lavalleé" identified by purple-red color and "Razaki" identified by green color were treated with preharvest spray in vineyard located in Gödence region of İzmir and harvested when grapes have reached commercial maturity. Grape clusters were immediately transported to laboratory after harvesting and postharvest coating was applied to the same clusters. Clusters of uniform size, color, and shape were selected for treatments.

2.2. Preharvest and postharvest treatments

The selection of vanillin concentration was determined by preliminary study based on the characterization of alginate-based edible films with or without vanillin. In the study, the effects of alginate-based edible films incorporating vanillin at 0.5, 1.0 and 1.5% (w/v) concentrations on *B. cinerea* growth were investigated. According to the results, it was found that 1.0 and 1.5% vanillin were effective on controlling *B. cinerea* growth and it was decided to use the lower concentration (1.0%) of vanillin due to its high cost (data not shown).

Alginate spray solution [1% (w/v)] for preharvest treatments and alginate coating solution [2% (w/v)] for postharvest treatments were prepared by dissolving sodium alginate (Sigma-Aldrich, St. Louis, USA) in distilled water under continuous stirring at 500 rpm. The solution was held overnight at 100 rpm to obtain homogeneous solution. Then, 1% (w/v) vanillin (Merck, Darmstad, Germany) was incorporated into the solution and stirred until the solution became clear. Glycerol (Merck) [1% (w/v)] was added to the solution as a plasticizer. Grape clusters were divided into three groups separately in vineyard for preharvest treatments. Alginate spray w/wo vanillin was sprayed on grape clusters by using a sprayer two weeks before harvesting. After spraying of alginate solution, 0.3 M $CaCl_2$ solution was also sprayed on the surface of fruits. Clusters without any treatment were used as control group. Table grapes in three groups consisting of control, alginate spray and alginate spray incorporating vanillin were harvested and these grapes were used for postharvest treatment. Postharvest treatment was performed by dipping of grape clusters into alginate solution w/wo vanillin for 30 sec and then the excess solution was removed. Then clusters were dipped in 0.3 M $CaCl_2$ solution for crosslinking. Coated grape clusters were dried at 10 °C and 75% RH for 6 h and then stored in plastic containers at 4 ± 2 °C for 35 days. Analyses were performed at 0, 7, 14, 21, 28, and 35 days of storage.

2.3. Total soluble solid content, pH, and titratable acidity

Total soluble solid content (SSC) of grapes was determined in juice obtained from grapes using a refractometer (Mettler Toledo, RE50 Refractometer, USA). Samples (10 g) were taken and homogenized with 100 ml water and pH was measured in suspensions using a pH meter (Inolab, Level 3, Weilheim, Germany). Titratable acidity (TA) was determined by titration of diluted grape juice (1 ml of grape juice in 25 ml of distilled water) with 0.1 N NaOH solution until the end of titration (pH 8.1) and expressed as equivalent concentration of tartaric acid. All analyses were performed in triplicate at the day of preharvest spraying, harvesting, and during storage.

2.4. Total Phenolic Content and Antioxidant Activity

Extraction method of phenolic compounds from grapes was determined using a method described by Melgarejo-Flores et al. (2013), with some modifications. Briefly, 10 g of grape was homogenized in 10 ml of methanol (Merck) [80% (v/v)] by using a homogenizer (IKA Ultraturrax T25, Germany) at 6000 rpm. Then, the extraction of phenolic compounds was carried out in an ultrasonic bath for 60 min at room temperature (25 ± 1 °C). In order to eliminate solid particles, extract was centrifuged at 3000 rpm for 15 min at room temperature using a centrifuge (Hettich Zentrifugen, Rotina 380R, Germany). The supernatant was used for analysis of total phenolic content and antioxidant activity. All experiments were performed in triplicate at the day of preharvest spraying, harvesting, and during storage.

Total phenolic content of samples was determined using the Folin-Ciocalteu method based on total phenol analysis (Slinkard & Singleton, 1977). Each extract (20 µl) was added into tubes, and 1.58 ml of distilled water was added. A total of 100 µl of Folin-Ciocalteu reagent (diluted 1:10 ratio with distilled water) was added and immediately mixed. After waiting for 8 min, 300 µl of sodium carbonate solution [7.5% (w/v) in distilled water] was added and mixed. Then, the solutions were incubated in a dark place for 2 h at room temperature. The absorbance of each solution was measured at 765 nm with a spectrophotometer (Shimadzu

UV-2450, Japan). The total phenolic content of samples was calculated as mg gallic acid equivalent (GAE) per kg fresh fruit by using a standard curve of gallic acid.

Antioxidant activity of samples was determined according to the spectrophotometric analysis of ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6sulphonic acid)] radical scavenging activity (Re et al., 1999). ABTS solution at 7 mM and potassium persulfate solution at 2.45 mM were prepared by dissolving in distilled water. $ABTS^+$ cation radical was produced by mixing equal amount of the ABTS and potassium persulfate solutions, then allowing the mixture to stand in the dark at room temperature for 16 h. The $ABTS^+$ solution was diluted with phosphate buffer (0.1 M, pH 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm. After addition of 1.0 ml of diluted $ABTS^+$ solution to 10 µl of sample, the reduction in absorbance at 734 nm was recorded 1 min after mixing up to 5 min. The percentage inhibition of absorbance at 734 nm was calculated and the antioxidant activity of samples was expressed as µmol Trolox equivalent (TEAC) per gram fresh fruit by using a standard curve of Trolox.

2.5. Lactic acid content

Lactic acid content of grapes was analysed by high performance liquid chromatography (HPLC) equipped with a Bio Rad Aminex HOX-87H column according to previously reported method with some modifications (Liu, Wang, Qin, & Tian, 2016). As a mobile phase, 5 mM sulfuric acid was used at a flow rate of 0.6 ml/min. Lactic acid was detected at 210 nm with DAD detector at 65 °C and volume of injected sample was 20 µl. Juices obtained from grape samples were diluted 1:4 ratio with ultrapure water. The standard solutions were prepared at different concentrations with ultrapure water. Standard solutions and grape juice samples were filtered through a 0.45 µm millipore membrane filter (Sartorius Minisart Syringe Filter, Germany). Concentration of lactic acid was evaluated using peak areas of standard solutions and results were expressed in mg lactic acid per liter juice (mg/l). The analysis was performed in duplicate at the day of preharvest spraying, harvesting, and during storage.

2.6. Yeast and Mold Counts

In order to understand the effect of treatments on yeast and mold growth in grapes, microbiological analysis was carried out according to the International Standard Organization Norms (ISO 21527-1, 2008). A representative sample of grapes (10 g) was transferred to a sterile stomacher bag and then homogenized for 1 min with 90 ml of 0.1% sterile peptone water using a stomacher (Bagmixer 400P, Interscience, France). Serial dilutions were prepared from homogenates and 0.1 ml from each dilutions was inoculated on potato dextrose agar (PDA) and the plates were incubated at 25 °C for 5 days. All tests were performed in duplicate at the day of preharvest spraying, harvesting, and during storage. Yeast and mold counts of grapes were determined periodically during storage and results were expressed as log CFU/g.

2.7. Weight loss, color, and firmness

Weight loss was measured as a percentage loss of initial weight by recording weight changes of three grape clusters selected from each treatment during storage.

Color values (CIE L^*, a^* and b^*) of grapes were determined by using a Minolta colorimeter (Konica Minolta Sensing Inc., Japan) during storage. Measurements were taken in the same samples previously selected for each treatment.

Firmness of grapes was measured by using a texture analyser (Texture Analyser TA-XT2 Stable Macro Systems Ltd., Godalming, Surrey, UK) with a 5 kg load cell and cylindrical probe having 2 mm diameter at a prespeed and postspeed of 2 mm/s. Measurements were performed on 10 grape berries for each treatment during storage and firmness was expressed as Newton (N).

2.8. Sensory analysis

Alphonse Lavalleé grapes without any treatment and treated with postharvest alginate coating w/wo vanillin were used for sensory analysis. Sensory panels were performed with 14 panelists selected from students and staff members of Food Engineering Department at IZTECH who consume table grapes frequently to evaluate the sensory properties of grapes during storage. Panelists were asked to rate given samples for appearance, color, odor, taste, texture, and overall acceptability by using a nine-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely). Three grape berries from each treatment were presented in individual white cups for observation and one grape sample (washed and prepared as a ready-to-eat sample) from each treatment was presented in a separate white cup for tasting. Short training on the basis of sensory properties of grapes was given to panelists at the beginning of sensory panel. Each panelist evaluated three different groups including four grape berries.

2.9. Statistical analysis

All data were analysed by using one-way analysis of variance (ANOVA). Significant difference was considered at $p < 0.05$ and Tukey's test was used to determine statistical significant difference between treatments with Minitab Software (Minitab Inc., State College, PA., USA).

3. Results and discussions

3.1. Effect of preharvest treatments

3.1.1. Total soluble solid content, titratable acidity, and pH

Total soluble solid contents (SSC) of grapes increased during maturation in vineyard. SSC of Alphonse Lavalleé and Razaki grapes were 10.88 and 11.30 °Bx at the day of spraying, respectively. At the day of harvesting, for Alphones Lavalleé grapes they were increased to 17.19, 17.51, 16.08 °Bx for control, alginate spray and alginate spray incorporating vanillin, and for Razaki grapes they were increased to 24.95, 24.67, 24.11 °Bx for control, alginate spray and alginate spray incorporating vanillin, respectively. In contrast, titratable acidity (TA) of grapes decreased with maturity. At the day of preharvest treatment, TA of Alphonse Lavalleé and Razaki grapes were 1.69 and 3.34 g/100 ml, respectively. When grapes were harvested, TA of Alphonse Lavalleé and Razaki grapes were 0.71, 0.73, 0.69 and 0.51, 0.51, 0.45 g/100 ml for control, alginate spray and alginate spray incorporating vanillin, respectively. Meng et al. (2008) showed also increasing pattern in SSC and decreasing pattern in TA for *Vitis vinifera* cv. Jingxiu table grapes during 10 days of maturation period before harvesting. There was no significant difference ($p > 0.05$) in SSC and TA between control and treatment groups in both cultivars. Thus, preharvest spray did not influence the development of soluble solids and organic acids during maturation. Correlatively, alginate solutions sprayed on the fruit surface did not affect pH of grapes significantly ($p > 0.05$).

3.1.2. Total phenolic content and antioxidant activity

Phenolic content of grapes from Alphonse Lavalleé and Razaki cultivars increased during ripening in vineyard. At the day of harvesting, total phenolic content of control group grapes, grapes

treated with alginate spray without vanillin and treated grapes with alginate spray incorporating vanillin were, respectively, 689.19, 702.94, 701.06 mg GAE/kg for Alphonse Lavalleé grapes and 684.19, 690.44, 686.06 mg GAE/kg for Razaki grapes. When the fruits were treated with preharvest spray, antioxidant activity was 77.46 and 79.42 μmol TEAC/g for Alphonse Lavalleé and Razaki grapes, respectively. At the day of harvesting, antioxidant activity of control group grapes, grapes treated with alginate spray without vanillin and grapes treated with alginate spray incorporating vanillin were, respectively, 80.88, 81.82 and 81.64 μmol TEAC/g for Alphonse Lavalleé grapes, 81.15, 81.60 and 81.51 μmol TEAC/g for Razaki grapes. In the study about postharvest enhancement of table grapes, total phenolic content of redglobe table grapes at the beginning of storage was around 600 mg GAE/kg while antioxidant activity of redglobe table grapes at the beginning of storage was between 40 and 60 trolox equivalents antioxidant capacity (TEAC) (Melgarejo-Flores et al., 2013). The results for total phenolic content and antioxidant activity were compatible with literature. Control group grapes, grapes treated with alginate spray and grapes treated with alginate spray incorporating vanillin did not show significant difference ($p > 0.05$) in total phenolic content and antioxidant activity in both cultivars. Briefly, the important point was that the application of preharvest treatment did not prevent synthesis of bioactive compounds in plant during maturation.

3.1.3. Lactic acid content

It was considered that preharvest alginate sprays remained as a very thin layer on the surface of grapes and the layer did not interfere with respiration process. Thus, changes in lactic acid content of grapes were determined as a precursor of anaerobic conditions. At the day of preharvest treatment, lactic acid content of Alphonse Lavalleé and Razaki grapes was 170 and 410 mg/l, respectively. At the day of harvesting, lactic acid content of control group grapes, grapes treated with alginate spray without vanillin and grapes treated with alginate spray incorporating vanillin were respectively 190, 140 and 140 mg/l for Alphonse Lavalleé grapes and 320, 340, 380 mg/l for Razaki grapes. It could be concluded that preharvest spray would not affect respiration of grapes. Lima et al. (2014) determined lactic acid content of grape juices obtained from fresh grapes (18–21 °Brix) between 190 and 643 mg/l while main organic acids were tartaric acid and malic acid. The results regarding the lactic acid content of grapes were consistent with previous reports.

3.1.4. Yeast and mold counts

Preharvest treatments by alginate spray w/wo vanillin were remarkable on controlling fungal decay of table grapes in vineyard. There was a significant difference ($p < 0.05$) in yeast and mold growth between control group and treated Alphonse Lavalleé grapes. At the day of preharvest treatment, Alphonse Lavalleé and Razaki grapes had 3.04 and 2.64 log CFU/g for yeast and mold counts, respectively. At the day of harvesting, yeast and mold counts of control group, treated with alginate spray and treated with alginate spray incorporating vanillin groups were, respectively, 3.01, 2.54 and 2.59 log CFU/g for Alphonse Lavalleé grapes, and 3.06, 2.89, 2.70 log CFU/g for Razaki grapes. There was no significant difference between yeast and mold growth of grapes treated with alginate spray w/wo vanillin. It was probably due to a rapid release of vanillin from grapes at high environmental temperatures which were between 35 and 37 °C in the vineyard. In literature, yeast and mold counts of harvested table grapes at the beginning of storage ranged between 3 and 4 log CFU/g. Preharvest spray treatment with Aloe-vera gel showed reduction in yeast and mold counts and the reduction was lower than 1 log CFU/g. However, considerable reduction in yeast and mold counts was observed by postharvest treatment with Aloe-vera gel coating during 35 days of storage at 2 °C (Castillo et al., 2010).

3.2. Effect of postharvest treatments

3.2.1. Total soluble solid content, titratable acidity, and pH during storage

SSC and TA are one of the most important quality characteristics of grapes during storage (Gao, Zhu, & Zhang, 2013). SSC of coated grapes during storage did not show any significant difference ($p > 0.05$) during storage. However, SSC of control group grapes gradually increased in the initial period of storage and then reflected a decrease similarly in Alphonse Lavalleé (Fig. 1a) and Razaki grapes (Fig. 1b). Increase in SSC in the initial period of storage is probably due to continuation of fruit maturation. Consumption of soluble solids by respiration process results in a decline in SSC for metabolic activities of fruit (Özden & Bayindirli, 2002). However, coated grapes showed regular pattern with slight changes. We concluded that alginate coating had a positive effect on SSC of grapes during storage. This positive effect of alginate coating could occur due to the reduction of respiration process by forming gaseous environment in fruit surface. As soluble solids, organic acids in fruit are also exhausted during storage. TA gradually decreased in control group grapes of Alphonse Lavalleé (Fig. 1c) and Razaki cultivars (Fig. 1d). The results indicated that alginate coating provided lower loss in both SSC and TA of grapes during storage. The decrease in TA is an index of increase in maturity. Meng et al. (2008) was also reported the decrease in TA content for grapes treated with preharvest chitosan spray and postharvest chitosan coating. The pH values of coated grapes changing between 3.66 and 4.00 for Alphonse Lavalleé, 4.11 and 4.28 for Razaki grapes did not show significant changes ($p > 0.05$) during storage.

3.2.2. Total phenolic content and antioxidant activity during storage

The changes in total phenolic content and antioxidant activity of grapes during storage are shown in Fig. 2. Concentration of total phenolic compounds at the end of storage was lower than initial concentration in control groups while postharvest coatings have displayed close values to the initial concentration at the end of storage (Fig. 2a and b). Decline in total phenolic concentration was efficiently prevented by incorporation of vanillin into the coating formulation. As a result, postharvest treatment by alginate coatings significantly ($p < 0.05$) prevented the loss of phenolic compounds and addition of vanillin into the coating ensured higher total phenolic concentration than alginate coating without vanillin throughout storage at 4 ± 2 °C. Total phenolic content of control group grapes slightly decreased during storage which was similar to total phenolic content changes reported for *Vitis vinifera* cv. Muscatel table grapes coated with hydroxypropylmethylcellulose containing an ethanolic extract of propolis (Pastor et al., 2011).

Antioxidant activity of the samples slightly increased during 14 days of storage (Fig. 2c and d). It was reported that formation of antioxidant compounds could occur as a result of enzymatic browning due to poylphenol oxidase (PPO) enzyme activity during postharvest storage of grapes (Meng et al., 2008). Therefore, the increase in antioxidant activity is probably due to formation of these compounds in the first period of postharvest storage. After 14 days of storage, antioxidant activity of grapes decreased from 82.67–78.69 μM TEAC/g in Alphonse Lavalleé grapes and from 80.88 to 77.48 μM TEAC/g Razaki grapes for control group, but antioxidant activity of grapes coated with alginate coating incorporating vanillin (84.33 and 82.58 μM TEAC/g for Alphonse Lavalleé and Razaki grapes) was higher than the grapes coated with alginate coating only (83.12 and 81.82 μM TEAC/g for Alphonse Lavalleé

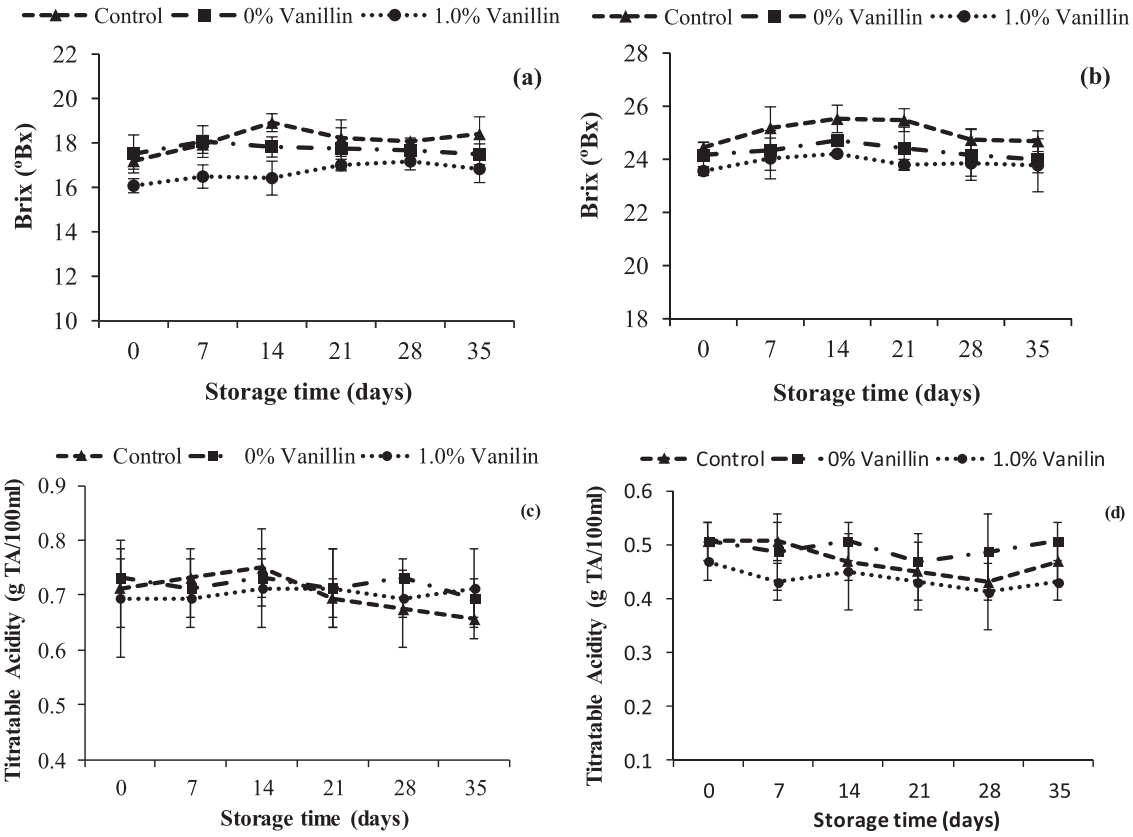

Fig. 1. Effects of alginate preharvest spray and postharvest coating treatment w/wo vanillin on total soluble solid (Brix) content and titratable acidity of Alphonse Lavalleé (a and c) and Razaki grapes (b and d).

and Razaki grapes) and control group grapes (83.12 and 81.82 μM TEAC/g for Alphonse Lavalleé and Razaki grapes). Alginate coating incorporating vanillin maintained initial antioxidant activity of grapes at the end of storage. The protective role of alginate coating incorporating vanillin on antioxidant activity was observed in both grape cultivars.

3.2.3. Lactic acid content during storage

Grapes could be spoiled by lactic acid bacteria which is naturally found on the surface of grape and grape leaves and produce lactic acid from sugars in anaerobic conditions (Bae, Fleet, & Heard, 2006). In that respect, the balance between O_2 and CO_2 provided by coating plays an important role. High lactic acid content can be an indicator of lactic acid fermentation due to anaerobic conditions. However, lactic acid is also one of the organic acids naturally presented in grapes. Lactic acid content of grape juices from different varieties of *Vitis labrusca* grapes varied from 190 to 643 mg/l (Lima et al., 2014). Lactic acid concentrations ranged from 100 to 290 mg/l for Alphonse Lavalleé grapes and from 190 to 460 mg/l for Razaki grapes. Lactic acid contents of grapes were compatible with literature and did not show significant ($p > 0.05$) changes in treated grapes during storage. It could be pointed out that alginate coatings w/wo vanillin were efficient for providing internal atmosphere with O_2 and CO_2 during storage.

3.2.4. Yeast and mold counts during storage

Deterioration in fresh fruit and vegetables by microorganisms caused high quality and quantity losses. Microbial spoilage of grapes is caused by fungi and it is the main reason for preharvest and postharvest decays. Changes in yeast and mold counts of grapes treated with preharvest spray and postharvest coating during storage are presented in Fig. 4. It was remarkable that alginate coatings incorporating vanillin were considerably effective in reducing growth of yeast and mold. Both control group and only alginate coated grapes showed increasing pattern in yeast and mold counts during storage while grapes coated with alginate coatings incorporating vanillin had diminishing pattern in yeast and mold counts until 35 days of storage. Compared with control groups, the addition of vanillin into alginate coating provided significant ($p < 0.05$) reduction in yeast and mold growth by 1.73 and 0.82 log CFU/g for Alphonse Lavalleé and Razaki grapes, respectively, at the end of storage. Consequently, application of alginate coating enriched with vanillin prolonged the shelf life of table grapes significantly ($p < 0.05$) during storage. Thus, postharvest edible alginate coating incorporating vanillin could be used as an alternative to prevent fungal decay and extend shelf life of grapes during storage.

A few studies in the literature have already demonstrated that vanillin had beneficial effect on reduction of yeast and mold growth in fresh fruits. Rojas-Graü et al. (2007) found that an alginate coating without incorporation of antimicrobial agents was not effective in reducing yeast and mold growth on fresh-cut apples. On the other hand, alginate coating incorporating lemongrass oil (1.0 and 1.5% w/v), oregano oil (0.5% w/v) and vanillin (0.3% and 0.6% w/v) inhibited the growth of yeast and mold. In addition, the growth rate of yeast and mold in fresh-cut apples coated with alginate coating incorporating vanillin did not exceed 3 log CFU/g at the end of 21 days of storage at 4 ± 2 °C. Our results were compatible with literature and indicated that yeast and mold growth significantly reduced by alginate coating when vanillin was added as a natural antimicrobial agent.

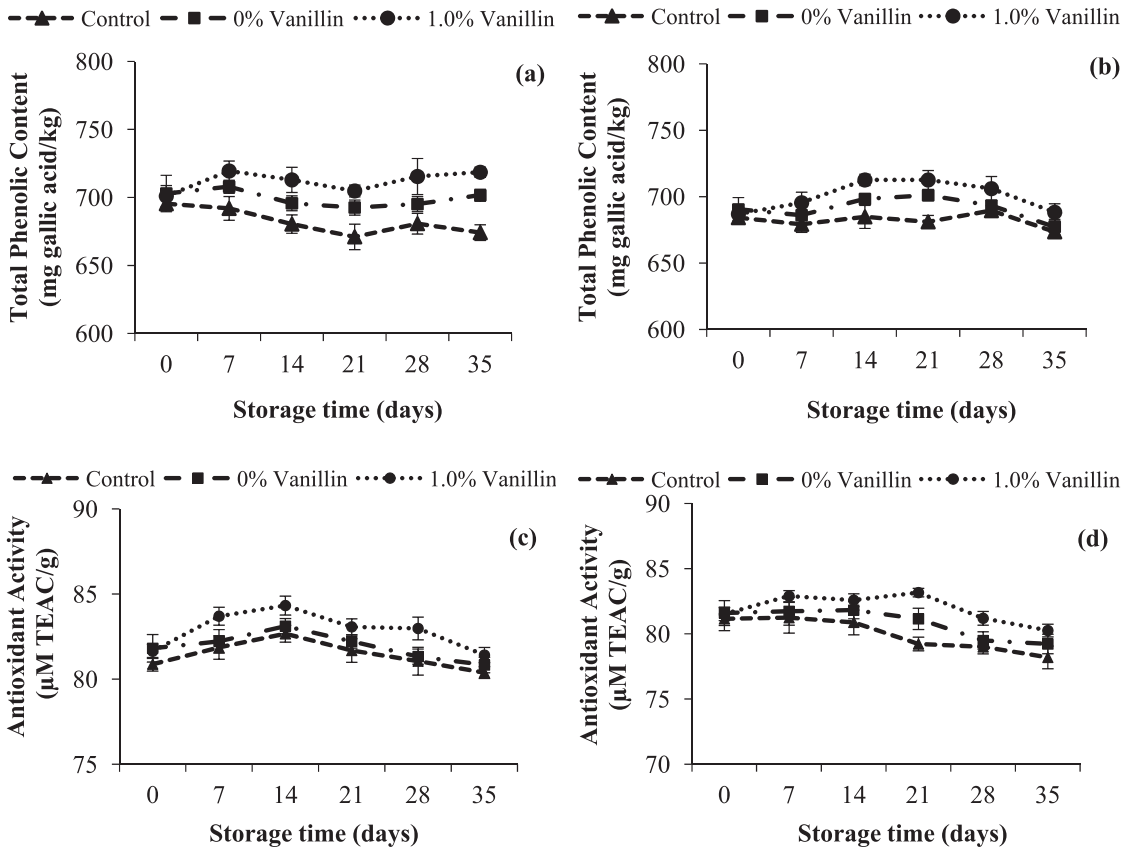

Fig. 2. Effects of alginate preharvest spray and postharvest coating treatment w/wo vanillin on total phenolic content and antioxidant activity of Alphonse Lavalleé (a and c) and Razaki grapes (b and d).

3.2.5. Weight loss, color and firmness during storage

Fruit and vegetables contain a relatively large amounts of water. However, when fruit and vegetables are harvested, water loss occurs naturally and its rate depends on temperature and relative humidity of medium (Embuscado & Huber, 2009). Coating application significantly ($p < 0.05$) prevented weight loss in table grapes during storage compared to control group (Fig. 3). At the end of 35 days of storage, control grapes lost 18.28 and 16.75% of their weight while weight loss of alginate coated grapes reached 10.92 and 11.39% for Alphonse Lavalleé (Fig. 3a) and Razaki (Fig. 3b) grapes, respectively. No significant ($p > 0.05$) difference was determined in weight loss among coatings w/wo vanillin. Although alginate coatings are considered to be not very effective to reduce weight loss, studies indicated that alginate–calcium coatings prevented water loss effectively through cross-linking alginate polymer with calcium ions (Olivas et al., 2007). Reduction in weight loss by alginate coating has also been demonstrated in fruit and vegetables including plum (Valero et al., 2013), apple slices (Olivas et al., 2007), fresh-cut pineapple (Azarakhsh et al., 2014), mushroom (Jiang et al., 2013), and strawberry (Fan et al., 2009). In the present study, results showed that alginate coatings w/wo vanillin significantly ($p < 0.05$) limited weight loss of Alphonse Lavalleé and Razaki grapes during storage at 4 ± 2 °C.

Color changes and softening occur during ripening and postharvest storage due to the degradation of cell wall components by hydrolitic enzyme activities (Serrano et al., 2008). Effects of alginate treatments on color values (CIE L^*, a^* and b^*) of grapes are given in Table 1 for both grape cultivars. Coating application was effective in maintaining color parameters of grapes during storage. The main effect of alginate coating incorporating vanillin was observed in the lightness of skin color. Lightness (L^*) values were higher in grapes coated with alginate coating incorporating vanillin than only alginate coating. This higher lightness values of grapes having alginate coating incorporating vanillin can be explained by a characteristic white color of vanillin. There was no significant ($p > 0.05$) difference between alginate coating without vanillin and control groups at the day of harvesting. However, lightness decreased with storage time and the decrease was significantly greater in control group. This is probably due to the enzymatic browning and coating had an important role in retarding enzymatic reactions which was reported in previous studies (Pastor et al., 2011). Chroma values of samples showed differences depending on the grape varieties during storage. Incorporation of vanillin into alginate coating decreased the chroma values of grapes having green color from Razaki cultivar whereas that increased the chroma values of grapes having red color from Alphonse Lavalleé cultivar. Hue values of grapes slightly decreased for Alphonse Lavalleé grapes and increased for Razaki grapes by incorporation of vanillin into alginate coating. No significant ($p > 0.05$) difference was observed in chroma and hue values of grapes coated with alginate coating and control group.

Firmness values of grapes as a function of storage time are presented in Fig. 3c and d. It is remarkable that grapes treated with preharvest spray showed greater firmness values compared to control group grapes at the beginning of storage. Firmness of control grapes sharply decreased with increasing storage time in all cultivars. Coating application maintained firmness of grapes during storage. ANOVA results also demonstrated that fruit deformation force (N) explained as fruit firmness of grapes was significantly ($p < 0.05$) affected by alginate coating w/wo vanillin. Addition of vanillin into coating did not significantly ($p > 0.05$) influence the firmness of grapes in all cultivars. The reduction in firmness loss

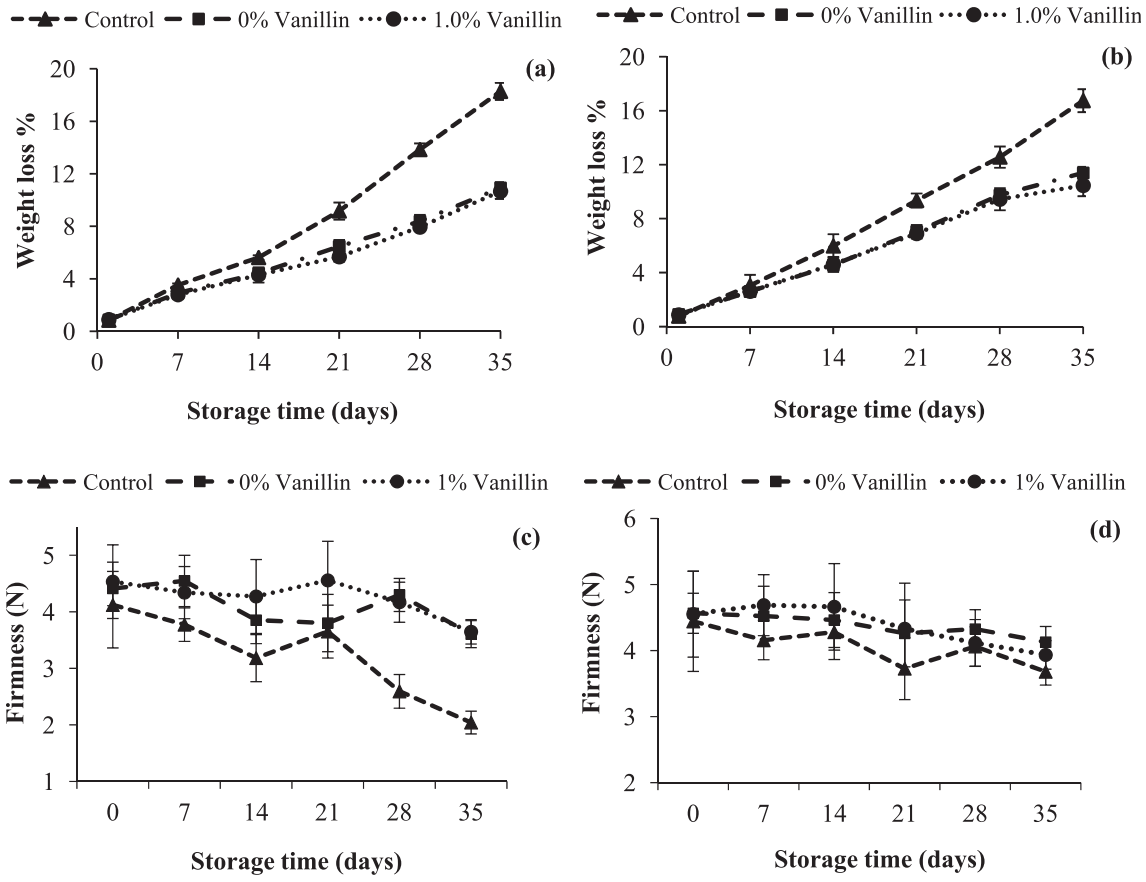

Fig. 3. Effects of alginate preharvest spray and postharvest coating treatment w/wo vanillin on weight loss and firmness of Alphonse Lavalleé (a and c) and Razaki grapes (b and d).

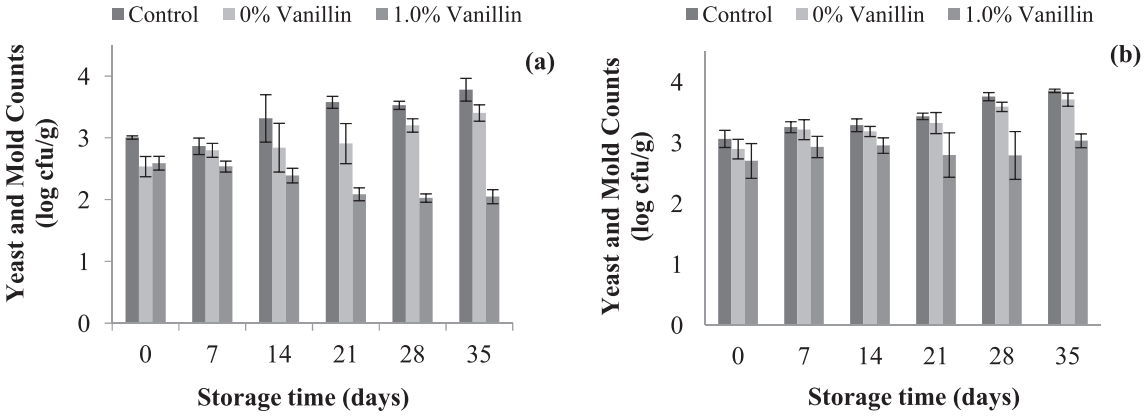

Fig. 4. Effects of alginate preharvest spray and postharvest coating treatment w/wo vanillin on yeast and mold counts of Alphonse Lavalleé (a) and Razaki grapes (b).

by alginate coating could be explained by minimizing weight losses through coating application that has been reported in table grapes (Sánchez-González et al., 2011), plum (Liu, Wang, & Young, 2014), and apples (Olivas et al., 2007) by different edible coatings. In addition, $CaCl_2$ treatment had good contribution to the fruit firmness. The positive effect of calcium in reduction of firmness loss during storage was reported in strawberries (Atress, El-Mogy, Aboul-Anean, & Alsanius, 2010).

3.2.6. Sensory evaluation during storage

Impact of edible film or coatings with active agents on sensory quality of fresh produce should be considered. Adaptability with sensory attributes of fresh produce and antimicrobial activity against targeted microorganisms are major considerations when making a decision about active agent selection. The results for sensory analysis are summarized in Table 2. Tasting of grapes was performed until 21 days of storage in order to be on the safe side due to the microbial spoilage especially in control group. All sensory attributes decreased during 28 days of storage in both control and coated grapes. However, panelists gave higher points for coated grapes than control groups. Appearance and color attributes of grapes were ranked as the highest in grapes coated with alginate coating without vanillin. Odor of grapes coated with alginate coating incorporating vanillin had the highest scores during storage.

Table 1
Color values of table grapes of Alphonse Lavalleé and Razaki cultivars with respect to alginate coating treatment w/wo vanillin.

Grape cultivar	Color value	Treatments	Days					
			0	7	14	21	28	35
Alfonse	L^*	Control	23.54^{a}	23.51^{a}	21.63^{b}	22.05^{b}	21.09^{a}	21.74^{b}
		0% Vanillin	25.55^{a}	25.26^{a}	24.96^{ab}	24.81^{ab}	25.24^{b}	24.82^{b}
		1.0% Vanillin	32.91^{a}	32.28^{a}	33.13^{a}	33.37^{a}	32.64^{b}	32.12^{a}
	a^*	Control	1.40^{a}	1.48^{a}	1.91^{a}	1.65^{a}	1.71^{a}	1.76^{a}
		0% Vanillin	1.69^{a}	1.45^{a}	1.43^{a}	1.82^{a}	1.59^{a}	1.63^{a}
		1.0% Vanillin	2.47^{a}	2.45^{a}	2.20^{a}	2.06^{a}	2.09^{a}	2.21^{a}
	b^*	Control	-1.06^{a}	-0.73^{a}	-0.45^{a}	-0.64^{a}	-0.53^{a}	-0.49^{a}
		0% Vanillin	-0.29^{a}	-0.81^{a}	-0.81^{a}	-0.51^{a}	-0.61^{a}	-0.53^{a}
		1.0% Vanillin	-2.31^{a}	-2.14^{a}	-1.51^{b}	-1.92^{b}	-1.67^{b}	-1.49^{b}
	C^*	Control	1.75^{b}	1.66^{b}	1.96^{b}	1.77^{c}	1.79^{b}	1.83^{b}
		0% Vanillin	1.71^{b}	1.66^{b}	1.64^{c}	1.89^{b}	1.70^{b}	1.72^{b}
		1.0% Vanillin	3.38^{a}	3.25^{a}	2.66^{a}	2.81^{a}	2.68^{a}	2.66^{a}
	h	Control	358.25^{a}	358.34^{a}	358.04^{b}	358.23^{a}	358.21^{a}	358.17^{a}
		0% Vanillin	358.29^{a}	358.34^{a}	358.36^{a}	358.11^{b}	358.30^{a}	358.28^{a}
		1.0% Vanillin	356.62^{b}	356.75^{b}	357.34^{c}	357.19^{c}	357.32^{b}	357.34^{b}
Razaki	L^*	Control	46.48^{a}	48.47^{a}	47.11^{a}	46.61^{a}	46.12^{a}	45.91^{a}
		0% Vanillin	46.77^{a}	48.30^{a}	48.42^{a}	47.78^{a}	45.91^{a}	45.00^{a}
		1.0% Vanillin	49.44^{a}	49.74^{a}	49.25^{a}	48.94^{a}	50.73^{a}	47.64^{a}
	a^*	Control	-4.90^{a}	-5.07^{a}	-5.11^{ab}	-4.44^{a}	-4.65^{a}	-3.98^{a}
		0% Vanillin	-6.20^{a}	-6.02^{a}	-5.97^{b}	-5.66^{a}	-5.83^{a}	-4.90^{a}
		1.0% Vanillin	-3.16^{a}	-3.69^{a}	-3.05^{a}	-3.42^{a}	-3.20^{a}	-3.25^{a}
	b^*	Control	18.68^{a}	19.15^{a}	20.60^{a}	21.40^{a}	20.02^{a}	20.96^{a}
		0% Vanillin	17.47^{a}	17.41^{a}	17.41^{a}	17.78^{a}	16.89^{a}	16.47^{a}
		1.0% Vanillin	9.77^{b}	10.49^{a}	10.86^{b}	10.23^{a}	10.72^{a}	11.79^{a}
	C^*	Control	19.31^{a}	19.81^{a}	21.23^{a}	21.85^{a}	20.56^{a}	21.33^{a}
		0% Vanillin	18.54^{b}	18.42^{b}	18.40^{b}	18.66^{b}	17.87^{b}	17.19^{b}
		1.0% Vanillin	10.27^{c}	11.12^{c}	11.28^{c}	10.78^{c}	11.19^{c}	12.23^{c}
	h	Control	160.69^{c}	160.19^{c}	158.77^{c}	158.15^{c}	159.44^{c}	158.67^{c}
		0% Vanillin	161.46^{b}	161.58^{b}	161.60^{b}	161.34^{b}	162.13^{b}	162.81^{b}
		1.0% Vanillin	169.73^{a}	168.88^{a}	168.72^{a}	169.22^{a}	168.81^{a}	167.77^{a}

Means of treatments in same column with different lowercase letters are significantly different ($p < 0.05$) for each color parameters.

Table 2
Sensory attributes of Alphonse Lavalleé grapes with respect to alginate coating treatment w/wo vanillin during 28 days of storage at 4 °C.

Sensory attribute	Treatments	Days				
		0	7	14	21	28
Appearance	Control	6.11^{b}	6.50^{b}	5.75^{b}	5.25^{b}	4.55^{b}
	0% Vanillin	8.33^{a}	7.50^{a}	6.00^{a}	5.88^{a}	5.15^{a}
	1.0% Vanillin	5.11^{c}	5.25^{c}	5.34^{c}	4.98^{c}	4.63^{b}
Color	Control	6.89^{b}	7.38^{b}	6.88^{a}	5.13^{b}	4.75^{b}
	0% Vanillin	8.44^{a}	8.25^{a}	7.50^{a}	6.23^{a}	5.69^{a}
	1.0% Vanillin	5.44^{c}	5.50^{c}	6.00^{b}	4.63^{c}	4.50^{c}
Odor	Control	6.00^{b}	6.38^{c}	6.38^{b}	5.01^{c}	4.28^{c}
	0% Vanillin	6.33^{ab}	7.25^{b}	6.25^{c}	5.25^{b}	4.63^{b}
	1.0% Vanillin	6.67^{a}	7.38^{a}	6.75^{a}	6.00^{a}	5.38^{a}
Taste	Control	7.89^{a}	7.00^{c}	6.50^{c}	n.d.	n.d.
	0% Vanillin	7.89^{a}	7.63^{b}	6.88^{b}	n.d.	n.d.
	1.0% Vanillin	7.11^{b}	8.00^{a}	7.50^{a}	n.d.	n.d.
Texture in mouth	Control	7.22^{c}	7.63^{c}	6.88^{c}	n.d.	n.d.
	0% Vanillin	7.67^{b}	7.75^{b}	7.38^{a}	n.d.	n.d.
	1.0% Vanillin	8.22^{a}	8.00^{a}	7.13^{b}	n.d.	n.d.
Overall acceptability	Control	7.33^{b}	7.13^{b}	6.25^{c}	5.13^{b}	3.75^{b}
	0% Vanillin	8.00^{a}	7.00^{c}	7.00^{a}	5.38^{a}	4.25^{a}
	1.0% Vanillin	6.56^{c}	8.00^{a}	6.63^{b}	4.63^{c}	3.65^{b}

n.d.: not determined.
Means of treatments in same column with different lowercase letters are significantly different ($p < 0.05$) for each sensory attributes.

Our results also showed that grapes coated with alginate coating incorporating vanillin had higher scores for taste than control groups and grapes coated with alginate coating. High scores in odor and taste were most probably related with desired taste and flavor of vanillin.

4. Conclusion

Preharvest treatment by alginate spray w/wo vanillin was effective to improve quality and safety of table grapes. Postharvest alginate coating incorporating vanillin maintained the quality of table grapes by retention of soluble solids, titratable acidity, firmness, and color, protected functional properties as total phenolic content and also extended the shelf life of grapes by diminishing fungal decay. Our work suggested that bioactive coating developed by addition of vanillin into alginate coating could be alternative of synthetic fungicides to prevent postharvest deteriorations and improve postharvest quality of grapes by avoiding harmful impact of chemicals on environment and human health.

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