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Composite Coating as a Carrier of Antioxidants Improves the Postharvest Shelf Life and Quality of Table Grapes (*Vitis vinifera* L. var. Thompson Seedless)

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ABSTRACT

Composite edible coating comprising xanthan gum (0.3%) and olive oil (0.1%) enriched with antioxidants (gallic acid 0.1%, ferulic acid 0.1% and ascorbic acid 0.1%) enhanced the postharvest storability and nutritional quality of table grapes. The quality characteristics of table grapes were monitored during storage at 10±2°C, (70–75% RH), at regular intervals of 6 days until 24 days of storage. Xanthan gum combined with olive oil reduced the weight loss, decay occurrence, accumulation of total soluble solids and total sugars by reducing the rate of respiration and metabolism in the coated fruit. Moreover, incorporation of antioxidants in coating enhanced the level of phenolics, ascorbic acid and total antioxidant activity in grapes. The activities of cell wall modifying enzymes such as Polygalacturonase (PG) and Pectate Lyase (PL) were reduced in the fruits of treated sets as compared to that of the control set. These results suggest that the composite coating delayed the ripening and softening process in grapes and thereby extended their shelf life up to 24 days, while the control grapes were decayed on the 12th day.

Keywords: Composite edible coating, Nutritional quality, Phenolic compounds, Shelf life, *Vitis vinifera* L.

INTRODUCTION

Grapes (*Vitis vinifera* L.) are an important fruit crop in India and the third most widely cultivated fruit after citrus and banana. Grapes contain various nutrient elements, such as vitamins, minerals, carbohydrates, edible fibres and phytochemicals. Unfortunately, table grapes show severe problems during postharvest storage and retailing. The losses of quality are based on weight loss, color changes, accelerated softening and rachis browning, and high incidence of berry decay (Crisosto *et al.,* 2002), which lead to a reduction of shelf life. Like many other fruits, table grapes undergo numerous physicochemical, biochemical, and microbiological changes during storage, accelerating the ripening process and reduction of their shelf life (Valverde *et al.,* 2005). These changes are accompanied by economical postharvest repercussions due to weight losses and occurrence of decay.

Therefore, it is very important to find a low-cost and efficient nontoxic preservation to improve the internal quality and commercial value of grapes. The increasing interest and research activity in edible packaging have been motivated by both increasing consumer demand for safe, convenient, and stable foods and also awareness of the negative environmental impacts of non biodegradable packaging waste. Edible coatings have long been known to protect perishable food products from deteriorations by retarding dehydration, suppressing respiration, improving textural quality, helping retain

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volatile flavor compounds and reducing microbial growth (Debeaufort *et al*., 1998).

Moreover, edible coatings have been presented as an excellent way to carry additives since they are able to maintain effective concentrations of the additives on the fruit surfaces, where they are mostly needed, reducing the impact of such chemicals on overall acceptability of the fruit (Oms-Oliu *et al.,* 2010). Composite films are in fact a mixture of these and other ingredients in varying proportions, which determine their barrier to gases and other mechanical properties. The presence of lipids in the composite formulations or film provides an appealing glassy finish over the commodity surface (Tharanathan, 2003).

Xanthan gum used in the present study is an extracellular polysaccharide produced by the bacterium *Xanthomonas campestris*. It is widely used in foods because of its good solubility in either hot or cold liquids, high viscosity even at very low concentrations, and excellent thermal stability. Xanthan gum forms very viscous solutions and at sufficiently high polymer concentration, it exhibits weak gel-like properties (Izydorczyk *et al.,* 2005). Therefore, lipid component can be incorporated to enhance the film forming property of the xanthan gum to be used as a coating material. One such lipid component is olive oil, which is composed of 56.3–86.5% MonoUnsaturated Fatty Acids (MUFA) and extensively consumed due to its nutritional value and its organoleptic characteristics. It is also rich in tocopherols and phenolic substances which act as antioxidants (Baraiya *et al.,* 2014). The antioxidant activity of ferulic acid, a natural hydroxycinnamic acid has been well recognized (Rice Evans *et al*., 1996). Gallic Acid (GA), a naturally occurring plant phenol, was also found to be a strong antioxidant in emulsion or lipid systems. GA was used in processed food, cosmetics and food packing materials to prevent rancidity induced by lipid peroxidation and spoilage even more effective than several water-soluble antioxidants (Madsen and Bertelsen, 1995). Yen *et al*. (2002) also reported that the gallic acid and ascorbic acid are natural antioxidants.

Therefore, the present study has been undertaken to examine the efficacy of xanthan gum and olive oil in combination with antioxidants in improving the postharvest storability and nutritional quality of green grapes.

MATERIALS AND METHODS

Raw Materials

Fresh table grapes (*Vitis vinifera* var. Thompson seedless) were procured from commercial fruit market, Anand, Gujarat (India) and immediately transported to the laboratory. They were sanitized by washing in 20 mL L^{-1} sodium hypochlorite solution for 10 minutes to remove residuals prior to coating and dried at room temperature. The grapes, selected for their uniformity in size, shape, colour and stage of maturity and without any signs of mechanical damage or fungal decay, were categorized into five sets, of these four sets were kept as experimental sets, while the 5^{th} was kept as a control. To obtain film-forming dispersions, xanthan gum, L-ascorbic acid, gallic acid and ferulic acid of Himedia brand, Mumbai (India) were procured through local chemical suppliers.

Methodology of Film-Forming Dispersions

Xanthan gum (0.3%, w/v) was initially dispersed in hot water and stirred at 80°C for 2 hours. After complete dispersion, a 0.1% (v/v) concentration of olive oil was added to the polymer solution and emulsified, using a magnetic stirrer (2 MLH, Remi equipments, India), at 80°C, for 30 minutes and labeled as T1 solution. To this composite coating of xanthan gum and olive oil, gallic acid (0.1% w/v), ferulic acid (0.1% w/v) and ascorbic acid (0.1% w/v) were added separately and labelled as T2, T3 and T4, respectively. The antioxidants added into the solutions were completely dissolved within 10 minutes with the help of magnetic stirrer (2 MLH, Remi

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equipments, India). 0.1% glycerol was added to all (T1, T2, T3 and T4) solutions.

Xanthan gum even at low concentration gives a high viscosity to the solution. To formulate the composite coating of xanthan gum and olive oil, their concentrations have been selected on the basis of lab trials. Xanthan gum 0.3% and olive oil 0.1% showed high viscosity even at low concentration and dispersed completely in the solution. The concentration of all three antioxidants has been selected on the basis of existing literature. At this concentration (0.1%), they showed the best antioxidant activity in improving the nutritional quality of fruits and vegetables.

Application of the Coatings

Selected clusters of 15–20 grapes were dipped in the following coating treatments for 5 minutes: T1 (xanthan gum 0.3%+olive oil 0.1%), T2 (xanthan gum 0.3%+olive oil 0.1%+gallic acid 0.1%), T3 (xanthan gum 0.3%+olive oil 0.1%+ferulic acid 0.1%), T4 (xanthan gum 0.3%+olive oil 0.1%+ascorbic acid 0.1%), and T5 (Control). Afterwards, they were hung up and dried at room temperature (65-70%) for 2–3 hours and then stored in plastic boxes at 10±2°C, (70-75% RH). The quality of these stored fruits was determined by analyzing the following parameters at 0 day and then after at regular interval of 6 days. All the analyses were performed without removing coating from their surface.

Weight Loss Percentage (WLP): Weight loss was expressed as the percentage loss of the initial total weight calculated by considering the difference between initial weight and final weight of presently tested grapes divided by their initial weight.

Shelf Life: The shelf life of grapes worked out under the current study was calculated by counting the days required for them to reach the last stage of ripening, but up to the stage when they remained still acceptable for marketing.

Total Soluble Solids (TSS): The TSS content of the grapes was determined as per the method of AOAC (1994). 1 g of fruit tissue was crushed in the motor-pastel with water and this homogenized sample was centrifuged. The TSS was measured from this sample by placing a few drops of it on the prism of refractometer (Atago Co., Tokyo, Japan) and the direct reading was taken as described in AOAC (1994).

Biochemical Analysis of Grapes

The total sugars were estimated by following the phenol-sulfuric acid method described by Thimmaiah (1999). The quantitative analysis of ascorbic acid was carried out by using 2, 6- dinitrophenyl hydrazine, as per the method of Roe (1954). Extraction and estimation of total phenols were carried out by FCR method as explained by Thimmaiah (1999). Total antioxidant activity was analysed by following the FRAP method (Benzie and Strain, 1996).

Enzyme Extraction and Assay

A 2 g sample of mesocarpic pulp tissue of grapes was homogenized in Tris-HCL (20 mM, pH 7.0) containing cysteine-HCl (20 mM), EDTA (20 mM) and Triton X-100 (0.05%). Then this homogenate was centrifuged at 15,000×g for 30 minutes at 4°C in a refrigerated centrifuge, Eppendorf 5430 R (Lohani *et al.*, 2004). The clear supernatant was collected and used for the enzyme assays. The protein content was measured using the Lowry's method (Lowry *et al.*, 1951).

Assay of Polygalacturonase

Polygalacturonase activity was assayed by following the method described by Pathak and Sanwal (1998). The reaction mixture contained 0.2 ml sodium acetate (200 mM, pH

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4.5), 0.1 ml NaCl (200 mM), 0.3 ml Polygalacturonic Acid (PGA, 1% aqueous solution adjusted to pH 4.5) and 0.05 ml of enzyme extract in a total volume of 1.0 ml. The reaction was initiated by the addition of PGA substrate. The mixture was incubated at 37°C for 1 hour followed by the addition of 3,5-Dinitro Salicylic acid (DNS). The reaction was terminated by heating the reaction mixture in a boiling water bath for 5 min. In control tubes, the substrate was added after the heat treatment. The formation of reducing groups was estimated against *D*-galacturonic acid as the standard after measuring the absorbance at 540 nm. One unit of PG enzyme is defined as the amount of enzyme required to liberate 1 nmol of galacturonic acid per min under the conditions of the enzyme assay.

Assay of Pectate Lyase

Pectate lyase activity was measured by using the method described by Moran *et al.* (1968) with some modifications. The assay was carried out in a mixture containing 4mM sodium acetate buffer (pH 4.5), 0.3 ml PolyGalacturonic Acid (PGA, 1% aqueous solution adjusted to pH 4.5) and 0.1 ml enzyme preparation in 1ml total reaction volume. The tubes containing the reaction mixture were incubated at 37°C for 30 minutes followed by boiling in a water bath for 2 minutes to stop the reaction. The absorbance of the reaction mixture was measured at 235 nm. The increase in the absorbance against the control with pre-boiled enzyme was taken as a measure of the pectate lyase activity. All calculations were carried out according to Moran *et al.* (1968) and 1 unit of pectate lyase activity was expressed as the amount of enzyme required to liberate 1 nmol of aldehyde groups from PGA per minute under the conditions of the enzyme assay.

Assay of Polyphenol Oxidase

Polyphenol Oxidase (PPO) (EC 1.10.3.1) activity was measured using the method described by Deng *et al.* (2009). A 1 g sample of fruit tissue was ground in 10 mL of 0.05 mol L^{-1} potassium dihydrogen phosphate buffer (pH 6.8) using a mortar and pestle. After rapid homogenization, the mixture was centrifuged at 8,000×g for 15 minutes at 4°C in an Eppendorf R5430 refrigerated centrifuge. The clear supernatant was used to determine PPO activity. The enzyme solution (0.2 mL) was added to a mixture of 3 mL of 0.05M phosphate buffer (pH 6.8) and 1.0 mL of 0.02M catechol (pH 7.0) as substrate. PPO activity was measured in a UV visible spectrophotometer (UV 1800, Shimadzu) at 398 nm. One unit of PPO activity was defined as the amount of enzyme which results in 0.01 increase in absorbance per minute under assay conditions. Each determination was run in triplicate.

Statistical Analysis

The data presented here were statistically analyzed by using SPSS 17 software. All performed analyses were carried out in triplicate. Mean and Standard Deviation (SD) were calculated. The statistical significance of the data was assessed by one way analysis of variance and LSD test. Mean comparisons were performed using HSD of Tukey's test to examine if differences between treatments and storage time were significant at $P\leq 0.05$. The overall least significance difference (LSD, $P\leq 0.05$) was calculated and used to detect significant differences among all treatments and the control set (Bico *et al.,* 2009).

RESULTS AND DISCUSSION

Effect on Weight Loss Percentage (WLP)

Fresh fruits undergo vigorous biological reactions after harvest and their respiration accelerates the natural loss of fruit tissue. It is commonly believed that the weight loss from fresh fruits and vegetables is through the peel by vapor pressure, which can cause flesh softening, fruit ripening, and senescence by metabolic reactions (Bai *et al.,* 2003). In this experiment, water content of grapes decreased with storage time due to loss from the surface (Figure 1) and thus the weight loss percentage increased rapidly within 12 days and then remained relatively constant at a high level. The results of the present study suggested that during the storage period, the least WLP was noticed on the 6th day in the fruits treated with T4 (2%), while the higher level of WLP was observed in the control set of fruit on the 6th day (7.2 %) and on the 12th day (12%). A similar pattern of WLP was observed in the grapes on the 12th and the 18th day of storage period. Normally, the weight loss occurs during the fruit storage due to its respiratory process, the transference of humidity and some processes of oxidation (Ayranci and Tunc, 2003). According to Pastor *et al.* (2011) an acceleration of weight loss can be attributed to an increase in the fruit metabolic activity, associated with tissue senescence at long storage times, which is slowed down by coatings. In the present study, addition of a lipid component such as olive oil and glycerol significantly enhanced the effectiveness of xanthan gum, indicating their regulation of the hydrophilic-hydrophobic balance, which would in turn, restrict the water loss from the fruit. These results are in accordance with Maqbool *et al.* (2011) who noted that the coating serves as a semipermeable barrier against oxygen, carbon dioxide, and moisture, thus reducing respiration, water loss, and oxidation reactions. Kittur *et al.* (2001) also reported the reduced weight loss in banana fruit coated with polysaccharide-based composite coatings as compared to that of control. Similarly, Gniewosz *et al*., (2014) found that the addition of meadowsweet flower extract to the pullulan coating contributed to reduction in weight losses during their storage of red peppers.

Effect on Shelf Life of Fruit

Decay is primarily caused by weight-loss, not only through direct quantitative loss but

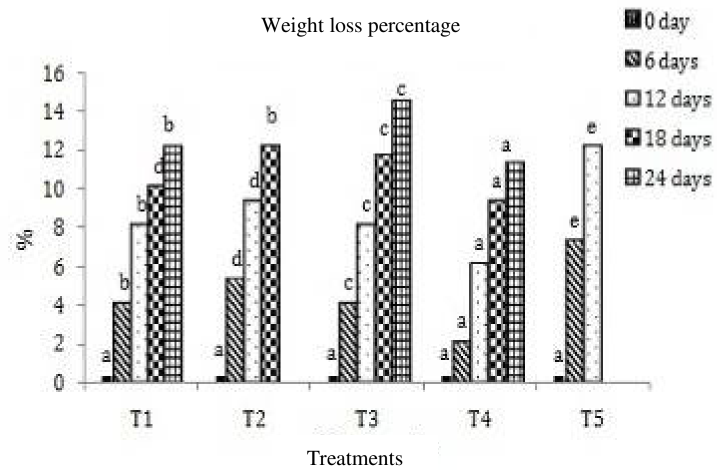

Figure 1. Effect of composite edible coatings on the weight loss in grapes during storage at 10± 2°C. [T1: (Xanthan gum 0.3%+olive oil 0.1%); T2: (Xanthan gum 0.3%+olive oil 0.1%+gallic acid 0.1%), T3: (Xanthan gum 0.3%+olive oil 0.1%+ferulic acid 0.1%); T4: (Xanthan gum 0.3%+olive oil 0.1%+ascorbic acid 0.1%), and T5: (Control). Different letters on the bars mean significantly different at P≤ 0.05].

also through the deterioration of appearance, textural quality (softness, loss of turgidity and juiciness) and nutritional quality (Moncayo *et al.,* 2013). During the course of the present study, the protective role of the composite coatings along with the antioxidants could be observed in reducing the decay incidence and extending the shelf life of grapes. The treated grapes were seen healthy, whereas the control grapes were found shrunk and decayed. Among all the treatments, the treatment T2 (xanthan gum + olive oil + gallic acid) was the best in improving the quality, whereas the T1, T3 and T4 showed the significant effect of decay control and thereby extended the shelf life until 24 days as compared to that of control (12 days). A similar kind of extension in shelf life of grapes was reported by Meng *et al*. (2008), with pre- and postharvest chitosan treatment. In this regard, Guilbert *et al.* (1996) explained that an edible coating was a thin film prepared from edible material that acted as a barrier to the external elements (factors like moisture, oil, vapor) and thus protected the product and extended its shelf-life. Maftoonazad and Ramaswamy (2005) also stated that the coating slows down the respiration rate, reduces the color changes of skin and flesh and increases the shelf life of fruits. Romanazzi *et al.* (2007) developed a composite coating using chitosan and ethanol to reduce postharvest decay of table grapes. Moreover, Synowiec *et al.* (2014) reported the advantage of Pullulan coatings in prolongation of the postharvest life of the cold stored red peppers.

Effect on Total Soluble Solids (TSS)

Data regarding changes in the TSS during the storage of currently analyzed grapes are presented in Table 1. TSS concentration significantly ($P\leq 0.05$) increased during cold storage in control grapes, from levels of 1.3 °Brix at harvest to 2.27 °Brix after 12 days at 10°C. This increase in TSS was more pronounced in the fruits of the control set and it was significantly delayed in coated fruits. Such progressive increases in TSS, explained as the consequences of water evaporation from berry surface, were widely reported in previous studies using different cultivars (Pretel *et al*., 2006). The onset of ripening can be clearly indicated by the relatively sudden increase in soluble solids and by the concomitant increase in berry deformability as explained by Nunan *et al*. (1998). However, at the 12th day of storage period, grapes treated with T1 exhibited a significantly lower accumulation of TSS (1.73 °Brix) compared with that of control (2.27 °Brix). Further, Sabir *et al.* (2011) also found that the TSS content in grapes *cv.* Razaki (*Vitis vinifera* L.) was

Table 1. Changes in TSS and total sugars of grapes during their storage at 10±2°C. [a]

Treatments [b]	Day 0	Day 6	Day 12	Day 18	Day 24
			Total Soluble Solids (°Brix)		
T1	1.30 ± 0.00a	1.50 ± 0.00d	1.73 ± 0.06a	1.83 ± 0.15a	2.20 ± 0.00b
T2	1.30 ± 0.00a	1.47 ± 0.06c	2.10 ± 0.00d	2.30 ± 0.00c	-
T3	1.30 ± 0.00a	1.30 ± 0.00a	1.80 ± 0.00b	2.07 ± 0.06b	2.13 ± 0.06a
T4	1.30 ± 0.00a	1.40 ± 0.00b	1.83 ± 0.06c	2.07 ± 0.06b	2.30 ± 0.00c
T5	1.30 ± 0.00a	1.73 ± 0.06e	2.27 ± 0.06e	-	-
			Total sugars (mg g^{-1})		
T1	161.2 ± 3.11a	268.9 ± 3.56a	400.0 ± 1.22c	466.0 ± 7.19b	437.5 ± 2.80c
T2	161.2 ± 3.11a	309.7 ± 1.41c	357.6 ± 4.00b	321.5 ± 1.25a	-
T3	161.2 ± 3.11a	305.4 ± 0.63b	430.4 ± 3.00e	587.9 ± 1.51d	326.2 ± 15.2a
T4	161.2 ± 3.11a	309.8 ± 1.91c	403.6 ± 2.46d	496.9 ± 17.2c	352.1 ± 2.44b
T5	161.2 ± 3.11a	335.4 ± 0.89e	304.6 ± 3.65a	-	-

[a] Different letters in the column means significantly different at $P\leq 0.05$. [b] T1: (Xanthan gum 0.3%+olive oil 0.1%); T2: (Xanthan gum 0.3%+olive oil 0.1%+gallic acid 0.1%), T3: (Xanthan gum 0.3%+olive oil 0.1%+ferulic acid 0.1%); T4: (Xanthan gum 0.3%+olive oil 0.1%+ascorbic acid 0.1%), and T5: (Control).

increased gradually along with the storage and at the end of the storage, TSS level of control berries was higher than those of treated ones. With this viewpoint, Debeaufort *et al.* (1998) explained that the edible coatings are selective barriers to O_2 and CO_2 modifying internal atmospheres and slowing down the respiration rate of fruit. Velickova *et al.* (2013) also attributed the decrease in the TSS content in strawberries coated with chitosan-beeswax at the end of storage to the lower respiration process.

Effect on Total Sugars

Total sugars are considered good indexes for the determination of storage life. An increase in content of total sugars was observed initially in both treated as well as untreated fruits (Table 1). However, throughout the storage period, fruit treated with T2 significantly ($P \leq 0.05$) delayed the sugar accumulation and showed lesser contents of sugars than that of the other treatments as well as control fruit. After 6 days of storage, the higher accumulation of sugars was found in the control fruits (335.4 mg g^{-1}), whereas it was declined after 12 days (304.6 mg g^{-1}) indicating the deterioration of the fruit quality. On the 18^{th} day of storage, the coated fruits showed high accumulation of sugars but on the 24^{th} day a slight decrease in sugar content was observed. Fruits treated with T1 showed higher sugar content (437.5 mg g^{-1}) suggesting the better quality of grapes. The delayed increase in coated fruit as compared to that of the control fruit was probably due to the effects of composite coatings which exerted a physical barrier to the gaseous exchange that helped in delaying the rate of sugar metabolism. Similar results were obtained by Zapata *et al.* (2008) who found lower sugar and organic acid concentrations in tomatoes coated with alginate and zein at the end of the experiment than that of the control fruits, which suggested a more advanced ripening stage in the control fruit than coated tomatoes.

Effect on Ascorbic Acid

Ascorbic acid is one of the most important nutritional quality factors, which are present in plant tissues undergoing active growth and development. Ascorbic acid is easily oxidized, especially in aqueous solutions, and greatly favoured by the presence of oxygen and the losses are enhanced by extended storage, higher temperature, low relative humidity, physical damage and chilling injury (Lee and Kader, 2000). The results of the current study represents the constancy of the decline in ascorbic acid content from 363.3 (0 day) to 70 µg g^{-1} at the end of storage period (Table 2), which could be related to its oxidation. The loss of vitamin C represents the conversion of dehydroascorbic acid to diketogulonic acid (Oms-Oliu *et al*., 2008). However, the T4 treatment was able to maintain the higher level of ascorbic acid in grapes throughout the storage and exhibited the highest level of it (134.2 µg g^{-1}) on the 24^{th} day probably due to the incorporation of ascorbic acid in the composite coating. On the contrary, the control fruit showed a higher reduction in the ascorbic acid content (139.1 µg g^{-1} on the 6^{th} day and 87.80 µg g^{-1} on the 12^{th} day of storage) as compared to treated grapes indicating the great loss of ascorbic acid. These findings suggest that the edible coating used in the present study helped in retaining the ascorbic acid content in grapes. These results are in agreement with those of Tapia *et al*. (2008) who found that the addition of ascorbic acid as antioxidant in coating material led to a better preservation of the natural ascorbic acid content in fresh-cut papaya, maintaining its nutritional quality. Wang *et al.* (2013) also found the reduced decrease of ascorbic acid in strawberries using chitosan treatments. In this regards, Ayranci and Tunc (2003) stated that the tightly packed network structure of film or coating exhibits limited oxygen permeability which has positive effects on the preservation of the quality and as the reduced oxygen availability in the coated product could reduce the oxidation of ascorbic acid. These authors found that a methyl cellulose-based edible coating containing ascorbic acid and citric acid reduced vitamin C loss of apricots and green peppers.

Effect on Total Phenols

Grape is a phenol-rich plant, and these phenolics are mainly distributed in the skin, stem, leaf and seed of grape, rather than their juicy middle sections (Pastrana -Bonilla *et al.,* 2003; Xia *et al.,* 2010). Recently, growing interests on phenolic compounds from grapes have focused on their biological activities linking to human health benefits, such as antioxidant, cardioprotective, anticancer, antiinflammation, antiaging and antimicrobial properties (Xia *et al.,* 2010). Increasing trends were observed in the phenol contents of both the treated and untreated grapes up to 18 days of storage, thereafter a gradual decrease occurred until the end of storage period. The least amount of total phenol content was observed in control fruits, whereas the T2 (xanthan gum + olive oil + gallic acid) coated grapes exhibited an acceleration of phenol accumulation on the 6th day (0.728 mg g^{-1}) and 12th day (0.878 mg g^{-1}) of storage period and reached the peak on the 18th day (1.33 mg g^{-1}) of storage, representing enhanced nutritional quality of grapes (Table 2). However, results of the current study suggest that higher levels of phenolics were observed in the coated grapes as compared with that of uncoated grapes (0.328, 0.344, and 0.460 mg g^{-1} on 0, 6th and 12th days respectively). Sanchez-Gonzalez *et al.,* (2011) also found in their study that at the end of the storage, the phenol level was highly declined in the control grapes in comparison with the grapes treated with hydroxypropyl methylcellulose (HPMC) and chitosan. At the end of the present experiment, the grapes treated with T3 showed a high content of phenolics (0.627 mg g^{-1}) compared to that of T1 and T4. The decrease of phenolic compounds at the end of storage might be due to breakdown of cell structure because of the senescence phenomena during storage (Macheix *et al*., 1990). In the present study, the enhancement and retention of the phenolics in coated grapes represents the beneficial effects of xanthan gum enriched with additives. The results of the present study are supported by the findings of Simoes *et al.* (2009) who reported the enhanced phenolic content in carrot sticks using combined application of edible coating containing chitosan and moderate O_2 and CO_2 levels.

Effect on Total Antioxidant Activity

As shown in Table 2, the total antioxidant activity was found to be enhanced in the treated fruits as compared to that of untreated fruits. On the 6th day of storage, the total antioxidant activity was notably higher in the fruits treated with T4 (xanthan gum + olive oil + ascorbic acid) (6.992 mg g^{-1}) and with T2 (xanthan gum + olive oil + Gallic acid (6.258 mg g^{-1}). On the contrary, reduced antioxidant activity (2.017 mg g^{-1}) was noticed in the control group and it declined further on the 12th day of storage (0.345 mg g^{-1}). At the end of the storage, the T4 and T3 treated fruits showed the highest antioxidant activity of 3.005 and 2.424 mg g^{-1}, respectively indicating the efficacy of coating (enriched with antioxidants) in enhancing fruit quality by means of antioxidant activity. Thus, the results of the present study are in accordance with the results of Oms-Oliu *et al.* (2008) who observed the enhanced antioxidant capacity in fresh-cut pears by using polysaccharide-based edible coatings incorporated with antioxidants. According to Davila-Avina *et al.* (2012), most post-harvest treatments involve altering the natural conditions of the fruit to prolong post-harvest life. Davila-Avina *et al.* (2012) suggested that the activation of the antioxidant system is a response to post-harvest stress which can be considered as a helpful response that improves the antioxidant status of tropical fruits.

Table 2. Changes in ascorbic acid, total phenols and total antioxidant activity of grapes during their storage at 10±2°C.[a]

	Day 0	Day 6	Day 12	Day 18	Day 24
Treatments[b]	Ascorbic acid (µg g^{-1})				
T1	369.3 ± 14.4e	168.9 ± 12.7c	112.0 ± 3.10b	97.60 ± 2.70a	70.00 ± 4.70a
T2	369.3 ± 14.4e	234.9 ± 16.2e	136.0 ± 16.2c	109.3 ± 3.10b	-
T3	369.3 ± 14.4e	144.0 ± 4.80b	156.0 ± 4.80d	126.0 ± 2.00c	83.30 ± 2.90b
T4	369.3 ± 14.4e	226.7 ± 23.1d	190.4 ± 3.70e	168.0 ± 4.20d	134.2 ± 2.30c
T5	369.3 ± 14.4e	139.1 ± 5.70a	87.80 ± 5.10a	-	-
	Total phenols (mg g^{-1})				
T1	0.329 ± 0.040a	0.436 ± 0.068c	0.560 ± 0.068c	0.693 ± 0.105a	0.234 ± 0.023a
T2	0.329 ± 0.040a	0.728 ± 0.080e	0.878 ± 0.018e	1.333 ± 0.090d	-
T3	0.329 ± 0.040a	0.432 ± 0.046b	0.515 ± 0.021b	0.633 ± 0.062b	0.627 ± 0.020c
T4	0.329 ± 0.040a	0.525 ± 0.038d	0.821 ± 0.010d	0.727 ± 0.079c	0.414 ± 0.028b
T5	0.329 ± 0.040a	0.344 ± 0.018a	0.460 ± 0.035a	-	-
	Total antioxidant activity (mg g^{-1})				
T1	0.997 ± 0.033a	2.671 ± 0.014c	1.537 ± 0.041c	2.339 ± 0.026c	3.005 ± 0.014c
T2	0.997 ± 0.033a	6.258 ± 0.099d	2.572 ± 0.049e	1.520 ± 0.045a	-
T3	0.997 ± 0.033a	2.318 ± 0.030b	1.216 ± 0.030b	1.975 ± 0.031b	2.424 ± 0.032b
T4	0.997 ± 0.033a	6.992 ± 0.035e	2.529 ± 0.061d	2.849 ± 0.068d	2.315 ± 0.191a
T5	0.997 ± 0.033a	2.017 ± 0.017a	0.345 ± 0.019a	-	-

[a] Different letters in the column means significantly different at $P \leq 0.05$. [b] T1: (Xanthan gum 0.3%+olive oil 0.1%); T2: (Xanthan gum 0.3%+olive oil 0.1%+gallic acid 0.1%), T3: (Xanthan gum 0.3%+olive oil 0.1%+ferulic acid 0.1%); T4: (Xanthan gum 0.3%+olive oil 0.1%+ascorbic acid 0.1%), and T5: (Control).

PolyGalacturonase (PG) and Pectate Lyase (PL) Activity

Softening is an important part of the ripening process in most fruit and it is widely recognized that changes in cell walls accompany fruit softening. During the ripening process, the progressive loss of firmness is the result of a gradual transformation of protopectin into pectin which is degraded by the enzyme, polygalacturonase, in the cell wall (Hobson *et al*., 1971).

During the course of the current study, a progressive increase in PG activity was observed in all treated as well as control grapes. The data presented in Figure 2-a reveals that the delayed PG activity occurs in the T1 coated grapes (0.052 U mg^{-1} proteins) on the 12th day of storage and in T3 coated grapes (0.074 U mg^{-1} proteins) on the 18th day of storage. On the contrary, enhanced activity was observed in the control grapes both on the 6th day (0.063 U mg^{-1} proteins) and on the 12th day (0.070 U mg^{-1} proteins) as compared to that of treated grapes. However, the grapes treated with T3 showed the lowest PG activity (0.087 U mg^{-1} proteins) in comparison with that of the other treatments. A similar kind of delayed increase in PG activity was reported by Gol *et al*. (2013) in strawberry fruit treated with carboxymethyl cellulose, hydroxypropylmethylcellulose and their combination with chitosan. Further, Zhou *et al*. (2011) reported that the relatively lower activity of PG in the shellac-coated pears contributed to the enhanced retention of firmness during their storage.

Pectate Lyase (PL) catalyses the cleavage of de-esterified or esterified galacturonate units by a trans *β*-elimination of hydrogen from the C-4 and C-5 positions of galacturonic acid. The dramatic changes in the pectin contents can be attributed to the

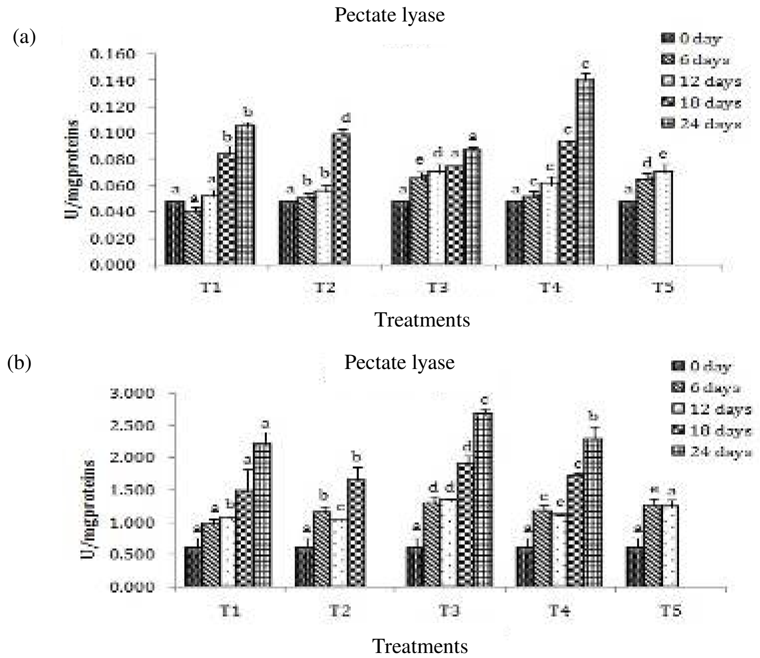

Figure 2. Effect of composite edible coatings on the specific activity of (a) PG and (b) PL in grapes during storage at 10±2°C. [T1: (Xanthan gum 0.3%+olive oil 0.1%); T2: (Xanthan gum 0.3%+olive oil 0.1%+gallic acid 0.1%), T3: (Xanthan gum 0.3%+olive oil 0.1%+ferulic acid 0.1%); T4: (Xanthan gum 0.3%+olive oil 0.1%+ascorbic acid 0.1%), and T5: (Control). Different letters on the bars mean significantly different at P≤ 0.05].

fact that pectin is most subject to enzymatic changes and shows the highest water solubility among these polysaccharides during ripening and storage (Fraeye *et al*., 2007). As explained by Conforti and Zinck (2002), the increased senescence most likely speeds up the metabolic process which in turn may increase the activity level of the endogenous pectin-degrading enzymes. Data presented in the Figure 2-b reveal that the pectate lyase activity had increased throughout the storage period in both the treated and untreated grapes. However, delayed activity of PL was observed in all treated fruits as compared with that of the control grapes. PL enzyme activity was found to be reduced in all treatments than that of the control set. However, the lower activity of PL in T1 treated fruits was due to the effect of xanthan gum + olive oil, whereas in other treatments addition of antioxidant did not show as much reduction in PL activity as in T1 treated fruits. The lowest activity of PL was detected in the T1 treated fruits on the 6th day (0.959 U mg^{-1} protein), 18th day (1.477 U mg^{-1} protein) and 2.203 U mg^{-1} protein at the end of the storage. The interpretation given by Yaman and Bayoundurh (2002) supports the results of the present study. According to these authors, low oxygen and high carbon dioxide concentrations reduce the activity of enzymes and allows retention of the firmness of fruits during storage.

Effect on PPO Activity

Figure 3 represents the data regarding changes in PPO activity of treated and untreated grapes during their storage. The PPO activity increased initially but subsequently a slight decline in activity of PPO was noticed in all fruits. However, on

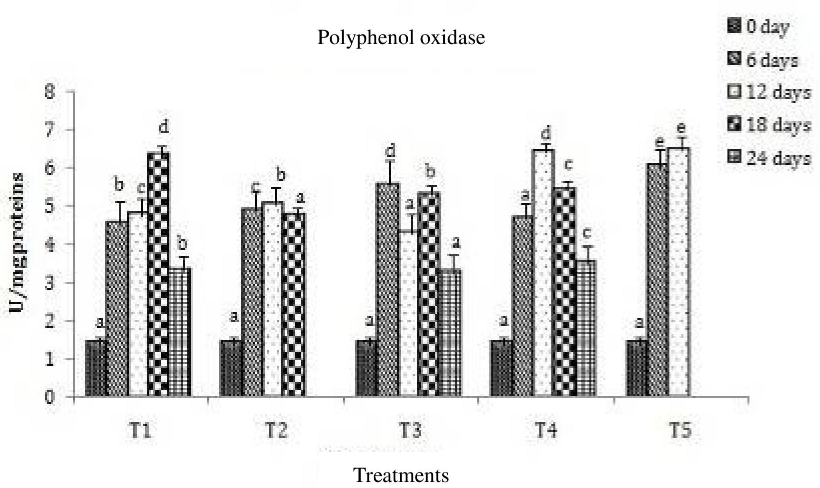

Figure 3. Effect of composite edible coatings on the specific activity of PPO in grapes during storage at 10±2°C. [T1: (Xanthan gum 0.3%+olive oil 0.1%); T2: (Xanthan gum 0.3%+olive oil 0.1%+gallic acid 0.1%), T3: (Xanthan gum 0.3%+olive oil 0.1%+ferulic acid 0.1%); T4: (Xanthan gum 0.3%+olive oil 0.1%+ascorbic acid 0.1%), and T5: (Control). Different letters on the bars mean significantly different at P≤ 0.05].

the 12^{th} day of storage, a lower activity of PPO was noticed in T3 treated grapes (4.25 U mg^{-1} proteins) followed by T1 (4.73 U mg^{-1} proteins) and T2 (4.99 U mg^{-1} proteins), whereas a higher activity was seen in control grapes (6.42 U mg^{-1} proteins). According to Ghasemnezhad *et al.* (2013), a lower activity can be interpreted as the inhibition of enzymatic browning and these authors found a lower activity of PPO enzyme in chitosan-treated arils than that of the control during their storage. Meng *et al.* (2008) stated that the browning of grapes was related to the action of polyphenol oxidase (PPO). Therefore, in the present study, the delayed PPO activity in treated grapes indicates the reduced browning of grapes. Previous studies have demonstrated the efficacy of chitosan-glucose coatings in inhibiting the PPO-mediated oxidation of phenols responsible to form melanin-like pigments, thus preventing the formation of a brown undesirable appearance and improving the visual appearance and color (Liu *et al*., 2007)

CONCLUSIONS

The present study indicated that grapes coated with a composite coating of xanthan gum and olive oil incorporated with antioxidants had prolonged the shelf life with better quality than that of the control fruit. Delayed increase in weight loss percentage, TSS and total sugars suggest that the xanthan gum, as a preservative material, was able to delay the ripening process by slowing down the respiration and metabolic rate in grapes. Moreover, xanthan gum enriched with antioxidants not only extended the storage life but also enhanced the antioxidant activity during their storage and delayed the browning and softening process in grapes. The best effect on quality improvement was achieved with the treatment of xanthan gum + olive oil incorporated with gallic acid. Therefore, the composite coating of xanthan gum and olive oil enriched with antioxidants is promising

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as a composite edible coating to be used to enhance the shelf life and quality of grapes.

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پوشش کامپوزیت حامل آنتی اکسیدان ها عمر مفید و کیفیت پس از برداشت انگور بی هسته تامپسون را بهبود می بخشد

ن. س. بارایا، ت. و. رامانا راو، و و. ر. تاکار

چکیده

پوشش کامپوزیت خوراکی شامل صمغ زانتان (۰.۳٪) و روغن زیتون (۰.۱٪) غنی شده با آنتی اکسیدان ها (اسید گالیک ۰.۱٪، فلوریک اسید ۰.۱٪ و اسید اسکوربیک ۰.۱٪) قابلیت نگهداری پس از برداشت و کیفیت غذایی انگور را افزایش داد. ویژگی های کیفی انگور در طول ذخیره سازی در دمای 2 ±۱۰ درجه سانتی گراد (رطوبت نسبی 75-70٪)، در فواصل منظم از ۶ روز تا 24روز ذخیره سازی تحت نظر قرار گرفتند. صمغ زانتان در ترکیب با روغن زیتون کاهش وزن، وقوع پوسیدگی، تجمع مواد جامد محلول و قند کل را با کاهش میزان تنفس و سوخت و ساز در میوه پوشش داده شده کاهش داد . علاوه بر این، اختلاط آنتی اکسیدان ها در پوشش، موجب افزایش سطح فنولیک، اسید آسکوربیک و فعالیت آنتی اکسیدانی کل در انگور گردید .فعالیت آنزیم های تغییردهنده دیواره سلولی مانند گالاکتوروناز (PG)وپکتات لیاز (PL)در میوه ها ی پوشش داده شده نسبت به نمونه شاهد کمتر بود .این نتایج نشان می دهد که پوشش کامپوزیتی باعث تاخیر در فرآیند رسیدن و نرم شدن در انگور و در نتیجه افزایش عمر نگهداری به ۲۴ روز گردید، در حالی که انگور شاهد در روز دوازدهم پوسیده شد.

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