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Use of *Aloe vera* Gel Coating Preserves the Functional Properties of Table Grapes

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Table grapes (*Vitis vinifera* L. cv. Crimson Seedless) were coated with *Aloe vera* gel according to our developed patent (SP Patent P200302937) and then stored for 35 days at 1 °C, and the subsequent shelf life (SL) was monitored at 20 °C. Uncoated clusters showed a rapid loss of functional compounds, such as total phenolics and ascorbic acid. These changes were accompanied by reduction of the total antioxidant activity (TAA) and increases in total anthocyanins, showing an accelerated ripening process. On the contrary, table grapes coated with *Aloe vera* gel significantly delayed the above changes, such as the retention of ascorbic acid during cold storage or SL. Consequently, *Aloe vera* gel coating, a simple and noncontaminating treatment, maintained the functional properties during postharvest storage of table grapes.

KEYWORDS: Anthocyanins; ascorbic acid; edible coating; phenolics; total antioxidant activity

INTRODUCTION

Polyphenols are the most important constituents of grapes related to nutrition and functional properties, in which catechins, flavonol glycosides, phenolic acids, stilbenes, and anthocyanins are present (*1*). All these compounds have been shown to exert antioxidant activity in vitro (*2*), and then beneficial effects on health have been claimed following the consumption of table grapes and other fruits (*3*). Thus, phenolics in grapes have been reported to inhibit lipid peroxidation of low-density lipoproteins and prevention heart and cancer diseases, among others (*4*). The recovery of polyphenols from grape products and their possible use as nutraceuticals have been recently reported (*5*, *6*). Some cultivars of table grapes contain high anthocyanin content, which is responsible for the red, violet, purple, and blue color of their skins (*7*). However, changes in anthocyanin profiles and loss of other phenolic compounds have been reported during advanced stages of ripening (*8*). In this sense, the ripening process in table grapes leads to a reduction in their functional properties, and then consumers may not achieve the benefits of the intake of these fruits.

For centuries, *Aloe vera* has been used for its medicinal and therapeutic properties (*9*). The two major liquid sources of *Aloe vera* are a yellow latex (exudate) and clear gel (mucilage), which proceeds from the large leaf parenchymatic cells (*10*). The oral ingestion of the gel juice has been shown to be effective against ulcerous, gastrointestinal, kidney, and cardiovascular problems and has also been used to reduce the cholesterol and triglyceride levels in blood. In addition, properties such as antiinflamatory, antibiotic, and against some diseases (diabetes, cancer, allergy, AIDS) have been reported (*9*, *11*). However, the main use of *Aloe vera* gel is in the cosmetic industry including treatment of burns and scars and wound healing (*12*).

Edible coatings are traditionally used to improve food appearance and conservation due to their environmentally friendly nature, since they are obtained from both animal and vegetable agricultural products (*13*). Generally, coatings can be divided according to their nature into protein, lipid, and polysaccharide based, alone or in combination. They act as barriers to moisture and oxygen during processing, handling, and storage, and not only solely retard food deterioration, but also improve safety, due to their natural biocide activity or to the incorporation of antimicrobial compounds, allowing edible coatings to be widely used (*14*). Other advantages of the use of edible coatings could be the reduction of packaging waste, since they are considered as biodegradable, and the development of new products. In table grapes, there are few evidences of the use of edible coatings, but chitosan was found to be effective to control storage gray mold, thus reducing decay and prolonging storage (*15*).

There is a recent increased interest in *Aloe vera* gel in the food industry for use as a functional ingredient food in drinks, beverages, and ice creams (*16*). In recent papers, we have demonstrated that *Aloe vera* gel was able to prolong the shelf life (SL) of sweet cherry and table grapes through a delay in the parameters related to deterioration from the point of view of quality and safety (*17*, *18*). The aim of this work was to study the effects of coated table grapes with *Aloe vera* on their functional properties during postharvest cold storage and

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subsequent SL and then to get a better knowledge about the effects of *Aloe vera* on maintenance of the beneficial compounds for human health of fruits and vegetables.

MATERIAL AND METHODS

Plant Material and Experimental Design. Table grapes (*Vitis vinifera* L. cv. "Crimson Seedless") were harvested from a commercial farm in southern Spain. At the laboratory, clusters were selected to obtain homogeneous batches based on color, size, absence of injuries, and healthy greenish rachises. Clusters were cut to obtain 120 samples ranging from 150 to 170 g. Half of them were treated with *Aloe vera* L. gel (pharmaceutical quality, 100% purity) manufactured by Roig Farma S.A. (Tarrasa, Barcelona, Spain). Treatment was performed by immersion for 5 min with a solution of *Aloe vera* gel diluted 1:3 with distilled water, according to previous experiments (*17*). The other half were immersed in distilled water and served as the control. Following treatment, all clusters were air-dried for 30 min before storage at 1 °C and 95% relative humidity (RH) in permanent darkness for 35 days. Ten samples of both treated and control clusters were taken after 0, 7, 14, 21, 28, and 35 days; half of them were immediately analyzed (cold storage), and the remainder were transferred to a chamber under controlled conditions at 20 °C and 90% RH and analyzed after 4 days, to simulate market operations (SL). From each cluster, 30 berries were taken, and then skin and pulp were obtained, frozen in liquid N_2, and milled to obtain homogeneous samples for analytical determinations.

The visualization of both clusters and grapes after 21 days of cold storage plus 4 days at 20 °C showed that control clusters were unmarketable, with a high incidence of decay and accelerated quality deterioration. For this reason, the predicted sampling schedule of SL was stopped at this moment. However, visual aspect of *Aloe vera*-treated clusters was monitored until the end of the experiment.

Total Antioxidant Activity, Phenolic Compounds, and Ascorbic Acid. For each sample, 5 g of table grape tissue (skin or pulp) was homogenized in 10 mL of 50 mM phosphate buffer pH = 7.8 and then centrifuged at 15 000 rpm for 15 min at 4 °C. The supernatant was used for total antioxidant activity (TAA) and total phenolic compounds quantification in duplicate, as previously described (*19*). For TAA, L-ascorbic acid was used for the calibration curve, and the results were expressed as mg ascorbic acid equivalent 100 g^{-1} fw. The total phenolic compounds were expressed as mg gallic acid equivalent 100 g^{-1} fw and were the mean of determinations made in duplicate in each one of the five samples. Ascorbic acid was measured in the pulp by HPLC analysis as previously described (*19*), and the results in duplicate were expressed as mg ascorbic acid 100 g^{-1} fw.

Total Anthocyanins. The method described by García-Viguera et al. (*20*) was adapted to table grape tissue. Two grams of fruit tissue was homogenized in 4 mL of methanol and left 1 h at −18 °C. Extracts were centrifuged at 15 000 rpm for 15 min at 4 °C. The supernatant was loaded onto a C18 Sep-Pak cartridge, previously conditioned with 5 mL of methanol, 5 mL of pure water, and then with 5 mL of 0.01 N HCl. The cartridge was washed with 5 mL of pure water and then eluted with acidified MeOH (0.01% HCl). The absorbance of the collected fraction was measured at 530 nm. Total anthocyanin was calculated using cyanidin-3-glucoside (molar absorption coefficient of 23 900 L cm^{-1} mol^{-1} and molecular weight of 449.2 g mol^{-1}), and results were expressed as mg 100 g^{-1} fw and were the mean of determinations made in duplicate in each one of the five samples.

Statistical Analysis. Data for the analytical determinations were subjected to analysis of variance (ANOVA). Sources of variation were storage and treatment. Mean comparisons were performed using HSD the Tukey's test to examine if differences were significant at $p < 0.05$. To know the compounds that contribute to TAA, linear regressions were performed among the functional parameters for all sampling data during cold storage and SL of control grapes. All analyses were performed with the SPSS software package version 11.0 for Windows (*21*).

RESULTS

Total Antioxidant Activity (TAA). The day of harvesting, TAA in the skin was 396.8 ± 21.1 mg equiv ascorbic acid 100 g^{-1} (**Figure 1**). During cold storage, a prolonged and significant decrease was observed for control berries reaching levels of 179.7 ± 9.7 mg equiv ascorbic acid 100 g^{-1} after 35 days. On the contrary, TAA in *Aloe vera*-treated grapes remained unchanged during 28 days of cold storage, and a slight diminution was detected at day 35, the values being significantly higher than those obtained in control fruits (316.8 ± 20.2 mg equiv ascorbic acid 100 g^{-1}). When table grapes were transferred 4 days at 20 °C (SL), the controls had lost approximately 50% of their initial TAA after 21 days at 1 °C + SL (211.8 ± 15.1 mg equiv ascorbic acid 100 g^{-1}), while this reduction was approximately 20% in *Aloe vera*-treated grapes (315.6 ± 23.4 mg equiv ascorbic acid 100 g^{-1}).

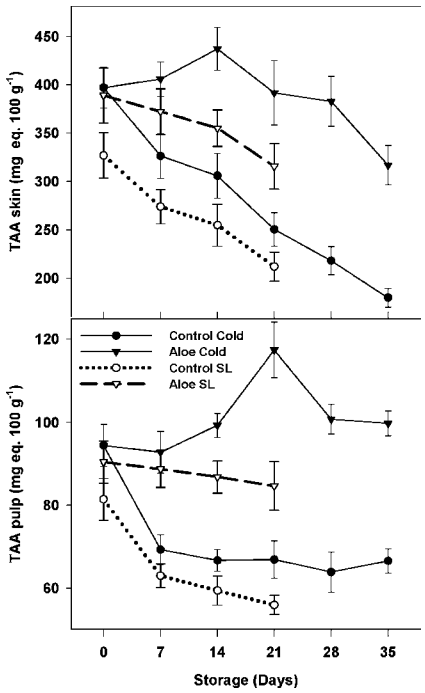

Figure 1. TAA of the skin and pulp during cold storage (1 °C) or after 4 days at 20 °C (SL) of control and *Aloe vera*-coated table grape clusters.

The measurement of TAA in the pulp revealed a similar behavior (**Figure 1**). There was a significant reduction of TAA in control fruits during cold storage, which was more pronounced during periods of SL. Interestingly, TAA in *Aloe vera*-treated grapes experienced a significant increase from the levels at harvest (94.4 ± 5.1 to 117 ± 7 mg equiv ascorbic acid 100 g^{-1} fw) at day 21 of cold storage followed by a slight decrease. During the SL periods, any significant reduction was observed in treated berries, since values of 84.6 ± 5.8 mg equiv ascorbic acid 100 g^{-1} were shown after 21 days at 1 °C + SL. However, the TAA capacity was 4-fold lower in the pulp than in the skin.

Total Phenolics. Skin total phenolics at harvest were 53.6 ± 3.1 mg equiv gallic acid 100 g^{-1} (**Figure 2**), which showed a pronounced decrease in control grapes throughout cold storage, reaching minimum levels at day 35 (7.0 ± 4.0 mg equiv gallic acid 100 g^{-1}). This loss of total phenolics was even greater during the SL periods, the concentration of total phenolics being 8.6 ± 0.6 mg equiv gallic acid 100 g^{-1} after 21 days at 1 °C plus 4 days at 20 °C. The treatment with *Aloe vera* gel led to maintenance of total phenolics during the first 14 days at 1 °C, showing a slight decrease from this time until the end of the cold storage, with levels significantly higher (40.7 ± 1.2 mg equiv gallic acid 100 g^{-1}) than those obtained in control berries. Similarly, the loss of total phenolics was reduced in *Aloe vera*-

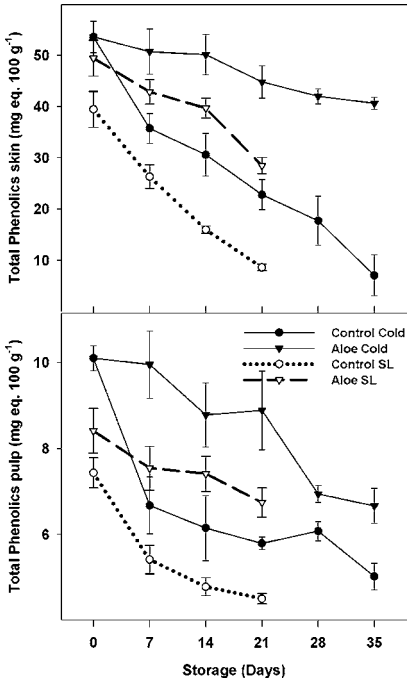

Figure 2. Total phenolics content of skin and pulp during cold storage (1 °C) or after 4 days at 20 °C (SL) of control and *Aloe vera*-coated table grape clusters.

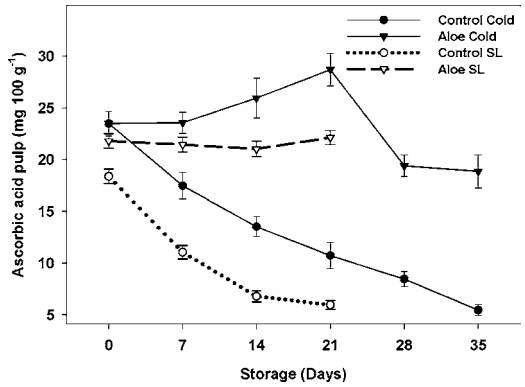

Figure 3. Ascorbic acid content of the pulp during cold storage (1 °C) or after 4 days at 20 °C (SL) of control and *Aloe vera*-coated table grape clusters.

treated grapes compared to that of controls during SL, with values of 28.4 ± 1.6 mg equiv gallic acid 100 g^{-1} at the last SL period.

The same effect was observed when total phenolics were analyzed in the pulp (**Figure 2**), although the concentrations of phenolic compounds were 5-fold lower than in the skin, similar to that observed for TAA. Control grapes exhibited a significant reduction during cold storage from the initial values (10.1 ± 0.3 mg equiv gallic acid 100 g^{-1}), showing a sharp decrease in just 7 days of storage (6.7 ± 0.7 mg equiv gallic acid 100 g^{-1}), which decreased slowly throughout storage. On the contrary, total phenolics in *Aloe vera*-treated grapes remained significantly unchanged during 21 days at 1 °C, with a slight diminution observed at day 35. During SL values were also significantly higher than those obtained in control fruits, with levels of 6.7 ± 0.3 mg equiv gallic acid 100 g^{-1} after 21 days at 1 °C plus 4 days at 20 °C.

Ascorbic Acid. The levels of ascorbic acid in the pulp at harvest were 23.5 ± 1.2 mg 100 g^{-1} (**Figure 3**). This concentration was significantly reduced in control berries

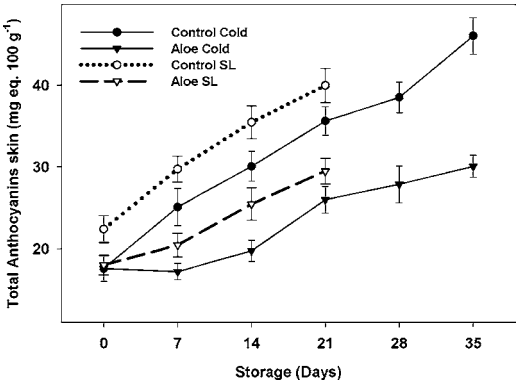

Figure 4. Total anthocyanins content of the skin during cold storage (1 °C) or after 4 days at 20 °C (SL) of control and *Aloe vera*-coated table grape clusters.

throughout storage reaching the lowest levels of 5.5 ± 0.5 mg 100 g^{-1} after 35 days. The loss of ascorbic acid was greater during the SL periods, the concentration being 5.9 ± 0.4 mg 100 g^{-1} after 21 days at 1 °C plus 4 days at 20 °C. Contrarily, *Aloe vera*-treated grapes showed a significant increase (28.7 ± 1.6 mg 100 g^{-1} fw) at day 21 of cold storage followed by a slight decrease. During the SL periods, any significant changes were observed in treated berries, since values of 22.1 ± 0.7 mg ascorbic acid 100 g^{-1} fw were shown after 21 days at 1 °C + SL.

Total Anthocyanin. "Crimson Seedless" table grapes are characterized by noncolored pulp and a light red color of the skin, showing a concentration of total anthocyanins at harvest in the skin of 17.6 ± 1.6 mg equiv cyanidin-3-glucoside 100 g^{-1} fw (**Figure 4**). As storage advanced, the levels of anthocyanin increased in both control and treated berries, the increase being significantly higher in the control than in the *Aloe vera*-treated grapes. Thus, after 35 days of cold storage the concentration of anthocyanins was 46.1 ± 2.2 and 30.1 ± 1.3 mg equiv cyanidin-3-glucoside 100 g^{-1} fw for control and treated berries, respectively. The accumulation of anthocyanins in the skin was enhanced during the SL periods, but levels were also higher in control than in treated grapes.

DISCUSSION

The compounds responsible for the beneficial properties for human health of fruit consumption are those with antioxidant activity, including carotenoids, ascorbic acid, flavonoids, and phenolic compounds such as anthocyanins (*3*, *5*). As we report in this paper, "Crimson Seedless" table grapes are rich in these compounds. For this cultivar, a rapid deterioration of organoleptic quality during postharvest storage was reported (*17*). This loss of quality was accompanied by a reduction of the functional properties, since either during cold storage or especially during the SL periods, significant losses of both total phenolics (both in skin and pulp) and ascorbic acid (pulp) occurred in control grapes (**Figures 2** and **3**). The reduction of these compounds was correlated to a decrease in TAA in both skin and pulp tissues (**Figure 1**). In addition, the higher anthocyanin accumulation in control berries (**Figure 4**) could be related to an advanced ripening process, as has been postulated in other grape cultivars (*22*). For this cultivar, only two anthocyanins have been found as predominant: cyanidin-3-glucoside and peonidin-3-glucoside (unpublished data).

In previous works we have found that grapes and sweet cherries coated with *Aloe vera* gel showed retardation of the ripening process by delayed evolution of the parameters related

to organoleptic quality, as well as by a reduction of fruit decay, with high acceptance from the sensory panel (*17*, *18*). In addition, the application of *Aloe vera* gel as an edible coating in table grapes imparted beneficial effects in terms of maintenance of TAA, for both skin and pulp, either during cold storage or after the periods of SL. It has been reported that the oral ingestion of *Aloe vera* was highly effective as an antiinflammatory due to its antioxidant properties (*23*). A large number of chemical compounds have been reported in the composition of *Aloe vera* gel (*10*, *11*), but it is thought that aloe-emodin, an anthraquinone derivative, is one of the main components that contributes to antioxidant activity, with an antioxidant capacity of 78% reported compared to the 96% value of the synthetic butylated-hydroxy anisole (*24*). Moreover, it has been recently postulated that extracts from *Aloe vera* have greater antioxidant activity than butylated-hydroxy toluene or α-tocopherol (*25*) and thus are highly effective in protecting against DNA damage (*26*) and render possible the use of aloe-emodin and aloin as anticarcinogenetics (*27*). In addition, the plant *Aloe vera* showed greater antioxidant activity as it advanced its developmental stage, the maximum antioxidant activity being reached in plants over 3 years old (*28*). In this sense, the higher TAA observed in *Aloe vera*-treated grapes as well as its maintenance during prolonged storage could be attributed to the presence of these compounds. The study of the TAA in extracts from *Aloe vera* leaf indicated that these compounds had a polar nature (*25*), although the exact composition is still unknown and deserves further investigation.

The evolution of total phenolics of fruits during storage could be different depending on the species cultivar, temperature, and climactic and environmental conditions during the growth period (*5*). Thus, total phenolics remained unchanged during storage of strawberry at 20 °C, while increases were observed for raspberry (*29*). In "Crimson Seedless" table grapes, total phenolics decreased during both cold storage and SL, but *Aloe vera* treatment was able to delay the loss of phenolic compounds during storage in both skin and pulp. For this cultivar (*30*), the analysis of phenolic compounds revealed that the main ones were anthocyanins (52%) and flavaan-3-ols (31%), followed by flavonols (10%) and hydroxycinnamates (7%). However, no information is available about total phenolic evolution during storage of table grapes. With respect to ascorbic acid, table grapes coated with *Aloe vera* gel maintained vitamin C content during the whole storage period, independent of the temperature. This retention of ascorbic acid has been observed in apricots and peppers coated with methylcellulose or polyethyleneglycol (*31*), but ascorbic acid was added in the formulation, while in the coating with *Aloe vera* the addition of exogenous vitamin C was not necessary.

TAA has been correlated to phenolic content in nectarine, peach, and plum (*32*) and small fruits such as blackberry, raspberry, and strawberry (*33*). In other table grape cultivars, TAA has been correlated to phenolic compounds too (*34*), these polyphenols being postulated as natural antioxidants in foods (*2*, *35*). However, in several cultivars of citrus, TAA was more correlated to ascorbic acid (*36*). Taking into account data for control grapes during both cold storage and SL, the results show that in "Crimson Seedless" table grapes, TAA of the skin was positively correlated to total phenolics and negatively correlated to total anthocyanins (**Figure 5**), while in the pulp TAA was correlated to both total phenolics and ascorbic acid (**Figure 6**). Thus, TAA of the skin was due to the presence of total phenolics, while in the pulp both phenolic compounds and ascorbic acid accounted for the TAA.

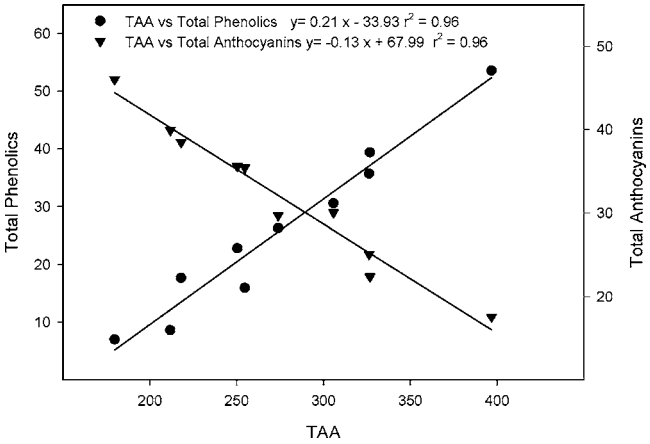

Figure 5. Correlation between TAA and total phenolics or total anthocyanins in the skin of "Crimson Seedless" table grapes.

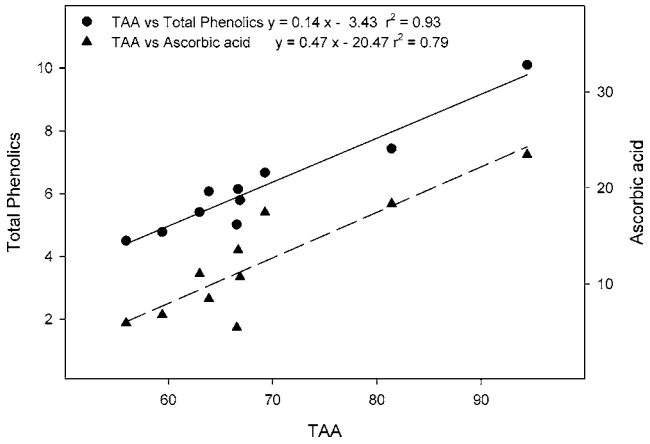

Figure 6. Correlation between TAA and total phenolics or ascorbic acid in the pulp of "Crimson Seedless" table grapes.

The overall results show that *Aloe vera* gel coating is an effective postharvest technology to maintain the functional properties of table grapes, since maintenance of total phenolics and ascorbic acid was obtained during storage with a higher retention of TAA. The loss of these compounds was clear in control table grapes, which developed a more advanced stage of ripening revealed by the increase in anthocyanin content. As far as we know, this is the first paper in which an edible coating based on *Aloe vera* gel was able to maintain the functional properties of table grapes during cold storage and subsequent SL.

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