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Aloe vera extract as a promising treatment for the quality maintenance of minimally-processed table grapes

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Abstract

The effect of an edible film obtained from a commercial *Aloe vera* extract, on the quality maintenance of minimally processed grapes belonging to three different cultivars (Sugar One, Victoria and Black Magic) was evaluated by enzymatic (PPO, PME, β-GAL), physicochemical (pH, acidity, °Brix), and sensorial methods. All the analyzed parameters were measured in extracts obtained from minimally processed grapes packaged in ordinary atmosphere and stored at 4 °C for 15 days. Samples dipped into *Aloe vera* showed significant differences ($p \leq 0.05$) compared to untreated ones. The determination of such parameters and the evaluation of consumer acceptability were helpful to determine the effectiveness of the post-harvest treatment with *Aloe vera* for a storage period of 15 days.

Keywords: *Aloe vera*; packaging; PPO; shelf life; table grape.

Practical Application: The use of *Aloe vera* as edible film for the treatment of minimally processed fruits could be an alternative to the use of chemical, also bearing an increase of the produce nutritional properties.

1 Introduction

Minimally processed fruits and vegetables are a category of foods that is subjected to few operations to get them ready for consumption. In the case of minimally processed table grapes, the fundamental processes required are washing, separation from the peduncle and packaging in trays or plastic bags. Minimally processed grape represents a promising product for distribution by vending machines, in school canteens and hospital, on airplanes or for household consumption, as an alternative to the current snacks. Indeed, this product fully meets the current market trends that require more and more high-quality products, rich from the nutritional point of view, containing only natural ingredients and minimally processed. However, minimally processed products are highly perishable, in fact, the main changes are caused by enzymatic activities (Phenylalanine ammonia-lyase (E.C. 4.3.1.5, PAL); Polyphenol oxidase (EC 1.14.18.1, PPO); β-galactosidase (EC 3.2.1.23, β-GAL), Pectin methylesterase (EC 3.1.1.11, PME)) that cause browning and the loss of consistency of the berries, decay of the sensory and nutritional properties and microbiological alterations (*Botrytis cinerea*). Enzymatic reactions are those mostly responsible for the loss of quality: specifically, cutting of the peduncle and physical damage determine the activation of PAL, which catalyses the transformation of phenylalanine into trans cinnamic acid which, in subsequent reactions, is transformed into other phenolic compounds such as chlorogenic acid, substrate of PPO. The latter enzyme oxidizes compounds synthesized by PAL to quinones which spontaneously polymerize, giving the molecules responsible for the enzymatic browning (Ke & Saltveit, 1989). Pectic enzymes take part in the softening of tissues of vegetable products. PME catalyzes the de-esterification of pectines, hydrolyzing the methoxyl groups and producing pectic and pectinic acids with high specificity for the methoxyl groups close to the non-reducing side of the polygalaturonic chain. Oxidative and pectic enzymes play a key role in the loss of freshness of minimally processed products (Mencarelli et al., 1989; Abe & Watada, 1991; O'Connor-Shaw et al., 1994; Paull & Chen, 1997). Many studies have been carried out to evaluate the effect of different operations and technological solution on the activity of such enzymes with the aim of inhibiting their effect and prolonging the shelf life of produces. Among the tested solutions, the use of fungicides (Zoffoli et al., 1999; Lydakis & Aked, 2003), immersion in hot water (Fallik, 2004; Del Nobile et al., 2008), in ethanol or chlorinated water (Ahvenainen, 1996; Soliva-Fortuny & Martin-Belloso, 2003), the use of biodegradable films (Del Nobile et al., 2008; Rojas-Graü et al., 2009). Apart from the well-known positive effects on human health (Eshun & He, 2004), Aloe products have the potential to be exploited in food formulations thanks to their antimicrobial and antioxidant properties, however, processing steps necessary to obtain such produces can compromise the natural qualities (Di Scala et al., 2013) and, consequently, decrease the effectiveness. The use of *Aloe vera* as edible coating for the treatment of minimally processed fruits could be a possible alternative to the use of chemicals and has already been evaluated for the quality maintenance and post-harvest ripening modulation of different fruits and vegetable produces, such as avocado (Maftoonazad & Ramaswamy, 2005),

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cut apples (Lee et al., 2003), table grapes (Valverde et al., 2005; Serrano et al., 2006), sweet cherries (Alonso & Alique, 2004; Martínez-Romero et al., 2006), nectarines (Ahmed et al., 2009; Navarro et al., 2011), raspberries (Hassanpour, 2015), tomatoes (Athmaselvi et al., 2013), and mushrooms (Mohebbi et al., 2012). Moreover, the use of Aloe as coating matrix may result in the increase of the nutritional and health-promoting properties of minimally processed fruits. The effectiveness of *Aloe vera* treatments on grapes may vary depending on the moment of its application, whether in the pre-harvest or post-harvest stage (Valverde et al., 2005; Serrano et al., 2006). These authors evaluated overall multiple effects of *Aloe vera* on different table grape cultivars (Crimson Seedless, Vitis vinifera), such as antimicrobial effect against *Botrytis cinerea,* ripening delay, reduction of the antioxidant activity and effect on respiration. The present work aimed at assessing the suitability of three table grapes cultivars for transformation into a minimally processed, packed product, and at evaluating the effect of post-harvest treatments with *Aloe vera* and SO_2 emitters on grapes quality maintenance. The aim was pursued by monitoring the evolution of PPO, PME and β-GAL responsible for the main degradative reactions occurring during refrigerated storage, and the changes in some sensory, chemical and physical parameters.

2 Materials and methods

2.1 Raw material

Table grapes belonged to cultivars Sugar One, Victoria and Black Magic. Vines were cultivated on uplands in the territory of Chiaramonte Gulfi (Ragusa, Italy) characterized by sandy-clay soils, and grapes were manually harvested in June, July and September, respectively. Samples were harvested at commercial ripeness, which was assessed in accordance with the minimum requirements defined by the European Regulations (EU Reg. 543/2011) (European Commission, 2011).

2.2 Sample processing, packaging and storage conditions

Samples were transported by refrigerated vans and stored at 4 ± 0.5°C. Grapes were separated from bunches, washed in cold chlorinated water (sodium hypochlorite 2% v/v), and rinsed with tap water. For each cultivar a batch of control was compared with a batch treated with a commercial *Aloe vera* (var. Barbadenisis Miller) extract obtained from the juice and pulp of the leaf fillet (Aloe Vera Extra, Zuccari, Trento, Italy) and one batch packed with a SO_2 emitter (Productos Quimicos Alimenticios Osku SA, El Guanaco 5212/Hunchuraba-Santiago-Chile, 7.0 grams) applied to the package just before sealing. Treated grapes were carefully air-dried and packed into plastic trays (Melinex 850, Pavia, Italy) containing 150 g product each, sealed with an antifog film (thickness: 35μm; soldering range: 110-140°C; water vapour permeability: 4.3 g/(m^2 24h); oxygen permeability: 1100 cm^3/(m^2 24h); carbon dioxide permeability: 3000 cm^2/(m^2 24h). Trays were stored in a refrigerated chamber at 4°C. Analyses were performed in triplicate after 3, 6, 9, 12 and 15 days. Sensory, chemical and enzymatic analyses were performed on each batch to monitor the qualitative changes during storage.

2.3 Headspace gas composition

O_2 and CO_2 variations inside packages were monitored by a Dansensor Checkmate portable gas analyzer (PBI Dansensor, Denmark) during storage at 4°C on three packages for each batch at each sampling time.

2.4 Determination of pH, Brix degrees and acidity

pH of samples was determined on the grape juice by a Gemini BV pH-meter model Inolab 720 (Gemini BV, Apeldoorn, The Netherlands), previously calibrated with buffer solutions at pH 4 and pH 7. Acidity was determined by titration with 0.1 N NaOH until pH 8.0, and expressed as mg/L tartaric acid. Soluble solids were measured on filtered grape juice by a refractometer (Zeiss, mod. 16531), and expressed as Brix degrees at 20 °C.

2.5 Enzymatic analyses

Determination of PPO

To assess the activity of PPO, 10 grams of grapes are homogenized with 0.1 M citrate-phosphate (C-P) buffer and stirred for 2 hours at 4 °C. The sample was then centrifuged at 10,000 *g* for 20 minutes at 4 °C. After centrifugation, the pellet was separated from the supernatant and was vacuum filtered with Whatman filters. The raw extract was purified by ultrafiltration membranes. The test was carried out inserting 0.05 ml of dimethylformamide, 0.05M of sodium acetate buffer pH 4.2 1.5 ml, MBTH (2% in methanol) 0.1 ml, 40 mM catechol in 3 mM phosphoric acid and the enzyme 0.5 ml. The reaction was stopped with H2SO4 to 8% and was determined by spectrophotometric readings at 505 nm at 20 °C. This method is in agreement with Espín et al. (1996) suitably adapted.

Determination of PME

To evaluate the performance of PME, 10 grams of grapes are homogenized with 40 ml of 0.2 M citrate-phosphate (C-P) buffer at pH 7.0, 1M NaCl, 1 mM dl-dithiothreitol (DTT). This sample preparation was homogenized for 2 hours at 4°, then centrifuged at 4 °C for 10 minutes, filtered and ultrafiltrates with a cut-off of 10 kDa (Millipore, Bedford, MA, USA). The assay was performed by inserting 2 ml of 0.6% apple pectin in citrate-phosphate (C-P) 0.05 M buffer at pH 3.6, 0.5 ml of the previous solution extracted continuously stirred in a water thermostatized bath at 20±0.5 °C. The reaction is stopped by using 0.5 ml of H_2SO_4 1 N. From each of these solutions was determined the methanol, produced by PE agent on pectin. The solution was brought to volume and read spectrophotometrically at 620 nm.

Determination of β-GAL

To assess β-GAL activity, 10 g of homogenised grape was used along with 40 ml of 0.2 M citrate–phosphate (C–P) buffer at pH 4, 1 M NaCl and 1 mM DL-dithiothreitol (DTT). Each of these mixtures was homogenised for 2 h at 40 °C, centrifuged at 4000g for 10 min, filtered and ultrafiltered with a cutoff of 50 kDa (Biomax Pellicon, Millipore Headquarters, Billerica, MA, USA). The β-GAL activity of the extract was evaluated by

determining the amount of p-nitrophenol released from the corresponding substrate, p-nitrophenol- β-D-galactopyranoside (Sigma–Aldrich, Milan, Italy). The assay reagents included 0.55 ml of substrate 0.0055 M, 10 ml of C–P buffer 0.1 M at pH 4, and 0.30 ml of enzyme extract. After 30 min at 30 °C, the reaction was stopped by adding 1ml of 1M Na_2CO_3. The free p-nitrophenol was measured at 400 nm and activities were assessed in relation to the internal standard, p-nitrophenol. All tests were performed in triplicate at 95% confidence. Protein concentration was determined according to the dye-binding method of Bradford (1976), with bovine serum albumin as the standard.

2.6 Sensory analysis

Sensory analysis was conducted by a panel of 10 trained assessors, according to the Quality Index method suggested by López-Gálvez et al. (1997). Samples were submitted to visual evaluation by the panelists immediately after opening the packages. A numerical scale from 1 (very low) to 9 (maximum) was used to measure the following parameters: general appearance and browning.

2.7 Statistical analysis

Statistical analysis was performed by SPSS® Statistics 13.0 (Armonk, NY, USA) One-way analysis of variance (ANOVA) followed by post-hoc comparison of means based on the Tukey test was used to explore the significant differences among storage times. A 5% significance level was used for all statistical comparisons.

3 Results and discussion

3.1 Enzyme activities

The enzymatic activities (PPO, PME and β-GAL) of table grapes (cv. Sugar One, Victoria, Black Magic) were characterized in the fresh product (Figures 1a-c). The three cultivars analyzed showed comparable initial values of PPO activity, with slightly higher values for the cultivar Victoria. In addition, the three cultivars showed significant differences in the activity of PME and β-GAL with values of enzyme activity decreasing in the order: Black Magic> Sugar One> Victoria. Such enzymatic parameters were related respectively with general appearance and degree of gilding of the grapes for a period of cold storage of 15 days. Figures 2 a-c, respectively, show the total enzymatic activity (PPO, PME and β-GAL) during storage time for minimally processed grapes in response to the different treatments, i.e. dipping in *Aloe vera* and packaging with SO_2 emitters. According to Figure 2a, the untreated cultivar Sugar One was characterized by the highest total PPO activity, while the cultivar Victoria presented the lowest enzymatic activity. Treatment with *Aloe vera* significantly reduced ($p \leq 0.05$) the enzymatic activity of PPO only in cultivar Sugar One, despite presented the highest total polyphenoloxidase activity without any treatment compared to other cultivars. Regarding PME (Figure 2b), untreated Sugar One and Victoria cultivars showed high values of total activity comparable during cold storage, while the Black Magic was characterized by a lower PME activity. Treatment with *Aloe vera* was ineffective at inhibiting PME in all three cultivars, even in the case of the cultivar Black Magic it resulted in a significant increase ($p \leq 0.05$) of PME activity. As can be inferred from Figure 2c, the untreated cultivar Victoria showed the highest total β-GAL activity, while Sugar One and Black Magic showed comparable activity. Treatment with *Aloe vera* significantly reduced activity of β-GAL ($p \leq 0.05$) in the cultivars Black Magic and Victoria. Valverde et al. (2005) hypothesized some role of *Aloe vera* gel in the reduction of the activity of β-galactosidase, polygalacturonase and pectinmethylesteras, which are considered as the main cell wall degrading enzymes responsible for table grape softening (Nunan et al., 1998). Our study confirms this hypothesis, even if the effectiveness of Aloe seems to be cultivar-dependant. Further research is needed in order to understand the mechanism of action and the reasons for its selective effectiveness.

Overall, the treatment with *Aloe vera* was effective in all tested cultivars, in particular for the inhibition of PPO and PME, mainly responsible for the process of browning and softening of grapes. The effectiveness of *Aloe vera* in minimally processed fruit had been demonstrated using different fruits such as the pomegranate and sweet cherries (Ahmed et al.,

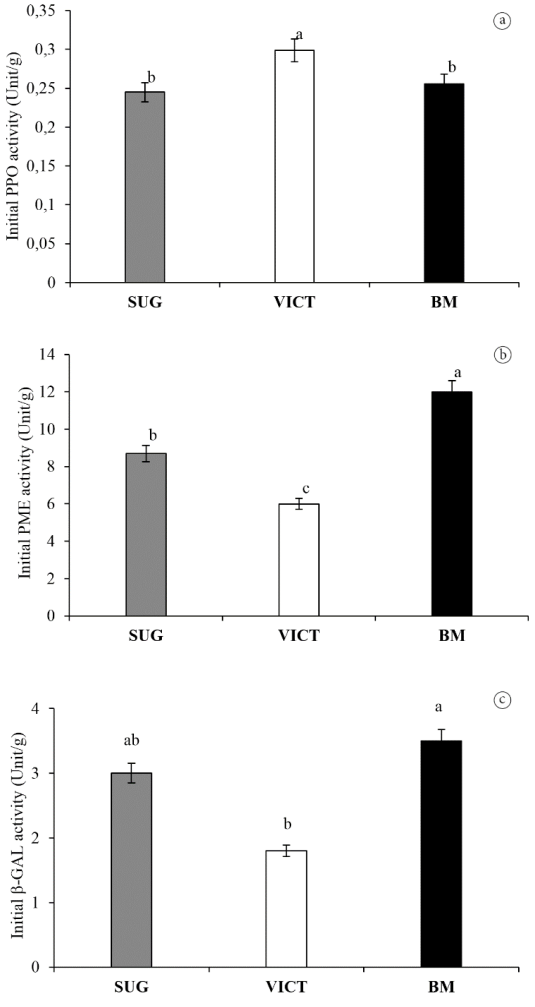

Figure 1. Initial PPO (a), PME (b) and β-GAL (c) activities in Sugar One (SUG), Victoria (VICT) and Black Magic (BM) table grape cultivars. Different letters indicate significant differences ($P \leq 0.05$) among cultivars.

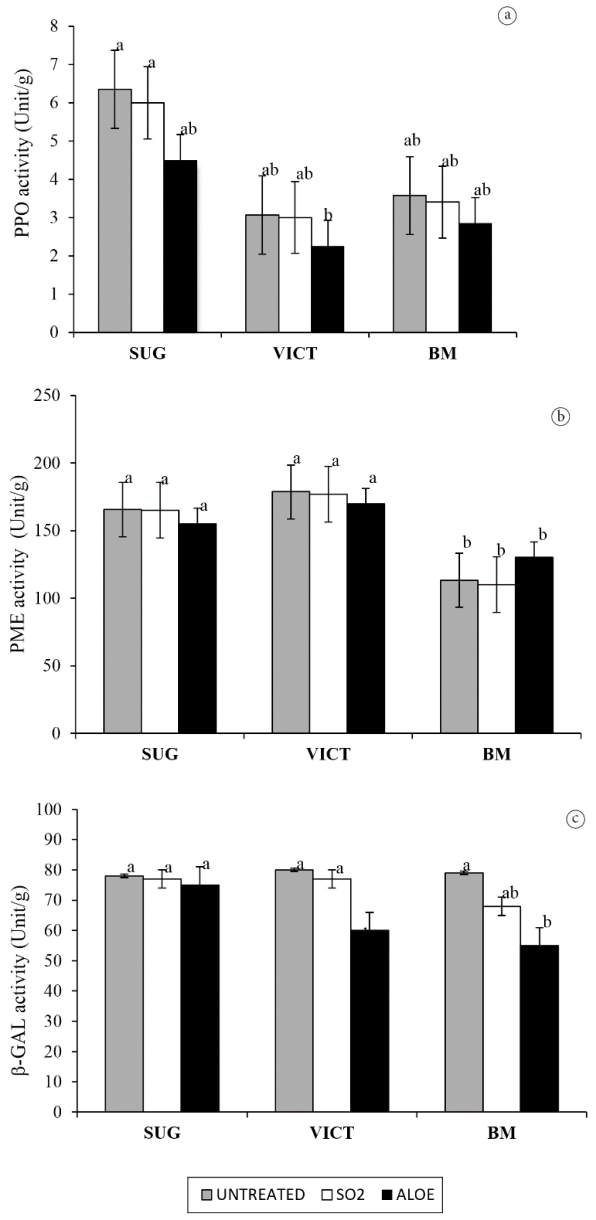

Figure 2. PPO (a), PME (b) and β-GAL (c) total activities during refrigerated storage for Sugar One (SUG), Victoria (VICT) and Black Magic (BM) table grape cultivars. ▒: untreated; □: SO_2 emitter; ■: Aloe-based dipping. Different letters indicate significant differences (P≤0.05) among cultivars.

2009; Conte et al., 2009). The addition of SO_2 emitters of as an alternative to the dipping did not give any advantage in terms of enzyme inhibition. Furthermore, the cultivar that, from the enzymatic standpoint, was found to be most suitable for minimal processing, was Black Magic.

3.2 Headspace gas composition

The gas composition inside packages of treated and control table grapes, which is a measure of the produce respiration extent, changed significantly particularly during the second week of refrigerated storage. Figures 3 a-c showed the increment of CO_2 and decrease of O_2 as a consequence of the respiration of the produce. The treatment with *Aloe vera* seems to reduce respiration in the Victoria and Black Magic cultivars, so that the CO_2 level after 15 days of storage did not exceed 25%, contrarily to untreated and SO_2-treated samples, and to Sugar One samples, irrespective of the treatment, for which the CO_2 level reached values as high as 35%. Studies in literature have led to controvertial results on the effect of modified and ordinary atmosphere for the preservation of fruits and vegetables (Siriphanick & Kader, 1985). The dipping with *Aloe vera* was effective at slowing down the production of CO_2 and depletion of O_2 in Victoria and Black Magic cultivars, this result is in agreement with a previous study (Valverde et al., 2005). The reduction of respiration determined by the application of coatings has been observed in various fruits, such as avocado (Maftoonazad & Ramaswamy, 2005), cut apples (Lee et al., 2003), table grapes (Valverde et al., 2005) and sweet cherry (Alonso & Alique, 2004; Martinez-Romero et al., 2006), and is due to the partial barrier to gas exchange which, in turn, allows the creation of an internal modified atmosphere (Banks et al., 1993). On the other hand, the respiration of the Sugar One cultivar was slightly reduced by the SO_2 treatment, which was ineffective on the other two cultivars.

3.3 Physicochemical parameters

The physicochemical features and statistical significance of differences among means are shown in Table 1. In particular, pH, acidity and °Brix were monitored during 15 days of refrigerated storage. All untreated samples were characterized by a pH around 3.5, and mean values did not differ significantly (p≤0.05) in the fresh produce, irrespective of the cultivar. The addition of *Aloe vera* did not determine significant pH differences in any of the cultivars during storage, as well as the use of SO_2 emitters. Acidity did not vary significantly among cultivars, and showed only a slight reduction during refrigerated storage.

Table 1 also shows the °Brix values during refrigerated storage. The value in the fresh produce was significantly lower for the Victoria cultivar (around 10%) compared to Sugar One and Black Magic varieties, which had a soluble solids content around 13%. The °Brix did not vary significantly during refrigerated storage with any of the treatments used.

3.4 Sensory analysis

At the beginning of the experiment all batches from each minimally processed table grape cultivars, irrespective of treatment, showed high overall quality scores (Figure 4). In particular, during the first week of storage the berries presented an extremely shiny, clearly defined color, without traces of browning in the zone of the petiole. This could be due to the fact that grapes were not cut and minimal damage was caused to the fruits before packaging. Indeed, browning, senescence and the increase of respiration rate depend on the extent of damage and cell ruptures (Böttcher et al., 2003; Del Nobile et al., 2007). In other fruit varieties such as mango, banana, strawberries and water melon, cutting accelerated the browning processes (Cocci et al., 2006). According to a study by Ke & Saltveit (1989), the PAL activity is stimulated by cutting much more than by ethylene (Spagna et al., 2005). Samples packed with SO_2

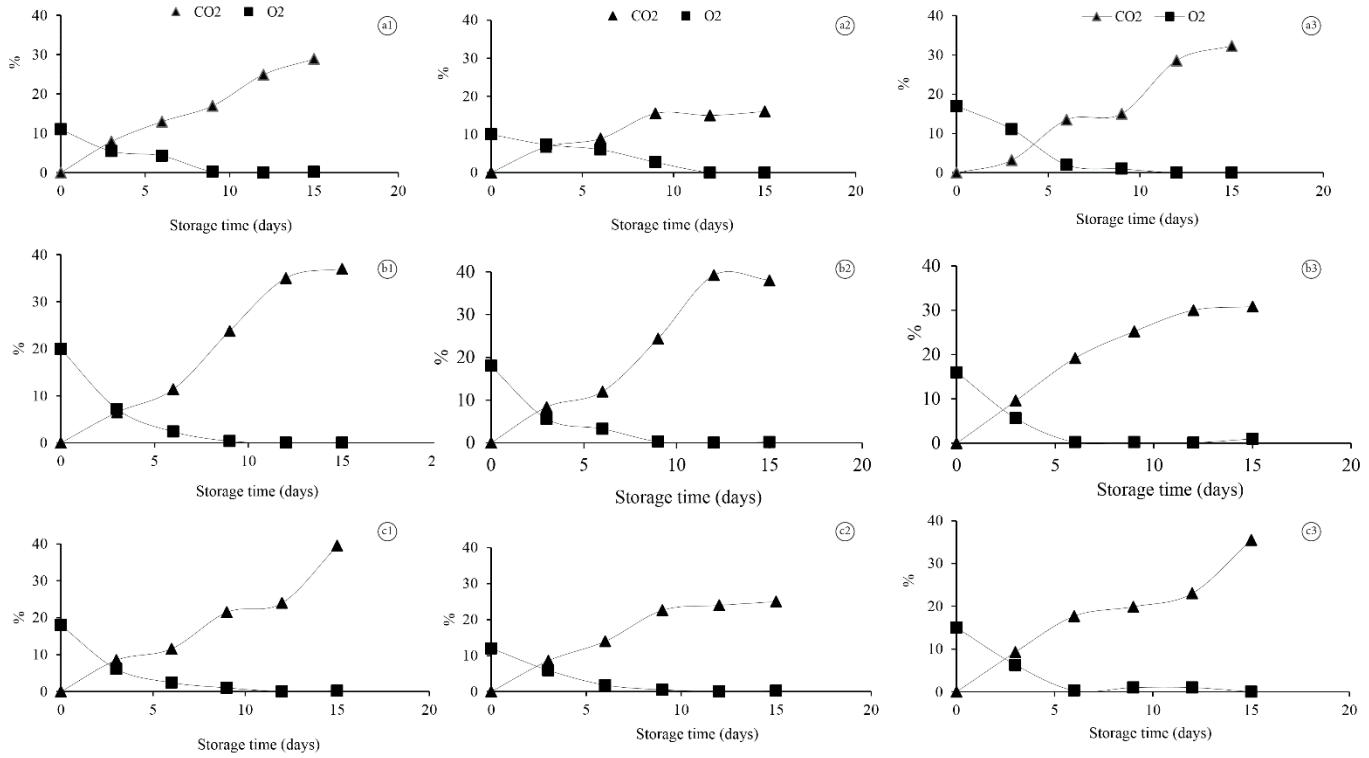

Figure 3. Gas evolution (O_2, CO_2) inside the packaging during cold storage for Victoria (figures a), Sugar One (figures b) and Black Magic (figures c). 1: no treatment; 2: Aloe-based dipping; 3: treatment with SO_2.

Table 1. Ph °BRIX and acidity of Sugar One (SUG). Black Magic (BM) and Victoria (VICT) table grape cultivars during refrigerated storage. as a function of treatment.

Time (days)	pH						°Brix						Acidity (mg/L)					
	0	3	6	9	12	15	0	3	6	9	12	15	0	3	6	9	12	15
SUG	3.67	3.76	3.566 ^{ab}	3.73 ^{b}	3.73 ^{b}	3.70 ^{b}	11.93 ^{bc}	12.13 ^{b}	12.00 ^{b}	12.07 ^{b}	12.03 ^{abc}	11.97 ^{bc}	1.20 $_{A}$	1.90 $_{B}$	1.97 $^{b}_{B}$	2.07 $^{c}_{B}$	2.00 $^{c}_{B}$	2.00 $^{b}_{B}$
SUG Aloe	3.60	3.60	3.67 ^{ab}	3.75 ^{b}	3.73 ^{b}	3.50 ^{ab}	13.93 ^{d}	13.97 ^{cd}	13.96 ^{c}	13.93 ^{c}	13.90 ^{cd}	13.97 ^{d}	1.52 $_{B}$	1.53 $_{B}$	1.07 $^{a}_{A}$	1.00 $^{a}_{A}$	1.07 $^{ab}_{A}$	1.03 $^{ab}_{A}$
SUG SO_2	3.53	3.73	3.30 ^{ab}	3.03 ^{a}	3.20 ^{a}	3.07 ^{a}	13.87 ^{d}	13.97 ^{cd}	13.83 ^{c}	13.97 ^{c}	13.90 ^{cd}	14.07 ^{d}	1.33	1.40	1.10 ^{a}	1.03 ^{a}	1.07 ^{ab}	1.00 ^{a}
BM	3.62 $_{B}$	3.53 $_{B}$	2.9 $^{a}_{A}$	3.63 $^{ab}_{B}$	3.90 $^{b}_{B}$	3.73 $^{b}_{B}$	11.97 ^{bc}	11.97 ^{b}	11.93 ^{b}	11.77 ^{b}	11.82 ^{ab}	12.40 ^{c}	1.40 $_{AB}$	1.47 $_{AB}$	1.00 $^{a}_{A}$	1.93 $^{c}_{B}$	1.03 $^{a}_{A}$	1.33 $^{ab}_{AB}$
BM Aloe	3.57	3.57	3.9 ^{b}	3.73 ^{b}	3.73 ^{b}	3.90 ^{b}	14.67 ^{d}	14.93 ^{d}	14.67 ^{c}	14.67 ^{c}	15.00 ^{d}	14.43 ^{d}	1.33	1.40	1.10 ^{a}	1.03 ^{a}	1.07 ^{ab}	1.00 ^{a}
BM SO_2	3.03 $_{A}$	3.90 $_{B}$	3.53 $^{ab}_{AB}$	3.60 $^{ab}_{AB}$	3.90 $^{b}_{B}$	3.10 $^{a}_{A}$	12.33 ^{c}	12.63 ^{bc}	12.53 ^{b}	12.13 ^{b}	12.67 ^{bc}	12.00 ^{bc}	1.40 $_{AB}$	1.47 $_{AB}$	1.00 $^{a}_{A}$	1.93 $^{c}_{B}$	1.03 $^{a}_{A}$	1.33 $^{ab}_{AB}$
VICT	3.50	3.52	3.43 ^{ab}	3.60 ^{ab}	3.63 ^{ab}	3.63 ^{ab}	10.27 ^{a}	9.93 ^{a}	10.00 ^{a}	10.50 ^{a}	10.67 ^{a}	10.33 ^{a}	1.33	1.40	1.10 ^{a}	1.03 ^{a}	1.07 ^{ab}	1.00 ^{a}
VICT Aloe	3.53	3.53	3.45 ^{ab}	3.50 ^{ab}	3.52 ^{ab}	3.50 ^{ab}	10.67 ^{ab}	11.67 ^{b}	11.33 ^{b}	11.63 ^{b}	11.33 ^{ab}	11.00 ^{ab}	1.40 $_{AB}$	1.47 $_{AB}$	1.00 $^{a}_{A}$	1.93 $^{c}_{B}$	1.03 $^{a}_{A}$	1.33 $^{ab}_{AB}$
VICT SO_2	3.52	3.47	3.53 ^{ab}	3.47 ^{ab}	3.93 ^{b}	3.97 ^{b}	12.33 ^{c}	11.97 ^{b}	12.27 ^{b}	12.00 ^{b}	11.87 ^{ab}	12.33 ^{c}	1.57	1.50	1.23 ^{a}	1.50 ^{b}	1.30 ^{b}	1.20 ^{ab}

Different small letters in columns indicate significant difference (P≤0.05) among average values relative to different treatments. Different capital letters in lines indicate significant difference (P≤0.05) among average values relative to different storage times.

emitters did not show significative differences compared with the untreated samples.

Treatment with *Aloe vera* was found to be more effective in its first week on the cultivar Black Magic, while from the second week this treatment was found to be ineffective.

Figure 5 shows that all samples maintained low browning scores until 6 days of storage, afterwards some signs of alteration appeared, with special regards for the zone of the insertion of the petiole, as consequence of the petiole removal. Aloe-based treatment was able to maintain the general visual quality of Sugar One and Victoria by significantly reducing browning (Figures 5 a, b). This effect confirms a protective effect of Aloe, which has been previously demonstrated and is to be mainly attributed to the antioxidant capacity of its components (Hu et al., 2005).

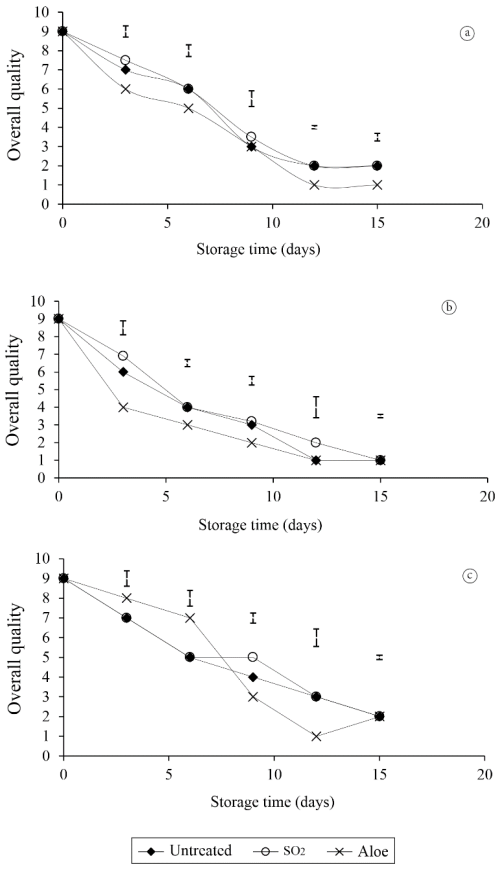

Figure 4. Overall quality for Sugar One (a), Victoria (b) and Black Magic (c) table grape cultivars as a function of treatment: –◆– untreated; –○– SO2 emitter; –×– Aloe-based dipping. Vertical bars indicate the least significant difference (multiple range test, $P \leq 0.05$).

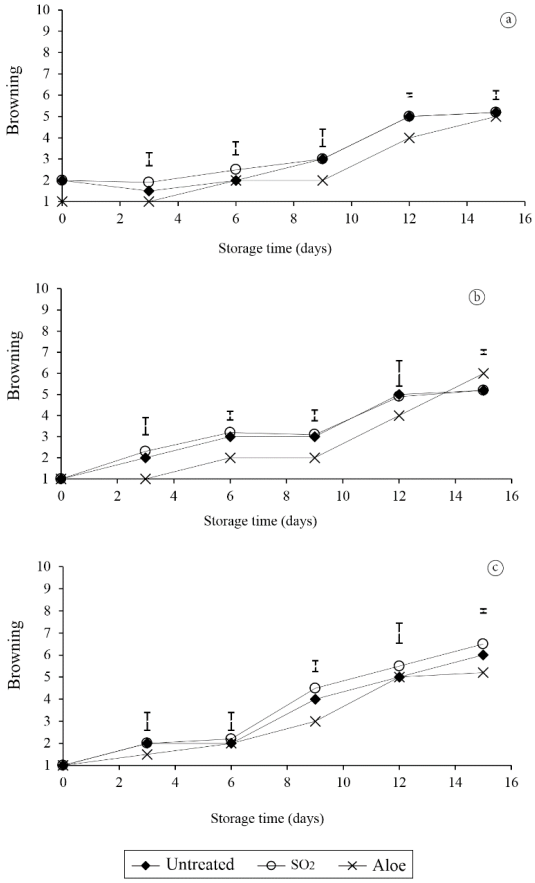

Figure 5. Browning degree of Sugar One (a), Victoria (b) and Black Magic (c) table grape cultivars as a function of treatment: –◆– untreated; –○– SO2 emitter; –×– Aloe-based dipping. Vertical bars indicate the least significant difference (multiple range test, $P \leq 0.05$).

4 Conclusions

Results point out some differences in the response of three table grape cultivars to minimal processing. Dipping into an *Aloe vera* extract allowed to reduce the respiration rate of Victoria and Black Magic cultivars and was effective at reducing the enzymatic activities commonly considered as responsible for the quality decay. Also, the general aspect and browning extent confirmed the ability of table grapes coating with *Aloe vera* to maintain better scores for at least 6 days compared to untreated table grapes. Dipping into *Aloe vera* represents a natural tool for the quality maintenance of minimally processed table grapes, improving the produce nutritional value and allowing to reduce the recourse to synthetic additives.

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